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UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Departamento de Ingeniería Química

Fate of Pharmaceutical and Personal Care Products (PPCPs) in Sewage Treatment Plants focusing on the anaerobic digestion of sludge

Memoria presentada por

Marta Carballa Arcos

Para optar al grado de Doctor por la
Universidade de Santiago de Compostela

Santiago de Compostela, Septiembre de 2005

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Departamento de Ingeniería Química

Juan Manuel Lema Rodicio, Catedrático de Ingeniería Química y Francisco Omil Prieto, Profesor Titular de Ingeniería Química de la Universidade de Santiago de Compostela,

Informan:

Que la memoria titulada “Fate of Pharmaceutical and Personal Care Products (PPCPs) in Sewage Treatment Plants focusing on the anaerobic digestion of sludge”, que para optar al grado de Doctor en Ingeniería Química, Programa de Doctorado en Ingeniería Química y Ambiental, presenta Doña Marta Carballa Arcos, ha sido realizada bajo nuestra inmediata dirección en el Departamento de Ingeniería Química de la Universidade de Santiago de Compostela.

Y para que así conste, firman el presente informe en Santiago de Compostela, el 19 de Septiembre de 2005.

Juan M. Lema Rodicio

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Vive tus sueños y no sueñes tu vida

Live your dreams and don't dream your life

A mis padres: Aquilino y Rosa

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Objetivos y Resumen

En las últimas décadas, el impacto de la contaminación química se ha enfocado casi exclusivamente en los contaminantes convencionales prioritarios, especialmente aquellas sustancias que, como los pesticidas u otros intermedios industriales descargados al medio ambiente, se consideran altamente tóxicos y con potencial carcinogénico. Sin embargo, este tipo de compuestos sólo constituye una parte muy pequeña del total de sustancias que se están vertiendo en el entorno.

En los últimos años, parece que la preocupación por la contaminación de las aguas producida como consecuencia de actividades industriales, sin dejar de ser un factor de permanente preocupación, poco a poco va dejando ver en el escenario otro tipo de contaminación derivada de los denominados "contaminantes emergentes", que incluyen tanto los fármacos de uso veterinario y humano, como los productos de limpieza y desinfección (antisépticos, utilizados en enormes cantidades en hospitales, limpieza doméstica y cría de ganado). Un problema obvio que diferencia ambos tipos de contaminación se deriva de la imposibilidad práctica de actuar en contra de la producción o utilización e incluso de la forma de eliminación de estos últimos. Mientras que para los compuestos derivados de la actividad industrial es posible una labor de vigilancia en etapas de producción y uso, política y socialmente, es impensable en la actualidad una regulación en este sentido para los productos destinados a la higiene y la salud.

Dentro de este extenso conjunto de sustancias, se encuentran los productos farmacéuticos y de cuidado personal ("Pharmaceutical and Personal Care Products", PPCPs), cuya existencia en el medio ambiente debe sus orígenes inmediatos al uso universal, frecuente, altamente disperso e individualmente pequeño pero acumulativo por parte de multitud de individuos, al contrario de lo que ocurre con la mayoría de los productos químicos industriales de gran

volumen. La introducción de PPCPs en el medio ambiente no tiene limitaciones geográficas ni climáticas como sucede con muchos otros productos sintéticos.

Los PPCPs constituyen un grupo que abarca una gran diversidad de compuestos químicos bioactivos, en el que se incluyen no sólo los medicamentos prescritos, sino también los agentes de diagnóstico (medios de contraste de rayos X), productos de limpieza, cosméticos, fragancias, agentes de protección solar y otros muchos.

Los PPCPs comprenden numerosas clases químicas, con estructuras muy variadas, de manera que abarcan un gran espectro de propiedades. Muchos de ellos son altamente bioactivos y ópticamente activos, siendo la mayoría de carácter polar. Los medicamentos se diferencian de los productos químicos de uso agrícola básicamente en que poseen múltiples grupos funcionales. Normalmente presentan unas dosis efectivas bajas (inferiores a $mg \cdot kg^{-1}$), lo cual dificulta no sólo su detección, que requiere de técnicas analíticas especiales, sino también su evolución en el medio. La mayoría de los productos farmacéuticos no son bioacumulativos, aunque muchos productos de cuidado personal, tales como las fragancias y los agentes de protección solar, suelen ser más lipofílicos y, por tanto, susceptibles a la bioconcentración.

Los productos farmacéuticos, como parte esencial de la medicina humana y veterinaria, son absorbidos, distribuidos, metabolizados de forma incompleta y finalmente, excretados sin cambios y en cantidades variables en la orina y las heces.

La mayoría de estos compuestos, bien por arrastre de aguas y escorrentías, porque son excretados y entran a formar parte de las aguas residuales domésticas, o porque son un desecho más de un hospital o proceso industrial, entran en el medio normalmente en las Estaciones de Depuración de Aguas Residuales urbanas (EDAR). En este sentido, las depuradoras modernas se han diseñado para ser herramientas muy efectivas en lo que respecta a tratar los problemas relacionados con la contaminación carbonada, nitrogenada y microbiana. Sin embargo, especialmente en las zonas urbanas, las aguas residuales pueden contener una multitud de compuestos sintéticos y naturales que no han sido considerados en el diseño y operación de los procesos de depuración, resultando en eliminaciones parciales y permitiendo finalmente la llegada de estos compuestos a los medios acuáticos receptores. La eficacia de eliminación en las

EDAR o en los sistemas de potabilización varía en función de la estructura y concentración del PPCP y del tipo de tratamiento empleado.

Por lo tanto, los PPCPs son capaces de atravesar los sistemas actuales de tratamiento de aguas residuales, difundiéndose de forma continua en el medioambiente a través de las descargas de las plantas de tratamiento municipales. Así, han sido detectados en aguas de abastecimiento, aguas superficiales y aguas subterráneas en niveles del orden de $ng \cdot L^{-1}$-$\mu g \cdot L^{-1}$.

El hecho de que los PPCPs puedan entrar continuamente en el medio acuático, les confiere la característica de "persistentes", ya que aunque tuviesen una baja estabilidad ambiental, su posible eliminación/transformación (mediante biodegradación, hidrólisis, fotolisis, etc.) está continuamente contrarrestada por su reposición continua.

Es especialmente importante la contaminación acuática, porque los organismos acuáticos están sometidos a una exposición multigeneracional durante su largo ciclo de vida. La posibilidad de efectos continuados pero indetectables o imperceptibles en los organismos acuáticos es particularmente preocupante, porque estos efectos podrían acumularse tan lentamente que el mayor cambio no sería detectado hasta que el nivel acumulado de estos efectos desencadenase finalmente un cambio irreversible, cambio que de alguna forma sería atribuido a la adaptación natural o a la sucesión ecológica.

Para reducir los riesgos de los efectos impredecibles a largo plazo de los PPCPs en el medioambiente y salud humana, y evitar el consumo durante largos periodos de tiempo de dosis bajas de PPCPs potencialmente tóxicos a través del agua de abastecimiento, en el contexto del V Programa Marco de investigación de la Comisión Europea, diversos grupos de investigación y empresas de diferentes países han puesto en marcha un proyecto que, bajo la denominación de POSEIDON (*Assessment of Technologies for the Removal of Pharmaceutical and Personal Care Products in Sewage and Drinking Water Facilities to Improve the Indirect Potable Water Reuse*), aborda el desarrollo de los métodos analíticos para determinar la presencia de diferentes grupos de PPCPs en aguas residuales y naturales, y evaluar la eficacia de los sistemas de tratamiento actuales en la eliminación, físico-química o biológica, de estos compuestos, reduciendo así las descargas incontroladas de PPCPs al medio natural. Es más, este proyecto intentará mejorar el aporte eficiente y limpio de agua y especificar los riesgos potenciales de los PPCPs en el medioambiente. Los compuestos objeto de estudio

han sido seleccionados de acuerdo con su utilización en la industria y en consumo familiar, su producción anual, las concentraciones encontradas en el medio ambiente y factibilidad de análisis a niveles por debajo del $ng \cdot L^{-1}$. Estos compuestos son: Galaxolide y Tonalide (fragancias), Diazepam (tranquilizante), Carbamazepina (antiepiléptico), Ibuprofen, Naproxen y Diclofenac (antiinflamatorios), Roxithromicina y Sulfamethoxazol (antibióticos), Iopromide (medio de contraste de rayos X) y los estrógenos (17β-estradiol, Estrona, 17α-ethinylestradiol).

Este trabajo, realizado bajo el marco del proyecto POSEIDON, tiene como objetivo global el estudio del comportamiento de los compuestos seleccionados a lo largo de las diferentes unidades de tratamiento de una EDAR urbana, prestando especial atención al tratamiento de lodos. Para la consecución de este objetivo global, se han propuesto los siguientes objetivos específicos:

i) Investigación de la existencia y niveles de concentración de estos compuestos en las aguas residuales tratadas en una EDAR urbana situada en Galicia (100.000 habitantes equivalentes, aproximadamente). Además, se estudió el comportamiento de estas sustancias en las diferentes unidades de tratamiento de la planta (Capítulo 3).

ii) Estudio del mecanismo (volatilización, adsorción/absorción y degradación) responsable de la eliminación de estos compuestos en EDAR (Capítulo 4).

iii) Estudio de la influencia de procesos físico-químicos, tales como coagulación-floculación y flotación, en la eliminación de estas sustancias durante el tratamiento primario en EDAR (Capítulo 5).

iv) Estudio del comportamiento de estas sustancias durante la digestión anaerobia de lodos en rango mesofílico y termofílico a diferentes tiempos de retención celular (Capítulo 6).

v) Estudio de la influencia de diferentes pretratamientos de lodos (alcalino, térmico y ozonización) en la dinámica del proceso de estabilización de lodos y en el comportamiento de PPCPs durante este tratamiento (Capítulo 7).

vi) Caracterización del lodo digerido en términos de materia orgánica, nutrientes, patógenos, metales pesados, compuestos sulfonados alquilbencénicos lineales (LAS) y propiedades de deshidratación (Capítulo 8).

En el Capítulo 1 se hace una revisión bibliográfica de los PPCPs, que incluye tasas de prescripción, farmacocinéticas de excreción, propiedades físico-químicas (peso molecular, coeficientes de distribución sólido-líquido y gas-líquido, solubilidad) orígenes y vías de expansión en el medioambiente, niveles de concentración en diferentes compartimentos ambientales, tales como aguas residuales, aguas superficiales (ríos, lagos, etc.), aguas subterráneas, aguas potables, lodos (primarios y biológicos), materia sólida particulada y sedimentos, comportamiento en EDAR urbanas (eficacias de eliminación) y efectos medioambientales. En la segunda parte de este capítulo, se realiza una revisión de los lodos residuales procedentes de EDAR urbanas. Esta revisión incluye definición y tipos de lodo, tasas de producción, principales características (materia orgánica, nutrientes, metales pesados, patógenos y contaminantes orgánicos), tecnologías de tratamiento (acondicionamiento, espesamiento, deshidratación, secado, estabilización y desinfección) y uso final (agricultura, incineración, vertedero y otras rutas).

En el Capítulo 2 se describen los métodos analíticos usados en el trabajo. Esto incluye tanto los parámetros convencionales de caracterización de aguas residuales y lodos (alcalinidad, producción y composición del biogás, demanda bioquímica de oxígeno, demanda química de oxígeno, compuestos inorgánicos, contenido en nitrógeno, contenido en grasa, pH, temperatura, sólidos, contenido en carbono y ácidos grasos volátiles) como los análisis de los PPCPs. En este último caso, la metodología analítica depende no sólo del tipo de matriz (líquido o sólido) sino también de las propiedades específicas de cada compuesto. Por lo tanto, los métodos han sido clasificados para fragancias (Galaxolide y Tonalide), compuestos neutros (Carbamazepina y Diazepam), compuestos ácidos (Ibuprofen, Naproxen y Diclofenac), antibióticos (Sulfamethoxazol y Roxithromicina), medios de contraste (Iopromide) y estrógenos (Estrona, 17β-estradiol y 17α-ethinylestradiol). Estos análisis incluyen una etapa previa de preparación de muestra (filtración para muestras líquidas y extracción para muestras sólidas), seguida de una etapa de concentración, realizada normalmente mediante una extracción en fase sólida, y limpieza (si es necesaria) con silica-gel. Finalmente, la detección se realiza por cromatografía gaseosa o líquida acoplada con espectrofotometría de masas.

En el Capítulo 3 se estudió la presencia y niveles de concentración de los compuestos seleccionados en una EDAR urbana situada en Galicia. Para ello, se

realizaron 4 campañas de muestreo integradas (primavera, verano, otoño e invierno) durante los años 2001 y 2002. De las 13 sustancias consideradas, han sido detectadas 8: Galaxolide, Tonalide, Ibuprofen, Naproxen, Iopromide, Sulfamethoxazol, Estrona y 17β-estradiol. Los otros compuestos (Carbamazepina, Diazepam, Diclofenac, Roxithromicina y 17α-ethinylestradiol) no fueron detectados o por debajo de los límites de cuantificación. Durante el tratamiento primario, solamente las fragancias (30-50%) y 17β-estradiol (20%) son eliminados parcialmente. Por el contrario, todos los compuestos detectados se eliminan durante el tratamiento biológico con eficacias entre 35 y 75%, a excepción de Iopromide, que no se ve afectado por la línea de tratamiento de la EDAR. Las eficacias globales de eliminación en la planta varían entre 70 y 90% para las fragancias, entre 40 y 65% para los antiinflamatorios y alrededor del 60% para Sulfamethoxazol y 17β-estradiol. Sin embargo, la concentración de Estrona aumenta a lo largo del tratamiento, probablemente debido a la ruptura de las formas glucurónicas y a la oxidación parcial del 17β-estradiol.

El siguiente paso es profundizar en el mecanismo responsable de la eliminación de estos compuestos en las EDAR: volatilización, adsorción/absorción ó degradación (Capítulo 4). La volatilización resultó despreciable para estos compuestos debido a los bajos valores del coeficiente de Henry (H). Para las fragancias, que son las sustancias con los valores de H más altos, como máximo se estima que se puede eliminar hasta un 5% por volatilización. Por lo tanto, los principales mecanismos a estudiar son adsorción/absorción y degradación. Para diferenciarlos, se realizaron balances de materia, incluyendo la fase sólida, de los compuestos detectados en la EDAR. Para la realización de estos balances, se proponen 2 métodos, cuya diferencia es la forma de determinar la fracción de PPCPs asociada a los sólidos. El método I usa valores de concentración *medidos* en la fase sólida, y en el método II, la concentración en la fase sólida se calcula a partir de la concentración en la fase líquida, usando los coeficientes de distribución sólido-líquido (K_d). Esto también nos permite comparar los resultados obtenidos por ambos métodos, analizando así la idoneidad de cada uno de ellos. Los resultados obtenidos indican que las fragancias están fundamentalmente absorbidas en el lodo (aunque también se observa degradación) y los productos farmacéuticos se degradan biológicamente en el tanque de aireación (lodos activos). De la comparación de los resultados obtenidos por ambos métodos, se concluye que para compuestos polares, con tendencia a permanecer en la fase líquida, no hay diferencias entre ambas

métodologías de cálculo. Por lo tanto, el método II sería el más adecuado para estos compuestos ya que evitaría los análisis de la fase sólida. Sin embargo, para sustancias con alta probabilidad de adsorción/absorción en sólidos, el método II puede dar lugar a infravaloraciones de la concentración en fase sólida, llevando a conclusiones erróneas en cuanto al mecanismo de eliminación. Por lo tanto, para estos compuestos, el método I parece el más conveniente.

En el Capítulo 5 se evalúan dos procesos físico-químicos, coagulación-floculación y flotación, para mejorar la eliminación de PPCPs durante el tratamiento primario en EDAR. Para este estudio se seleccionaron 8 compuestos representativos de los 3 grupos principales de PPCPs de acuerdo con sus propiedades físico-químicas: compuestos lipofílicos (fragancias), compuestos neutros (Carbamazepina y Diazepam) y compuestos ácidos (antiinflamatorios). Durante los ensayos de coagulación-floculación, se ha analizado la influencia del tipo de aditivo (cloruro férrico, sulfato de aluminio y policloruro de aluminio), su dosis y la temperatura (12 y 25ºC, intentando simular condiciones de invierno y verano, respectivamente). Las mayores eficacias de eliminación se obtuvieron para las fragancias y para Diclofenac (70%). Los otros compuestos se eliminaron en menor medida (<25%). Las excepciones fueron Carbamazepina e Ibuprofen, que no se vieron afectados por ninguna de las condiciones ensayadas. En general, no se observó influencia alguna de la temperatura y de la dosis de aditivo, resultando el cloruro férrico el coagulante más eficaz. Durante los ensayos de flotación, se ha estudiado el efecto de la concentración inicial de grasa en las aguas residuales y la temperatura. De nuevo, las mayores eliminaciones se obtuvieron para las fragancias (35-60%), seguidas de Diazepam (40-50%) y Diclofenac (20-45%) y, en menor medida, Carbamazepina (20-35%), Ibuprofen (10-25%) y Naproxen (10-30%). Se lograron mayores eficacias de eliminación a 25ºC, aunque para algunos compuestos, los resultados fueron similares a ambas temperaturas. La eliminación de fragancias y compuestos neutros fue mayor cuando se usaron aguas con alto contenido en grasa (sobre 150 $mg \cdot L^{-1}$).

En el Capítulo 6 se estudió el comportamiento de los PPCPs seleccionados durante la digestión anaerobia convencional de lodos procedentes de EDAR. Los parámetros considerados fueron la temperatura (condiciones mesófilas y termófilas) y el tiempo de retención celular (10, 20 y 30 d para el digestor mesófilo; 6, 10 y 20 d para el digestor termófilo). Esto también permitió hacer una comparación entre la operación de digestión anaerobia de lodos a diferentes

temperaturas y tiempos de retención. Los resultados indican eliminaciones de sólidos y materia orgánica entre 50 y 70% en ambos digestores, obteniéndose mejores resultados cuando se opera a velocidades de carga orgánica baja (altos tiempos de retención celular), independientemente de la temperatura. Cuando ambos digestores se operaron con el mismo tiempo de retención, se lograron mayores eficacias de eliminación en el digestor termófilo, indicando esto el efecto de la temperatura en el grado de estabilización del lodo. En lo referente a PPCPs, las conclusiones obtenidas de la operación convencional de digestión anaerobia son: i) eliminación muy alta (>80%) de Naproxen, Sulfamethoxazol y Roxithromicina; ii) eliminación alta (60-80%) de Galaxolide, Tonalide y los estrógenos naturales (Estrona y 17β-estradiol); iii) eliminación intermedia (30-60%) de Ibuprofen; iv) eliminación baja (<40%) de Iopromide; y v) no eliminación de Carbamazepina. La eliminación de Diazepam, Diclofenac y 17α-ethinylestradiol tuvo lugar tras un proceso de adaptación del lodo. En general, no se observó influencia ni del tiempo de retención celular ni de la temperatura.

Recientemente, se han desarrollado nuevas tecnologías de tratamiento para mejorar el reciclaje y reuso de lodos. Debido a que la hidrólisis es la etapa limitante del proceso de digestión anaerobia de lodos, estas tecnologías se basan fundamentalmente en pretratamientos antes del proceso biológico de estabilización con el objetivo de promover y mejorar la solubilización de la materia orgánica. En el Capítulo 7 se estudia la influencia de estos pretratamientos en la dinámica del proceso de estabilización de lodos mediante digestión anaerobia y en el comportamiento de los PPCPs durante este tratamiento. Entre los diferentes métodos disponibles, se han elegido para este estudio tres procesos: químico (alcalino), térmico y oxidativo (ozonización). A igual que en el Capítulo 6, los parámetros considerados fueron la temperatura (condiciones mesófilas y termófilas) y el tiempo de retención celular (10 y 20 d para el digestor mesófilo; 6 y 10 d para el digestor termófilo). Los porcentajes de solubilización de materia orgánica obtenidos con el proceso térmico y oxidativo con ozono son similares (60%). Sin embargo, el tratamiento alcalino incrementa este valor hasta un 80%. Las eficacias de eliminación de sólidos y materia orgánica varían entre 40 y 70% en ambos digestores, con pequeñas influencias de la temperatura, tiempo de retención celular y tipo de pretratamiento. En cuanto a los PPCPs, la eliminación de Naproxen, Iopromide y Sulfamethoxazol no se ve afectada por ningún pretratamiento. Por el contrario, con el proceso oxidativo con ozono se obtiene una eliminación de Carbamazepina entre 20 y 50%, mientras

que con los otros pretratamientos y en el proceso convencional, esta sustancia no se ve afectada. Para el resto de los compuestos se observan pequeñas influencias: i) la eliminación de Roxithromicina en el digestor mesófilo se ve afectada negativamente por el proceso alcalino; ii) la eliminación de Ibuprofen en el digestor mesófilo se ve afectada positivamente por el proceso térmico; y, iii) el tratamiento con ozono influye positivamente en la eliminación de las fragancias en condiciones mesófilas y negativamente en la eliminación de Tonalide (en el digestor termófilo) e Ibuprofen (en ambos digestores). De nuevo, la eliminación de Diazepam, Diclofenac y los estrógenos está más relacionada con la adaptación del lodo que con las condiciones de operación de los digestores.

Debido a la existencia de una creciente preocupación acerca de los riesgos para la salud humana y para el medio ambiente que conlleva el uso agrícola de los lodos de depuradora, el debate acerca del reciclaje y uso/disposición final de lodos ha sido un tema de gran interés en los últimos años. Por ello, el lodo digerido obtenido en las diferentes condiciones experimentales ensayadas en este trabajo se ha caracterizado en términos de materia orgánica, nutrientes, patógenos, metales pesados, contaminantes orgánicos y propiedades de deshidratación (Capítulo 8). La mayoría de las condiciones ensayadas han resultado eficientes para no alcanzar los valores límite propuestos en el Documento de Trabajo sobre lodos y el uso de los pretratamientos seleccionados asegura este logro.

Finalmente, se han añadido dos anexos al final de este trabajo con los siguientes objetivos: i) Estudiar la influencia del parámetro más crucial del balance de PPCPs en los digestores (la alimentación) en las eficacias de eliminación obtenidas (Anexo I); y, ii) Indicar los datos usados en los Capítulos 6 y 7 (Anexo II).

Obxectivos e Resumo

Nos últimos anos, varios estudios en Europa e USA indican a presencia de productos farmacéuticos e de coidado persoal (Pharmaceutical and Personal Care Products, PPCPs) en diferentes compartimentos líquidos (augas residuais, augas superficiais, augas subterráneas) e sólidos (lodos, materia particulada, sedimentos). En xeral, as concentracións de PPCPs en augas e sólidos varían dende $ng \cdot L^{-1}$ ata $\mu g \cdot L^{-1}$ nivel e dende $ng \cdot g^{-1}$ ata $\mu g \cdot g^{-1}$, respectivamente. Sen embargo, a eliminación destas sustancias ó longo dos diferentes procesos de tratamento de augas residuais e lodos en plantas de tratamento (EDAR) foi pouco estudiada.

O obxectivo xeral deste traballo é estudiar o comportamento dos PPCPs seleccionados nunha EDAR urbana, poñendo especial atención no tratamento de lodos. Seleccionáronse 13 sustancias pertencentes a diferentes clases terapéuticas de acordo coas súas prescricións, existencia no medioambiente, metodoloxía analítica dispoñible e relación dose-efecto alta. Estes compostos son: dúas fragrancias (Galaxolide e Tonalide), un tranquilizante (Diazepam), un antiepiléptico (Carbamazepina), tres anti-inflamatorios (Ibuprofen, Naproxen e Diclofenac), un medio de contraste de raios X (Iopromide), dous antibióticos (Sulfamethoxazol e Roxithromicina) e tres hormonas (Estrona, 17β-estradiol e 17α-ethinylestradiol). Para conseguir este obxectivo, considéranse os seguintes obxectivos específicos:

i) Estudiar a existencia dos PPCPs seleccionados nas augas residuais tratadas nunha EDAR urbana de Galicia (100.000 habitantes equivalentes) xunto co comportamento a través das diferentes unidades de tratamento da planta (Capítulo 3).

ii) Estudiar o mecanismo (volatilización, sorción e degradación) responsable da eliminación dos PPCPs nas EDAR (Capítulo 4).

iii) Estudiar a influencia de dous procesos físico-químicos, coagulación-floculación e flotación, na eliminación dos PPCPs durante o tratamento primario (Capítulo 5).

iv) Estudiar o comportamento dos PPCPs seleccionados durante a dixestión anaerobia convencional de lodos en condicións mesofílicas e termofílicas a diferentes tempos de retención celular (Capítulo 6).

v) Estudiar a influencia de varios pretratamentos de lodos (alcalino, térmico e con ozono) na dinámica do proceso de estabilización de lodos e no comportamento dos PPCPs (Capítulo 7).

vi) A caracterización do lodo dixerido en termos de materia orgánica, nutrientes, patóxenos, metais pesados, compostos sulfonados alquilbencénicos lineais (LAS) e propiedades de deshidratación (Capítulo 8).

No Capítulo 1 levouse a cabo unha revisión bibliográfica dos PPCPs, incluíndo prescricións, porcentaxes de excreción, propiedades físico-químicas, orixes e vías de expansión no medioambiente, existencia en diferentes compartimentos ambientais, comportamento en EDAR e efectos ambientais. A segunda parte é unha revisión dos lodos procedentes de EDAR urbanas. Esta revisión inclúe definición e tipos de lodo, índices de producción, características, tecnoloxías de tratamento e reciclaxe e uso final.

No Capítulo 2 descríbense os métodos analíticos usados neste traballo. Esto inclúe tanto os parámetros convencionais de caracterización de augas residuais e lodos (alcalinidade, composición e producción de biogás, materia orgánica, nitróxeno, fosfato, sulfato, cloruro, graxa, pH, temperatura, sólidos, carbono e ácidos graxos volátiles) coma as análises dos PPCPs.

O primeiro paso foi o estudio da existencia destas sustancias na EDAR seleccionada. Para iso, realizáronse varias campañas de mostreo integrado (2001-2002). Entre as sustancias consideradas, soamente se detectaron concentracións importantes no influente das dúas fragrancias (HHCB e AHTN), de dous antiinflamatorios (IBP e NPX), dos estróxenos naturais (E1 e E2), dun antibiótico (SMX) e do medio de contraste (IPM), atopándose os outros compostos por debaixo do límite de cuantificación. Durante o tratamento primario, soamente se eliminan parcialmente as fragrancias (30-50%) e E2 (20%). Pola contra, todos os compostos atopados se eliminan no tratamento biolóxico (35-75%), excepto IPM, que permanece na fase líquida. As eliminacións globais na planta varían entre 70-

90% para as fragancias, 40-65% para os antiinflamatorios e sobre 60% para E2 e SMX. Sen embargo, a concentración de E1 aumenta ó longo do tratamento debido a ruptura das formas glucurónicas e a oxidación parcial de E2 (Capítulo 3).

O obxectivo do Capítulo 4 é afondar no mecanismo responsable da eliminación dos PPCPs ó longo das diferentes unidades de tratamento da EDAR. Para iso, realizáronse balances de materia incluíndo a fase sólida. Propóñense dous métodos para o cálculo da fracción adsorbida no lodo. O primeiro usa as concentracións medidas no lodo e o segundo calcula a concentración no lodo a partir da concentración disolta na fase líquida, usando os coeficientes de distribución sólido-líquido (K_d). Observouse que o mecanismo de eliminación das fragrancias é a sorción, mentres que as sustancias polares son degradadas biolóxicamente.

Como este traballo está principalmente enfocado no tratamento de lodos mediante dixestión anaerobia, pensouse que procesos físico-químicos, como coagulación-floculación e flotación, poderían mellorar a eliminación dos PPCPs da fase líquida durante o tratamento primario (Capítulo 5). Escolléronse 8 compostos representativos dos tres grupos principais de acordo coas súas propiedades físico-químicas: compostos lipofílicos (HHCB e AHTN), neutros (CBZ e DZP) e ácidos (IBP, NPX e DCF). Analizáronse varios parámetros: tipo e dose de coagulante, contido inicial de graxa nas augas residuais e a temperatura. Observouse que o uso de aditivos mellora a eliminación dos PPCPs durante o tratamento primario. Como se esperaba, os compostos con propiedades de sorción (absorción e adsorción) altas (HHCB, AHTN e DCF) eliminároonse de xeito importante (sobre 70%) durante os experimentos de coagulación-floculación. Sen embargo, os compostos hidrófilos víronse afectados en menor medida (por debaixo do 25%). Non se observou ningún efecto nin da dose ou tipo de coagulante nin da temperatura no rango ensaiado. Todos os compostos se eliminaron en certa medida durante os experimentos de flotación, acadándose os mellores resultados a 25ºC e con augas con alto contido en graxa.

No Capítulo 6 estudiouse o comportamento dos PPCPs durante a dixestión anaerobia de lodos. Analizouse a influencia da temperatura (rango mesófilo e termófilo) e do tempo de retención celular (10, 20 e 30 d no dixestor mesófilo; e 6, 10 e 20 d no dixestor termófilo). Deste xeito púidose facer tamén unha comparación entre a operación mesófila e termófila a diferentes tempos de retención. As eliminacións máis altas foron acadadas para antibióticos, estróxenos

naturais, fragrancias e NPX. Para os outros compostos, as eficacias varían entre 20 e 60%, excepto para CBZ, que non se elimina (<20%). A eliminación de DZP, DCF e EE2 ocorre despois dun período de adaptación do lodo. En xeral, non se observou ningunha influencia da temperatura nin do tempo de retención.

Recentemente desenvolvéronse novas tecnoloxías para mellorar a reciclaxe e o reuso do lodo, que normalmente implican un pretratamento antes do proceso de estabilización biolóxico para promover a solubilización da materia orgánica. No Capítulo 7 estudiouse a influencia dun pretratamento químico (alcalino), térmico e oxidativo (con ozono) na dinámica do proceso de dixestión anaerobia e no comportamento dos PPCPs durante esta operación. De novo, os parámetros analizados foron a temperatura e o tempo de retención celular. Acadáronse porcentaxes de solubilización de materia orgánica similares cos procesos térmico e oxidativo con ozono (60%); sen embargo, o tratamento alcalino aumenta este valor ata 80%. As eliminación de sólidos e materia orgánica varían entre 40 e 70% nos dous dixestores, con pequenas influencias do tempo de retención, temperatura ou tipo de pretratamento. A eliminación de NPX, IPM e SMX non se ve afectada por ningún pretratamento. O tratamento con ozono inflúe na eliminación de CBZ, HHCB, AHTN e IBP, e os tratamentos alcalino e térmico afectan a ROX e IBP, respectivamente. A eliminación de DZP, DCF e dos estróxenos está de novo máis afectada pola adaptación do lodo que polas condicións de operación.

Finalmente, debido á preocupación recente polos riscos potenciais do uso do lodo na agricultura para a saúde humana e para o medioambiente, o cal levou á revisión da política e lexislación vixente, o lodo dixerido obtido nos diferentes experimentos foi caracterizado en termos de patóxenos, propiedades de deshidratación, metais pesados e LAS (Capítulo 8). A maioría das condicións ensaiadas foron suficientes para non acadar os valores límite propostos no Documento de Traballo sobre lodos e o uso de pretratamentos asegura este logro.

Ademais preséntanse ó final do traballo dous anexos co obxectivo de: i) Estudiar a influencia do parámetro máis importante dos balances de PPCPs nos dixestores (a alimentación) nas eficacias obtidas (Anexo I); e, ii) Mostrar os datos usados nos Capítulos 6 e 7 (Anexo II).

Objectives and Summary

In recent years, several studies in Europe and USA reported the occurrence of Pharmaceutical and Personal Care Products (PPCPs) in different water (wastewaters, surface waters, groundwaters) and solid (sludge, particulate matter, sediments) compartments. In general, the concentrations of PPCPs in waters and solids range from the $ng \cdot L^{-1}$ to the low $\mu g \cdot L^{-1}$ level and from the $ng \cdot g^{-1}$ to the low $\mu g \cdot g^{-1}$ level, respectively. However, the elimination of these substances through the different wastewater and sludge treatment processes in a Sewage Treatment Plant (STP) has scarcely been studied.

The overall objective of this work is to study the fate of selected PPCPs in an urban STP, focusing on the sludge treatment. 13 substances belonging to different therapeutical classes have been selected according to their prescriptions, reported occurrence in the environment, analytical methodology available and high effect doses. These compounds are: two musks (Galaxolide and Tonalide), one tranquilliser (Diazepam), one antiepileptic (Carbamazepine), three anti-inflammatories (Ibuprofen, Naproxen and Diclofenac), one X-ray contrast medium (Iopromide), two antibiotics (Sulfamethoxazole and Roxithromycin) and three hormones (Estrone, 17β-estradiol and 17α-ethinylestradiol). For this purpose, the next specific objectives were considered:

i) The occurrence of the selected PPCPs in the wastewaters treated by a municipal STP located in Galicia (100,000 population equivalents) as well as the fate through the different units of the plant (Chapter 3).

ii) The mechanism of elimination (volatilization, sorption and degradation) involved in PPCPs removal in STPs (Chapter 4).

iii) The influence of two physico-chemical processes, coagulation-flocculation and flotation, on PPCPs removal during primary treatment (Chapter 5).

iv) The fate of selected PPCPs during conventional anaerobic digestion of sewage sludge under mesophilic and thermophilic conditions at different Sludge Retention Time (Chapter 6).

v) The influence of several sludge pre-treatments (alkaline, thermal and ozonation) on sludge stabilization dynamics and PPCPs behaviour (Chapter 7).

vi) The characterisation of digested sludge in terms of organic matter, nutrients, pathogens, heavy metals, Linear Alkylbenzene Sulfonates (LAS) and dewatering properties (Chapter 8).

In Chapter 1, a literature overview related to PPCPs, including prescription rates, pharmacokinetics, physico-chemical properties, sources and pathways in the environment, occurrence in different environmental compartments, fate in STPs and environmental effects is presented. The second part is a review related to sewage sludge, including types of sludge, generation and production rates, characteristics, treatment technologies and disposal routes.

In Chapter 2, the analytical methods used in this work are described. It comprises the conventional parameters used for wastewater and sludge characterisation (alkalinity, biogas composition and production, organic matter, nitrogen, phosphate, sulphate, chloride, oil and grease, pH, temperature, solids, carbon and volatile fatty acids) and the PPCPs analysis.

The first step was to study the occurrence of these substances in the STP selected. For that purpose, several integrated sampling campaigns (2001-2002) were carried out. Among all the substances considered, significant concentrations in the influent were only found for the two musks (HHCB and AHTN), two anti-inflammatories (IBP and NPX), the natural estrogens (E1 and E2), one antibiotic (SMX) and the X-ray contrast medium (IPM), being the other compounds found below the limit of quantification. During primary treatment, only the fragrances (30-50%) and E2 (20%) are partially removed. In contrast, the biological treatment causes an important reduction (35-75%) in all compounds detected, with the exception of IPM, which remains in the water phase. The overall removal efficiencies within the STP range between 70-90% for the fragrances, 40-65% for the anti-inflammatories, around 65% for E2 and 60% for SMX. On the other hand, the concentration of E1 increases along the treatment due to the cleavage of glucuronides and the partial oxidation of E2 (Chapter 3).

The objective of Chapter 4 was to get into more insight in the mechanism responsible for the PPCPs removal through the different treatment units of the STP. Therefore mass balance calculations were performed including also the sludge phase. To determine the fraction sorbed onto sludge, two methods have

been considered. The first method uses measured data in the sludge and in the second one, the concentration in the sludge is calculated from that in the aqueous phase using the solid-water distribution coefficients (K_d). It was observed that the removal mechanism for musks is sorption, while the hydrophilic substances are biologically degraded.

As this work was mainly focused on the sludge treatment by anaerobic digestion, it was thought that physico-chemical processes, such as coagulation-flocculation and flotation, could improve the PPCPs removal from the liquid phase during primary treatment (Chapter 5). Eight compounds representative of three main groups of PPCPs according to their physico-chemical properties have been selected: lipophilic (HHCB and AHTN), neutral (CBZ and DZP) and acidic compounds (IBP, NPX and DCF). Several parameters have been analysed, such as the type and dose of coagulant, the initial content of fat in the wastewaters and the temperature of operation. The use of additives improved PPCPs removal during primary clarification. As expected, those compounds with high sorption (absorption or adsorption) properties (HHCB, AHTN and DCF) were removed significantly during coagulation-flocculation experiments (around 70%). However, the hydrophilic compounds were less affected (below 25%). No influence of the type and dose of coagulant and temperature in the selected range was observed. During the flotation assays, all the PPCPs considered were eliminated in some extent, with the best results being achieved at 25ºC using wastewaters with high fat content.

In Chapter 6, the behaviour of selected PPCPs during anaerobic digestion of sewage sludge has been studied. The influence of temperature (mesophilic vs. thermophilic) and SRT (10, 20 and 30 d for the mesophilic process; and 6, 10 and 20 d for the thermophilic one) has been analysed. In this way, a comparison between the performance of the anaerobic digestion in mesophilic and thermophilic conditions at different SRT was also performed. The higher removal efficiencies were achieved for the antibiotics, natural estrogens, musks and NPX. For the other compounds, the values ranged between 20 and 60%, except for CBZ, which showed no or very low elimination (<20%). The removal of DZP, DCF and EE2 occurred after sludge adaptation. In general, no influence of SRT and temperature on PPCPs removal was observed.

Many novel treatment technologies, usually representing a pre-treatment prior to the biological degradation process to promote solubilisation of organic

matter, have been developed in order to improve the recycling and reuse of sewage sludge. In Chapter 7, the influence of a chemical (alkaline), thermal and oxidative (ozone) treatment on anaerobic digestion operation and PPCPs removal has been studied. Once again, two parameters have been analysed: temperature and SRT. Thermal and ozonation processes led to similar organic matter solubilization (60%), while the alkaline treatment increased this value up to 80%. Solids and organic matter removal efficiencies ranged between 40 and 70% in both digesters, with small influences of SRT, temperature or type of pre-treatment. The removal of NPX, IPM and SMX was not affected by any pre-treatment. Ozonation influenced the elimination of CBZ, HHCB, AHTN and IBP and the alkaline and thermal processes affected ROX and IBP removals, respectively. The elimination of DZP, DCF and estrogens were again related to sludge adaptation rather than to operational conditions.

Finally, due to the fact that some concern was expressed about the potential risks of the agricultural use of sludge for health and the environment, which led to revisions in government policy and regulations, the digested sludge obtained from the different experimental conditions tested was characterized in terms of pathogens, dewatering properties, heavy metals and LAS (Chapter 8). Most of conditions tested proved to be efficient to reach the requirements proposed in the Working Document on Sludge and the use of pre-treatments assures this achievement.

In addition, two annexes were added at the end of the work with the aim of: i) Study the influence of the most crucial parameter of the PPCPs mass balances in the anaerobic digesters (the feeding) on the removal efficiencies obtained (Annex I); and, ii) Present the data used in Chapter 6 and 7 (Annex II).

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Notation

AAS:	Atomic Absorption Spectrophotometry
AD:	Anaerobic Digestion
AES:	Atomic Emission Spectrophotometry
AHTN:	Tonalide
A/O:	Anaerobic-Oxic process
A_2/O:	Anaerobic-Anoxic-Oxic process
AO:	Advanced Operation
AOX:	Sum of organohalogenous compounds
APCI:	Atmospheric Pressure Chemical Ionization
AS:	Activated Sludge
B:	Biofilter
BFB:	Biological Filter Bed
BOD:	Biochemical Oxygen Demand
C:	Carousel
CBZ:	Carbamazepine
CFU:	Colony Forming Units
CI:	Chemical Ionization
COD_s:	Soluble Chemical Oxygen Demand
COD_t:	Total Chemical Oxygen Demand
CST:	Capillary Suction Time
DAF:	Dissolved Air Flotation
DCF:	Diclofenac
DDD:	Defined Daily Dose
DEHP:	Di(2-ethylexyl)phthalate
DO:	Dissolved Oxygen
DT_{50}:	Degradation Time for 50%
DZP:	Diazepam
E1:	Estrone
E2:	17β-estradiol
EA:	Extended Aeration
EDCs:	Endocrine Disrupting Chemicals

EE2:	17α-ethinylestradiol
EI:	Electron Ionization
ERA:	Environmental Risk Assessment
ERT:	Estrogen Replacement Therapy
ESI:	ElectroSpray Ionization
FB:	Fixed Bed
F/M:	Feed Microoganisms ratio
GC:	Gas Chromatography
GPC:	Gel Permeation Chromatography
GPR:	Gas Production Rate
H:	Henry's law constant
HF:	High Fat wastewaters
HHCB:	Galaxolide
HPLC:	High Performance Liquid Chromatography
HRTh:	Hormone Replacement Therapy
HRT:	Hydraulic Retention Time
HS-SPME:	Head Space Solid Phase MicroExtraction
IA:	Intermediate alkalinity
IBP:	Ibuprofen
IBP-OH:	Ibuprofen-Hydroxyl
IBP-CX:	Ibuprofen-Carboxyl
IC:	Inorganic Carbon
IN:	Inorganic Nitrogen
IPM:	Iopromide
K_a:	Dissociation constant
k_{biodeg}:	Biodegradation rate constant
K_d:	Solid/water distribution coefficient
K_{oc}:	Organic carbon partition coefficient
K_{ow}:	Octanol/water partition coefficient
La:	Lagoon
LAS:	Linear Alkylbenzene Sulfonates
LF:	Low Fat wastewaters
LOD:	Limit of Detection
LOQ:	Limit of Quantification

MBR:	Membrane Biological Reactor
MPN:	Most Probable Number
MS:	Mass Spectrometry
MW:	Molecular Weight
NAS:	Nitrifying Activated Sludge
NOM:	Natural Organic Matter
NPE:	Nonylphenol and Nonylphenolethoxylates
NPX:	Naproxen
NSAIDs:	Non-Steroid Anti-Inflammatory Drugs
OD:	Oxidation Ditch
OLR:	Organic Load Rate
OM:	Organic Matter
PA:	Partial alkalinity
PAC:	Powdered Activated Carbon
PAH:	Polynuclear Aromatic Hydrocarbons
PCB:	PolyChlorinated Biphenils
PCDD/F:	PolyChloroDibenzoDioxins/Furans
PEC:	Predicted Environmental Concentration
pK_a:	Dissociation constant
PMF:	Polycyclic Musk Fragrances
PNEC:	Predicted Non Effect Concentration
PNLD:	Plan Nacional de Lodos de Depuradora
PPCPs:	Pharmaceutical and Personal Care Products
PS:	Primary Sludge
RBC:	Rotating Biological Contactor
ROX:	Roxithromycin
S:	Concentration in the liquid phase
SAF:	Submerged Aerated Filter
SD:	Standard Deviation
SGP:	Specific Gas Production
SMX:	Sulfamethoxazole
SPE:	Solid Phase Extraction
SPME:	Solid Phase MicroExtraction
SRF:	Specific Resistance to Filtration

SRT:	Sludge Retention Time
SS:	Suspended Solids
STP:	Sewage Treatment Plant
T:	Temperature
TA:	Total alkalinity
TC:	Total Carbon
TF:	Trickling Filter
TKN:	Total Kjeldahl Nitrogen
TN:	Total Nitrogen
TOC:	Total Organic Carbon
TS:	Total Solids
TSS:	Total Suspended Solids
USE:	Ultrasonic Solvent Extraction
UV:	Ultraviolet
VFA:	Volatile Fatty Acids
VS:	Volatile Solids
VSS:	Volatile Suspended Solids
WAS:	Waste Activated Sludge
X:	Concentration in the solid phase
YES:	Yeast Estrogen Screening

Chapter 1

Introduction

Summary

Environmental pollution has become an important issue in the development of our society. Virtually any human activity leads to an environmental contamination with substances of anthropogenic origin. While many substances are released purposely, i.e. pesticides, others enter the environment unintentionally. In general, contaminants which cause adverse impacts on humans and on the environment due to their properties, amounts released or environmental concentrations are defined as pollutants.

For the last decades, the research activity has been mainly focused on pollutants which were applied in high volumes by industry and agriculture, such as pesticides and industrial chemicals. However, substances which are suspected to interfere with the hormone system of human and animals (endocrine disruptors) and pharmaceutical active compounds are of increasing importance in the recent years. These compounds are widely used and distributed in the environment. Their disposal in the environment means that a huge number of different substances in different amounts, products and modes of action has to be considered. Besides, the information available related to their fate, occurrence and effects in the aquatic and terrestrial environment is still scarce and not sufficient for their assessment and decision-making.

In this chapter, an overview of Pharmaceutical and Personal Care Products (PPCPs) is presented. Prescription rates, pharmacokinetics, physico-chemical properties, sources and pathways in the environment, occurrence in different environmental compartments, fate in Sewage Treatment Plants (STPs) and environmental effects are described. Besides, a review related to sewage sludge, including types of sludge, generation and production rates, characteristics, treatment technologies and disposal routes is presented.

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1.1. Pharmaceutical and Personal Care Products as pollutants

The 20th century has introduced more than 100,000 chemicals that are used in our every day life, either in households, industries or agriculture, without realising the consequences for the environment and directly and indirectly for human health. EU started to list these chemicals in the late 1970s, and since the mid 1980s it has been compulsory to set up an environmental risk assessment (ERA) for all new chemicals.

It is strange that drugs were not included in this compulsory ERA because they have properties which make them suspicious of environmental effects. Drugs are biologically active, quite mobile and not readily biodegradable. Today, an ERA is required for all new medical compounds used for veterinary purposes, but it is expected that this will be also required for human drugs in the near future due to three main reasons:

- They are used in high quantities throughout the world.
- They enter into the environment by different routes.
- Concern is growing over their effects on humans and animals health.

However, the main problem for a correct environmental assessment of drugs is the lack of information. In that sense, the EU project POSEIDON (*Assessment of Technologies for the Removal of Pharmaceuticals and Personal Care Products in Sewage and Drinking Water Facilities to Improve the Indirect Potable Water Reuse*) was launched in 2001 in order to establish a basic knowledge on the occurrence, fate and behaviour of Pharmaceutical and Personal Care Products (PPCPs) during waste and drinking water treatment processes. As a major innovation, POSEIDON tackled this problem adequately at source, by focusing on the development of alternative methods of wastewater collection (i.e. separate collection of urine) in addition to advanced innovative treatment technologies (i.e. membrane technology) and optimization of the conventional processes (i.e. activated sludge system). In addition, POSEIDON addressed the efficiency of conventional sludge treatment processes (i.e. anaerobic digestion) as well as advanced approaches (i.e. anaerobic digestion combined with different sludge pre-treatments). The final outcome is a comprehensive scheme for the implementation of administrative measures regarding the elimination of persistent domestic chemicals as contaminants of reclaimed Sewage Treatment Plants (STPs) discharges.

1.1.1. Selection of PPCPs

Due to the large numbers of registered pharmaceuticals, which is further enlarged by a huge number of excreted metabolites, it appears to be nearly impossible to perform in-depth ERAs or to develop analytical methods for all compounds. Therefore, a pre-selection step is essential to focus on compounds with potential environmental relevance. The selection was done within Poseidon project and the criteria considered were: i) elevated annual prescriptions, ii) high effect doses/concentrations, and iii) pharmacokinetic behaviour (e.g. metabolism, urinary/fecal excretion rates). Table 1.1 shows the selected compounds.

Polycyclic musk fragrances

In the 1950s and 60s, a new class of synthetic musks, the so-called polycyclic musk compounds, was developed. These compounds are widely used as important fragrance ingredients in perfumes, lotions, soaps, shampoos, detergents, cleaning agents, air fresheners and other scented household products, as additives in cigarettes and fish baits, and in technical products such as herbicide formulations and explosives. Among them, Galaxolide (HHCB) and Tonalide (AHTN) are the most commonly used.

Tranquillisers and Antiepileptics

Tranquillisers are drugs prescribed to relieve anxiety, depression and insomnia. The most common form of tranquillisers is a group called benzodiazepines, which includes Temazepam and Diazepam (most commonly known as Valium).

Antiepileptics are drugs used for the treatment of epilepsy, which is the second most common central nervous system disease. Carbamazepine has replaced both Phenytoin and Phenobarbitone as the first-choice anticonvulsant for a number of paediatric seizure disorders. In addition, Carbamazepine is used for the treatment of trigeminal neuralgia, as a psychotropic agent and in clinical psychiatry for the treatment of schizophrenia.

Anti-inflammatories

There are several drugs that suppress inflammation in a manner similar to steroids, but without their side effects, referred to as non-steroid anti-inflammtory drugs (NSAIDs). NSAIDs are commonly used to relieve symptoms of arthritis, bursitis, gout, swelling, stiffness and joint pain. Many of these pharmaceuticals

also have analgesic (pain killing) and/or antipyretic (fever reducing) activities. There are many different types of NSAIDs available over the counter, such as Ibuprofen, and also under prescription, such as Naproxen and Diclofenac.

Antibiotics

Antibiotics are used in human and veterinary medicin, farming and aquaculture for prevention and treatment of diseases, but also as antimicrobially active substances to improve nutrient uptake in the gastrointestinal tract (growth promoters). The classes of antibiotics most investigated include macrolides, quinolones, quinoxaline-dioxides, sulfonamides, tetracyclines and trimethoprim.

Macrolides are a group of compounds that contain a 12-, 14- or 16-membered macrocyclic lactone ring to which sugar moieties (including amino- and deoxy-sugars) are attached. Among them, Roxithromycin is active against gram-positive and gram- negative cocci, gram-positive bacilli and some gram-negative bacilli, but has no significant effect on the predominant faecal flora. It also displays good activity against atypical pathogens. In vivo, Roxithromycin is as effective or more effective than other macrolides in a wide range of infections.

Sulfonamides have become the most widely used class of antimicrobials in the world since their development. The sulfonamides are synthetic bacteriostatic, broad-spectrum antibiotics, effective against most gram-positive and many gram-negative bacteria. Among them, Sulfamethoxazole was selected.

X-ray contrast media

Contrast media are used to get detailed images of soft issues in X-ray radiography. Among them, the group of iodinated organics is widely used. They consist of a benzene ring carrying three iodine atoms. The remaining positions of the aromatic ring are used to couple side chains determining hydrophilicity, pharmaceutical tolerance and pharmacokinetic behaviour. Among them, Iopromide was selected.

Estrogens

Natural estrogens, i.e. Estrone (E1) and 17β-estradiol (E2), are excreted by humans and animals through their urine and faeces principally as inactive polar conjugates such as glucuronides and sulphates.

Table 1.1. Selected PPCPs.

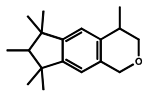
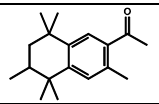
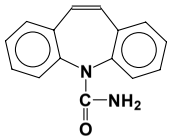
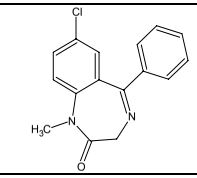
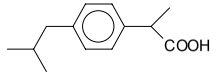
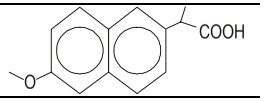
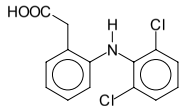
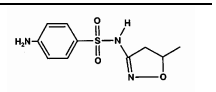
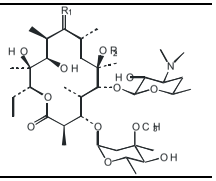
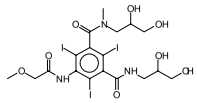
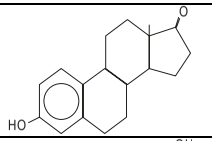
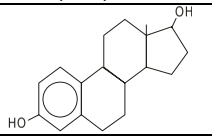
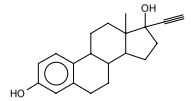
PPCP	IUPAC name	Trade name	CAS number	Use/ origin	Sum formula	MW[a]	Structure
HHCB	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-(g)-2-benzopyran	Galaxolide Abbalide	1222-05-5	Polycyclic musk	$C_{18}H_{26}O$	258.4	
AHTN	7-acetyl-1,1,3,4,4,6-hexa-methyletralin	Tonalide Fixolide	1506-02-1	Polycyclic musk	$C_{18}H_{26}O$	258.4	
CBZ	5H-Dibenz-(b,f)-azepine-5-carboxamide	Carbamazepine	298-46-4	Analgesic; antiepileptic	$C_{15}H_{12}N_2O$	236.3	
DZP	7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin -2-one	Diazepam	439-14-5	Psychiatric drug	$C_{16}H_{13}ClN_2O$	284.7	
IBP	α-Methyl-4-(2-methylpropyl)-benzeneacetic acid	Ibuprofen	15687-27-1	Analgesic/anti-inflammatory	$C_{13}H_{18}O_2$	206.3	
NPX	(S)-6-Methoxy-α-methyl-2-naphthaleneacetic acid	Naproxen	22204-53-1	Analgesic/anti-inflammatory	$C_{14}H_{14}O_3$	230.3	
DCF	2-[(2,6-Dichlorophenyl)-amino] benzeneacetic acid-Na	Diclofenac	15307-79-6	Analgesic/anti-inflammatory	$C_{14}H_{10}Cl_2NO_2Na$	318.1	

Table 1.1. Selected PPCPs. *Cont.*

PPCP	IUPAC name	Trade name	CAS number	Use/ origin	Sum formula	MW[a]	Structure
SMX	4-amino-N-(5-methyl-3-isoxazolyl)benzenesulfon-amide	Sulfamethoxazol	723-46-6	Sulfonamide Antibiotic	$C_{10}H_{11}N_3O_3S$	253.3	
ROX	Erythromycin-9-(-o-[2-ethoxyethoxy] methyloxime)	Roxithromycin	80214-83-1	Macrolide Antibiotic	$C_{41}H_{76}N_2O_{15}$	837.1	
IPM	N,N-Bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-[(methoxy-acetyl)amino]-N-methyl-1,3-benzenedicarboxamide	Iopromide	73334-07-3	X-ray contrast media	$C_{18}H_{24}I_3N_3O_8$	791.1	
E1	3-Hydroxyestra-1,3,5(10)-trien-17-one	Estrone, Estrol Femidyn	53-16-7	Steroid	$C_{18}H_{22}O_2$	270.4	
E2	(17β)-Estra-1,3,5(10)-triene-3,17-diol	17β-estradiol, Estrace, Estraderm	50-28-2	Steroid	$C_{18}H_{24}O_2$	272.4	
EE2	17-ethynyl-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopent a[a]phenanthrene-3,17-diol	17α-ethinylestradiol	57-63-6	Contraceptive	$C_{20}H_{24}O_2$	296.4	

[a]MW: Molecular weight ($g \cdot mol^{-1}$).

The synthetic estrogen, 17α-ethinylestradiol (EE2) is used as the active ingredient in birth control pills and in drugs to relieve the symptoms associated with menopause. It is also mainly excreted as conjugates in the urine and faeces.

1.1.2. Prescription rates

For most compounds, the total amounts sold/consumed are not available, but they have been estimated following several approaches.

Polycyclic musk fragrances

The average use per capita of musks can be estimated on the basis of the consumption of HHCB and AHTN (1427 and 343 t, respectively) and the inhabitants in Europe ($3.77x10^8$ according to European Union, 2002). Since the variation in use of musks between the different countries is relatively low (Van de Plassche and Balk, 1997), the estimation of local consumptions is based on the per capita use in the EU extrapolated to the number of inhabitants of the local country (Balk and Ford, 1999). Local differences may be a factor of 2 higher or lower. For Spain (43.2 million inhabitants), the results are 163.5 and 39.3 tons for HHCB and AHTN, respectively.

Pharmaceuticals

For drugs, a good estimate of the annual amounts prescribed can be obtained based on the accessible number of prescription items multiplied by the average defined daily dose (DDD) of a particular compound (Schwabe and Paffrath, 2002). Table 1.2 indicates the number of prescribed items in Spain in 2003, the number of doses of each item, the amount of active drug per dose and the estimated total amounts used (Carballa *et al.,* 2006a). It can be observed that annual consumption rates range from a few kilograms (i.e. for hormones) up to more than hundred tons (i.e. for Ibuprofen). However, these quantities underestimate the total usage of drugs which can be purchased without a pharmacy prescription and those procured illegally.

Steroid estrogens

For the estimation of the natural steroid estrogens excreted by humans, the model described by Johnson and Williams (2004) was used. These authors differentiate 5 groups of population: men, menstrual women, pregnant women, menopausal women and menopausal women using Hormone Replacement

Therapy (HRTh). They indicate the excretion rates of E1 and E2 corresponding to each population group. Therefore, the total amounts excreted can be calculated multiplying the excretion rates by the number of persons belonging to each group (Table 1.3).

Table 1.2. Annual prescription items of selected pharmaceuticals and estimated amounts in Spain in 2003.

Compound	Number of items ($x10^6$)	Number of doses per item	Active compound per dose (mg)	Amount used ($ton \cdot y^{-1}$)
CBZ	1.0	100	200	19.9
DZP	6.1	30	5	0.9
IBP	27.6	20	500	276.1
NPX	-	30	375	-
DCF	16.2	40	50	32.3
IPM	0.06	-	-	-
ROX	0.2	14	150	0.3
SMX	1.6	20	400	12.7
EE2	19.0	21	0.03	0.012

The total population of Spain in 2003 was 43.2 million inhabitants (National Institute of Statistics, INE), with a percentage of males and females of 49.3 and 50.7%, respectively. Menstruating females were assumed to be between the ages of 15-59, which from the Spain population census data for 2003 represents 61.5% of female population (31.2% of total population). The average age of menopause (McKinlay *et al.*, 1992) is 51, which on the bases of the Spain population census data for 2003 represents about 35.5% of the female population (18.0% of total population). The number of births in Spain in 2003 was 439,863, which leads to a pregnancy/birth rate of 1 in 50 females (1.0% of total population).

Table 1.3. Excreted amounts of natural estrogens by the Spanish population.

Population group	% total population	Amount excreted ($\mu g \cdot person^{-1} \cdot d^{-1}$)		Total amount ($g \cdot d^{-1}$)	
		E1	E2	E1	E2
Males	49.3	2.6	1.8	55.4	38.3
Menstrual	31.2	11.7	3.2	157.6	43.1
Pregnant	1.0	550	393	241.9	172.9
Menopausal	18.0	1.8	1.0	14.0	7.8
HRTh	2.8	28.4	56.1	34.2	67.6
TOTAL	-	-	-	*503.1*	*329.7*

A range of hormone replacement therapies (HRTh), which contains estrogen and progesterone compounds and estrogen replacement therapies (ERT) are used by some postmenopausal women. The average age of women starting HRTh treatments is 54-55 (Ettinger *et al.,* 1998). Data representative for an industrialized Western country indicate that around 5-6% of all females are receiving HRTh treatment. Extrapolating this data to the whole population of Spain, the results are that 2.8% of total population follows HRTh therapies.

A wide range of different products are available on the market, but according to Johnson and Williams (2004), the most common one contains 2 mg $E2 \cdot d^{-1}$, which would generate a release of about 28.4 $\mu g \cdot d^{-1}$ and 56.1 $\mu g \cdot d^{-1}$ of E1 and E2, respectively, in the urine.

In total, it is estimated that worldwide consumption of active compounds amounts to some 100,000 tons or more per year and this use may vary from country to country (Table 1.4). Similarly to Spain, the annual consumption rates of frequently prescribed pharmaceuticals in the different countries range from a few kilograms (i.e. for hormones) up to more than hundred tons (i.e. for Ibuprofen and Iopromide). Once again, these quantities underestimate the total usage of drugs which can be purchased without a pharmacy prescription and those procured illegally.

1.1.3. Pharmacokinetics

The pharmacokinetic behaviour of drugs directly influences the potential for environmental contamination. For a drug which is only excreted as metabolites, the parent compound should not generally be expected to be found in sewage and the environment and it makes more sense to monitor the major stable excreted metabolites.

Many pharmaceuticals are biotransformed in the body. Biodegradation modifies the chemical structure of their active molecules, which in turn often results in a change in their physicochemical and pharmaceutical properties. Metabolism may lower activity or enhance water solubility; however, it is frequently incomplete.

Table 1.5 shows the human excretion rates (%) and the excreted/used amount for human application of selected PPCPs. It can be observed that excretion rates range from 0 to 100%.

Table 1.4. Annual consumption (ton·y^{-1}, except EE2 in kg·y^{-1}) of selected PPCPs in different countries.

PPCP	*Australia*[1]	*Austria*[2]	*Denmark*[3]	*France*[4]	*Germany*			*Spain*[8]	*Switzerland*[9]	*Europe*	
					1995[5]	1997[6]	2000[7]			1992-1998[10]	2000[11]
HHCB	-	-	-	-	-	-	-	163.5	35.7[b]	2,400-1,473	1427
AHTN	-	-	-	-	-	-	-	39.3	8.6[b]	885-385	343
CBZ	10.0	6.3	-	37.8	80	80	-	20.0	4.1	-	-
DZP	-	0.1	0.2	-	-	-	-	0.9	0.04	-	-
IBP	14.2	6.7	33.2	166.6	105	180	150 (31)	276.1	15.7	-	-
NPX	22.9	-	-	38.7	-	-	-	-	-	-	-
DCF	4.4	6.1	-	-	75	75	(44)	32.3	3.9	-	-
ROX	3.8	-	-	-	3.1-6.2[a]	-	-	0.3	0.2	-	-
SMX	7.3	1.0	-	-	16.6-76[a]	60	-	12.7	2.6	-	-
IPM	-	5.4	-	-	-	130	-	-	11.0	-	-
EE2	-	4.0	-	-	-	50	-	12.0	4.0	-	-

[1]Khan and Ongerth, 2004 (year 1998); [2]Clara *et al.*, 2004a; Clara *et al.*, 2004b; Kreuzinger *et al.*, 2004 (year 1997);
[3]Jorgensen and Halling-Sorensen, 2000 (year 1995); [4]Duguet *et al.*, 2004 (Year 1998);
[5]Ternes, 1998. [a]Hirsch *et al.*, 1999; [6]Ternes, 2001a;
[7]Weigel *et al.*, 2004; in brackets, only pharmacy (Wiegel *et al.*, 2004); [8]Carballa *et al.*, 2006a (year 2003);
[9]Huber, 2004. [b]Buerge *et al.*, 2003 (year 2000); [10]Schure, 2000; OSPAR, 2000;
[11]Kupper *et al.*, 2004.

There are two important pathways of metabolism. Phase I metabolites result from the modification of the active compound itself by hydrolysis, oxidation, reduction, alkylation and dealkylation. Phase II metabolites are Phase I metabolites which have been modified by glucuronation or sulfation ("coupling reactions") to enhance excretion. There is evidence that glucuronides are capable of being deconjugated to parent compound during municipal sewage treatment (Ternes *et al.,* 1999; Möhle and Metzger, 2001).

Table 1.5. Human excretion rates (%) and excreted/used amounts for human application of selected PPCPs.

PPCP	Excretion rate			Excreted/ Used	Reference
	U	M	G		
HHCB				11.1[a]	Balk and Ford, 1999
				10.4[a]	Kupper *et al.,* 2004
				13.4[a]	Buerge *et al.,* 2003
AHTN				4.4[a]	Balk and Ford, 1999
				2.5[a]	Kupper *et al.,* 2004
				3.2[a]	Buerge *et al.,* 2003
E1			50		Adler *et al.*, 2001
				0.002-0.550[b] (w)	Johnson and Williams, 2004
				0.003[b] (m)	
				0.01-0.10[b] (w)	Baronti *et al.,* 2000
				0.003-0.020[b] (w)	Belfroid *et al.,* 1999
				0.005[b] (m)	
E2			50	5[b] (pregnant)	Duguet *et al.*, 2004
				0.4[b] (w)	OSPAR, 2000
				0.001-0.393[b] (w)	Johnson and Williams, 2004
				0.002[b] (m)	
				0.01-0.10[b] (w)	Baronti *et al.,* 2000
				0.002-0.012[b] (w)	Belfroid *et al.,* 1999
				0.4[b]	Ternes *et al.,* 1999
EE2				0.001[b]	Larsson *et al.*, 1999
				0.026[c]	Johnson and Williams, 2004
				0.0009[b]	
				0.03[c]	Cargouët *et al.,* 2004
				0.01-0.10[b] (w)	Baronti *et al.,* 2000
				0.025-0.050[c]	Rudder *et al.,* 2004
				0.010[c]	Webb *et al.,* 2003

U: Unmetabolised; M: Metabolised; G: Glucuronides.
[a]Used amount/person ($mg \cdot d^{-1}$); [b]Excreted amount/person ($mg \cdot d^{-1}$).
(w): women, (m): men; [c]Therapeutic dose ($mg \cdot d^{-1}$).

Table 1.5. Human excretion rates (%) and therapeutic dose for human application of selected PPCPs. *Cont.*

PPCP	Excretion rate			Used	Reference
	U	M	G		
	1-2	YES	YES		Daughton and Ternes, 1999
	3		0	27.3*	Khan and Ongerth, 2002
	31		0	27.3*	Khan and Ongerth, 2004
CBZ				600-1,200[a]	Drewes *et al.*, 2002
	2-3				Clara *et al.*, 2004a
				>100[a]	Stackelberg *et al.*, 2004
				400[a]	Webb *et al.*, 2003
DZP				6[a]	Webb *et al.*, 2003
		>9	>17		Ternes, 2001a
	1-8		14		Ternes, 1998
	15	26 (OH)			Weigel *et al.*, 2004
IBP		43 (CX)			
	10		5	38.9*	Khan and Ongerth, 2004
				600-1,200[a]	Buser *et al.*, 1999; Zwiener *et al.*, 2002
				1,200[a]	Jorgensen, 2000; Webb *et al.*, 2003
NPX	0		95	62.6*	Khan and Ongerth, 2002
	10		60	62.6*	Khan and Ongerth, 2004
	15		<1		Ternes, 1998
				100-150[a]	Buser *et al.*, 1998
DCF	2		15	12*	Khan and Ongerth, 2004
	<1		60		Strenn *et al.*, 2004
				25[a]	Webb *et al.*, 2003
	>60			150-300[a]	Hirsch *et al.*, 1999
ROX	74		0	10.3*	Khan and Ongerth, 2004
				150[a]	Webb *et al.*, 2003
	15			400-1,600[a]	Hirsch *et al.*, 1999
SMX	30		0	20.1*	Khan and Ongerth, 2004
				800[a]	Webb *et al.*, 2003
	95				Daughton and Ternes, 1999
IPM	90			300,000*[a]	Ternes and Hirsch, 2000
				20,000[a]	Webb *et al.*, 2003

U: Unmetabolised; M: Metabolised; G: Glucuronides.
[a]Therapeutic dose ($mg \cdot d^{-1}$); *Total amount used per day ($kg \cdot d^{-1}$).

1.1.4. Physico-chemical properties

To predict and understand the fate of this type of compounds in the environment, it is crucial to consider their physico-chemical properties. Moreover, these characteristics will also influence the development of the analytical methodology for their determination in environmental samples.

Molecular structure

In general, pharmaceuticals are comparatively large and chemically complex molecules (Table 1.1). Contrary to other organic pollutants, PPCPs do not represent any sort of homogeneous group of compounds since they vary widely in molecular weight, structure, functionality, salt forms, polymorphs, etc.

Molecular weight

The molecular weight is important for the selection of the detection methods (e.g. gas chromatography (GC) or high performance liquid chromatography (HPLC)) and for the selection of the potential clean up steps. GC is based on the evaporation of the analytes into the gas phase without decomposition. With increasing molecular weights (higher than 500 Da) that becomes more and more unlikely due to the elevated evaporation enthalpy. HPLC analysis is independent of the molecular weight and in general it is appropriate for large molecules.

From Table 1.1, it can be observed that the molecular weight of selected PPCPs range from 200 to 300 $g \cdot mol^{-1}$, except for Roxithromycin and Iopromide, which have higher size, around 800 $g \cdot mol^{-1}$.

Partitioning

For the assessment of an organic compound behaviour in natural and artificial systems, it is necessary to consider the distribution of the chemical between the different system compartments/phases. The partitioning of a compound between several phases is a result of intermolecular interactions between the compound's molecules and its molecular environment. These intermolecular interactions can be divided into the non-specific and specific interactions. Non-specific attractive forces, also referred to as *Van der Waals* interactions, occur between all molecules and are responsible for the interactions between non-polar molecules. Specific interactions result from electron donor-acceptor interactions between polar compounds and are attractive forces of significantly higher strength than *Van der Waals* (Schwarzenbach *et al.*, 2003).

Octanol/water distribution coefficient

Non specific interactions can be predicted by the octanol/water distribution coefficient, K_{ow}, defined as the ratio of the concentration of a compound in two phases, *n*-octanol and water, when the phases are in equilibrium at a specific temperature and the test compound is in dilute solution in both phases (Eq.1).

$$K_{ow} = \frac{C_{C_8H_{17}OH}}{C_{water}} \qquad \text{Eq. 1.1}$$

The distribution of neutral organic compounds between water and natural solids, such as soils, sediments, suspended matter and organisms, can be considered in many cases as a partitioning process between the aqueous phase and an organic phase. Roughly, it can be predicted that sorption/accumulation based on non specific interactions is appreciable high for values of log K_{ow} > 3 (Schwarzenbach *et al.*, 2003).

Dissociation constant

Since specific interactions are mainly based on ionic interactions and hydrogen bonds, they will be dependant on the ionisation potential of the compound.

In the presence of water, acids HA and bases B are in equilibrium with their conjugated bases A^- and acids BH^+. The alignment of these equilibriums can be expressed by the acidity (K_a) and basicity (K_b) constants, respectively. However, to compare acids and bases on a uniform scale it is convenient to use the acidity constant of the conjugated acid BH^+.

The dissociation constant (K_a), defined as an equilibrium constant representative of the relative proton transfer for a substance, is a measure of the acid strength and describes the degree of ionisation of a compound at a known pH (Eq. 1.2). It is usually expressed by its negative decadal logarithm pK_a (Eq. 1.3).

$$K_a = \frac{[H^+][A^-]}{[HA]} = \frac{[H^+][B_{con}]}{[B_{con}H^+]} \qquad \text{Eq. 1.2}$$

$$pK_a = -\log(K_a) = pH - \log\frac{[A^-]}{[HA]} = pH - \log\frac{[B_{con}]}{[B_{con}H^+]} \qquad \text{Eq. 1.3}$$

For organic acids and bases, the degree of ionisation is strongly dependant on the pH. These compounds can undergo proton-transfer reactions, resulting in the formation of charged species which have different properties and reactivities as compared to their neutral counterparts.

Sorption properties

The association of chemicals with solid phases is generally referred to as sorption. In the environment, with water as most relevant solvent, sorption is very often a combination of multiple non-polar and polar interactions.

Historically, the octanol/water distribution coefficient has been used to predict the sorption of chemicals onto soil, sediments, biomass and sludge. However, they do not appear to be applicable to PPCPs and their metabolites since they are large, complex, multifunctional organic compounds which are ionised in the aquatic environment at environmentally relevant pH levels.

Therefore, the solid-water distribution coefficient (K_d), defined as the ratio between the concentrations of a given compound in the solid (X) and in the aqueous phase (S) at equilibrium conditions (Eq. 1.4), is used to describe the sorption of PPCPs. It takes into account the two main sorption mechanisms: absorption and adsorption (Ternes *et al.*, 2004).

$$K_d = \frac{X}{S} \qquad \text{Eq. 1.4}$$

Vapour pressure/air-water partitioning

The vapour pressure and the air-water partitioning influence the losses of the analytes by evaporation. This fact is important for the analytical determination since compounds with an extreme high vapour pressure should be determined with procedures avoiding a solvent evaporation.

The fraction of the analytes transferred from the water phase into the air can be predicted using the Henry's law constant (H), which describes the partition between the fractions dissolved in water (C_w) and those in the air (C_{air}) at equilibrium, as shown in Equation 1.5.

$$H = \frac{C_{air}}{C_w} \qquad \text{Eq. 1.5}$$

Water solubility

Water solubility is crucial for higher concentrations of analytes in aqueous samples. Frequently low water solubility is associated with an elevated sorption property.

Table 1.6 shows the physico-chemical properties of selected PPCPs.

Table 1.6. Physico-chemical properties of selected PPCPs.

PPCP	Solubility in water ($mg \cdot L^{-1}$)	log K_{ow}	Henry's law constant ($atm \cdot m^3 \cdot mol^{-1}$)	pK_a	Log K_d ($L \cdot kg^{-1}$)		
					Primary	***Biological***	***Digested***
HHCB	1.8	5.9 - 6.3	$1.1 \cdot 10^{-4}$	-	3.7	3.3	3.9 - 4.1
AHTN	1.2	4.6 - 6.4	$1.2 \cdot 10^{-4}$	-	3.7	3.4	3.9 - 4.2
CBZ	17.7	2.3 - 2.5	$1.1 \cdot 10^{-10}$	13.9	< 1.3	0.1	1.5 - 1.7
DZP	50	2.5 - 3.0	$3.6 \cdot 10^{-9}$	3.3 - 3.4	1.6	1.3	
IBP	21	3.5 - 4.5	$1.5 \cdot 10^{-7}$	4.9 - 5.7	< 1.3	0.9	1.4
NPX	16	3.2	$3.4 \cdot 10^{-10}$	4.2	-	-	1.3 - 1.4
DCF	2.4	4.5 - 4.8	$4.7 \cdot 10^{-12}$	4.0 - 4.5	2.7	1.2	1.7
SMX	610	0.5 - 0.9	$6.4 \cdot 10^{-13}$	5.6 - 6.0	-	2.3 - 2.6	1.2 - 1.4
ROX	0.02	2.1 - 2.8	$2.5 \cdot 10^{-26}$	9.2	-	2.3 – 2.6	1.5 - 1.9
IPM	23.8	-	$1.0 \cdot 10^{-28}$	-	< 0.7	1.0	1.0 - 1.2
E1	30	3.1 - 3.4	$3.8 \cdot 10^{-10}$	10.4	-	-	2.4 - 2.6
E2	3.6	3.9 - 4.0	$3.6 \cdot 10^{-11}$	10.4	-	-	2.3 - 2.5
EE2	11.3	2.8 - 4.2	$7.9 \cdot 10^{-12}$	10.5 - 10.7	2.4	2.5	2.3 - 2.6

Liebig, 2004; Syracuse Research Corporation (SRC); Ternes *et al.*, 2004; Kummerer, 2000; Jones *et al.*, 2002; Carballa *et al.*, 2006b; Theiss, 2004.

1.1.5. Sources and pathways into the environment

Most pharmaceuticals or their metabolites are excreted or discarded into urban wastewaters and eventually make their way to municipal STPs (Figure 1.1). There are other sources of pharmaceuticals in the environment, such as veterinary drugs or feed additives for livestock, but these sources are beyond the scope of this work.

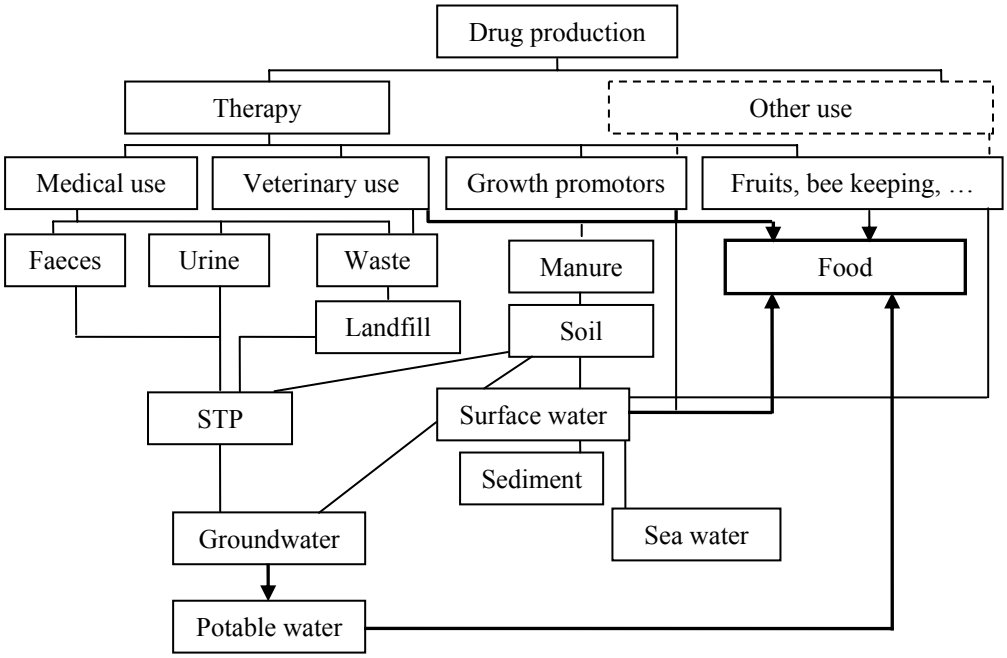


Figure 1.1. Sources, distribution and pathways of PPCPs in the environment (from Kummerer, 2000).

The main exposure routes of PPCPs into the municipal wastewaters are expected to be through their intentional use by patients in private households, either via excretion or disposal of pharmaceuticals through toilets. Hospital wastewaters may also contribute to the total environmental loading of PPCPs. In Spain, most hospitals do not individually treat their wastewaters and accordingly hospital effluents are treated by municipal STPs. A third route into the environment is point source discharges from pharmaceutical manufactures which might cause locally elevated levels of contamination.

Those PPCPs not readily biodegradable enter the receiving waters as dissolved pollutants via sewage treatment plants discharges or, those sorbed onto

sludge (i.e. biosolids), in agricultural fields when digested sludge is applied as fertilizer. Leakages from landfill sites, sewer drains, septic tanks and STPs are potential routes for groundwater contamination. Other pathway for soil and groundwater contamination includes spray irrigation of raw and treated wastewater onto agricultural fields.

As PPCPs are continuously discharged into the environment during the whole year, potential steady-state concentrations can be therefore expected in surface waters.

1.1.6. Occurrence and fate in the environment

Systematic studies have been conducted to investigate the occurrence of PPCPs in different environmental compartments, such as hospital effluents, influents and effluents of STPs, surface, ground and drinking water. The concentrations range from $ng \cdot L^{-1}$ levels in surface waters to $\mu g \cdot L^{-1}$ level in STP influents. Table 1.7 gives an overview of the concentrations of selected PPCPs detected in various aqueous samples and the sources of these data.

Wastewater

The levels of PPCPs in STP influents range from few $ng \cdot L^{-1}$ to high $\mu g \cdot L^{-1}$. The highest concentrations correspond to Iopromide (8 - 46 $\mu g \cdot L^{-1}$), followed by the musks (0.2 - 20 $\mu g \cdot L^{-1}$) and the anti-inflammatories (0.3 – 10 $\mu g \cdot L^{-1}$). The concentrations of Carbamazepine vary between 0.3 and 4 $\mu g \cdot L^{-1}$ and the content of antibiotics is 1 $\mu g \cdot L^{-1}$, approximately. The lowest levels were found for the estrogens (< 0.5 $\mu g \cdot L^{-1}$).

In STP effluents, the content of PPCPs ranges from few $ng \cdot L^{-1}$ to low $\mu g \cdot L^{-1}$. The highest concentrations of musks were 13.3 $\mu g \cdot L^{-1}$ for Galaxolide (Fromme *et al.,* 2001) and 6.8 $\mu g \cdot L^{-1}$ for Tonalide (Heberer, 2002a). Anti-inflammatories were found in the concentrations as high as 85 $\mu g \cdot L^{-1}$ for Ibuprofen (Farré *et al.,* 2001), 6.3 $\mu g \cdot L^{-1}$ for Naproxen (Drewes *et al.,* 2002) and 4.7 $\mu g \cdot L^{-1}$ for Diclofenac (Heberer, 2002b). The maximum levels of Carbamazepine and Iopromide were 6.3 $\mu g \cdot L^{-1}$ and 11 $\mu g \cdot L^{-1}$, respectively (Ternes, 1998). The antibiotics are found up to 2 $\mu g \cdot L^{-1}$ (Hirsch *et al.*, 1999), while the levels of estrogens are in the lower $ng \cdot L^{-1}$-range, corresponding to Estrone the maximum concentration found, 180 $ng \cdot L^{-1}$ (Komori *et al.*, 2004).

Table 1.7. Occurrence of selected PPCPs in different aquatic compartments (μg·L^{-1}).

PPCP	Wastewater		Surface water		Ground water	Drinking water	Reference
	Influent	Effluent	River	Lake			
HHCB		0.37-0.51[a]					García-Jares *et al.*, 2002
			0.036-0.152				Winkler *et al.*, 1998
			0.136				Müller *et al.*, 1996
		1-6					Schure, 2000
		7.58 (13.33)	0.02-12.5				Heberer, 2002a[*]
	9.7-16.6	0.98-4.62					Simonich *et al.*, 2002
		2.98					Noser *et al.*, 2000
	6.4 (14.5)	1.1-5.6	0.01-0.61	0.026-0.206			OSPAR , 2000[*]
		1.64		4.7			Peck *et al.*, 2004
	3.26-4.30	1.43-2.22					Artola-Garicano *et al.*, 2003a[a]
	(1.18-1.63)	(1.44-1.97)					
	7.8-19.2	1.1-6.4					Kanda *et al.*, 2003
		0.034-0.098		0.0001-0.001			Osemwengie *et al.*, 2004
	(0.97-16.6)	0.16-1.30					Ricking *et al.*, 2003[b]
		(0.04-4.6)					
		6.9 (13.3)		0.1-1.6 (0.3-3.2)			Fromme *et al.*, 2001[*]
		6.3-10.8	0.02-12.5				Heberer *et al.*, 1999
		0.6-2.5	0.37				Eschke *et al.*, 1994; 1995
		1-6					Paxeus, 1996
		0.72-1.95	0.005-0.564	<0.002-0.047			Buerge *et al.*, 2003
			(0.056-0.26)				Kolpin *et al.*, 2004[*]
	1.42-4.30	1.25-2.22					Artola-Garicano *et al.*, 2003b[a]
	(0.79-1.63)	(1.21-1.97)					
	1.41-2.33	0.65-0.80					Bester, 2004
	2.5-4.5	1.0-1.3					Fahlenkamp *et al.*, 2004
	6.4-13.6 (14.5)	0.23-2.3 (6.0)	0.0002-0.4 (0.8)				Balk and Ford, 1999[*]

[*]Mean (maximum) values; [a]Total (free) concentrations; [b]Values of literature review in brackets.

Table 1.7. Occurrence of selected PPCPs in different aquatic compartments (μg·L^{-1}). *Cont.*

PPCP	Wastewater		Surface water		Ground water	Drinking water	Reference
	Influent	Effluent	River	Lake			
AHTN		0.10-0.15[a]					García-Jares *et al.,* 2002
			0.024-0.088				Winkler *et al.*, 1998
			0.075				Müller *et al.*, 1996
		2.64 (6.80)	0.03-6.8				Heberer, 2002a[*]
	6.0-12.5	0.89-2.67					Simonich *et al.*, 2002
		2.04					Noser *et al.*, 2000
	4.0 (8.7)	0.5-2.4	0.01-0.4	0.015-0.043			OSPAR , 2000[*]
		1.15		1.0			Peck *et al.*, 2004
	1.24-1.76	0.57-1.20					Artola-Garicano *et al.,* 2003a[a]
	(0.36-0.48)	(0.45-0.55)					
	2.2-8.1	0.31-2.7					Kanda *et al.,* 2003
		0.022-0.050		0.0001-0.0006			Osemwengie *et al.*, 2004
	(0.32-12.5)	0.04-0.52					Ricking *et al.,* 2003[b]
		(0.04-1.9)					
		2.24 (4.36)		0.02-0.5 (0.1-1.1)			Fromme *et al.*, 2001[*]
		1.95-5.8	0.03-6.8				Heberer *et al.,* 1999
			0.20				Eschke *et al.,* 1994; 1995
		0.31-0.76	0.0023-0.186	<0.001-0.018			Buerge *et al.,* 2003
			(0.11-1.2)				Kolpin *et al.,* 2004[*]
	0.54-1.76	0.42-1.20					Artola-Garicano *et al.,* 2003b[a]
	(0.21-0.48)	(0.31-0.45)					
	0.43-0.71	0.20-0.24					Bester, 2004
	0.7-1.0	0.20-0.25					Fahlenkamp *et al.,* 2004
	4.0-8.7 (10.7)	0.23-1.4 (1.8)	0.0002-0.09 (0.5)				Balk and Ford, 1999[*]

[*]Mean (maximum) values; [a]Total (free) concentrations; [b]Values of literature review in brackets.

Table 1.7. Occurrence of selected PPCPs in different aquatic compartments (μg·L^{-1}). *Cont.*

PPCP	Wastewater		Surface water		Ground water	Drinking water	Reference
	Influent	Effluent	River	Lake			
CBZ		2.1 (6.3)	0.25 (1.1)				Ternes, 1998; 2001a*
			0.1-0.9				Sacher *et al.*, 1998
	3.23	0.471					Snyder, 2002
	2.2	2.0	(1.3)		(1.1)	<LOQ (0.03)	Ternes, 2001b[a]
			1.075				Heberer, 2002b
		0.007-0.126	0.020-0.185 (0.65)				Metcalfe *et al.*, 2003*
	n.d.	n.d.					Weigel *et al.*, 2004
			<0.02-7.1				Wiegel *et al.*, 2004
			(0.002-0.263)				Kolpin *et al.*, 2004*
	2	1					Khan and Ongerth, 2004[b]
		0.155-0.445					Drewes *et al.*, 2002
	0.5-2.75	0.94-1.51					Clara *et al.*, 2004a
	0.33-3.67	0.47-3.87					Clara *et al.*, 2005
	1.78 (3.8)	1.63 (5.0)	0.025-1.075				Heberer *et al.*, 2002; 2002c*
		0.1-1.2					Paxeus, 2004
	0.3-0.9	0.5-0.7					Strenn *et al.*, 2004
	1.25	1.25					Fahlenkamp *et al.*, 2004
DZP		<LOQ (0.04)	<LOQ (<LOQ)				Ternes, 1998; 2001a*[c]
					10 – 40		Daugthon and Ternes, 1999
			<LOD-0.0012			<LOD-0.024	Zuccato *et al.*, 2000[d]
						0.01	Christensen, 1998

n.d.: not detected; *Mean (maximum) values; [a]LOQ: 0.01 μg·L^{-1}; [b]Predicted values; [c]LOQ: 0.030 μg·L^{-1}; [d]LOD water: 0.02 ng·L^{-1}.

Table 1.7. Occurrence of selected PPCPs in different aquatic compartments (μg·L^{-1}). *Cont.*

PPCP	Wastewater		Surface water		Ground water	Drinking water	Reference
	Influent	Effluent	River	Lake			
IBP	2.81-5.77	0.91-2.10					Rodríguez *et al.*, 2003
		0.37 (3.40)	0.07 (0.53)				Ternes, 1998; 2001a[*]
	0.3	0.6 (3.8)	0.02 (0.19)				Stumpf *et al.*, 1999[*]
	0.99-3.3	0.002-0.081	0.0015-0.0078				Buser *et al.*, 1999
	4.4	0.45	0.2			<LOQ (0.003)	Ternes, 2001b[*a]
		0.87-85	2.7				Farré *et al.*, 2001
			<LOD-0.092			<LOD	Zuccato *et al.*, 2000[b]
		0.12					Janex *et al.*, 2002[c]
		0.079-1.885	<0.005-0.141 (0.79)				Metcalfe *et al.*, 2003[*]
		<LOD	<LOD	<LOD		<LOD	Boyd *et al.*, 2003[d]
	2-11	LOD-4.7					Kanda *et al.*, 2003[e]
	0.6	0.16-0.68					Weigel *et al.*, 2004
			0.2 (1.0)				Kolpin *et al.*, 2002[*]
			<0.002-0.146				Wiegel *et al.*, 2004
			(<0.018)				Kolpin *et al.*, 2004[*]
	1	0.6					Khan and Ongerth, 2004[f]
		0.005-3.38					Drewes *et al.*, 2002
	1.2-3.68	0.02-2.40					Clara *et al.*, 2005
			n.d.-0.055				Heberer *et al.*, 2002
		0.02-1.96					Paxeus, 2004
	>3.5	0.25					Fahlenkamp *et al.*, 2004
		0.1			n.d.-0.2		Heberer, 2002c

n.d.: not detected; [*]Mean (maximum) values; [a]LOQ: 0.001 μg·L^{-1}; [b]LOD: 0.50 ng·L^{-1}; [c]Maximum concentrations; [d]LOD: 13 ng·L^{-1}; [e]LOD: 6.7 ng·L^{-1}; [f]Predicted values.

Table 1.7. Occurrence of selected PPCPs in different aquatic compartments (μg·L^{-1}). *Cont.*

PPCP	Wastewater		Surface water		Ground water	Drinking water	Reference
	Influent	Effluent	River	Lake			
NPX	3.50-4.50	1.87-2.56					Rodríguez *et al.*, 2003
	1.3	0.30 (0.52)	0.07 (0.39)				Ternes, 1998; 2001a[*]; 2001b
	0.6	0.6 (3.0)	0.02 (0.21)				Stumpf *et al.*, 1999[*]
		0.12					Janex *et al.*, 2002[a]
		0.021-0.524	<0.005-0.207 (0.55)				Metcalfe *et al.*, 2003[*]
		0.081-0.106	0.037-0.039	0.022-0.107			Boyd *et al.*, 2003
			<0.001-0.032				Wiegel *et al.*, 2004
	8	4					Khan and Ongerth, 2004[b]
		0.11-6.28					Drewes *et al.*, 2002
	0.44	0.08	n.d.-0.095				Heberer *et al.*, 2002; 2002c
		<0.05-1.51					Paxeus, 2004
	0.6	0.35					Fahlenkamp *et al.*, 2004
DCF	n.d	n.d.					Rodríguez *et al.*, 2003
	1.9	0.81 (2.10)	0.15 (1.20)		(3.5)	<LOQ (0.006)	Ternes, 1998; 2001a[*]; 2001b[*c]
	0.47-1.92	0.31-0.93	<0.001-0.37	0.001-0.012			Buser *et al.*, 1998
	0.8	0.4 (1.5)	0.02 (0.45)				Stumpf *et al.*, 1999[*]
		0.3	0.05				Janex *et al.*, 2002[a]
		0.005-0.35	<0.005-0.194 (0.19)				Metcalfe *et al.*, 2003[*]
	n.d	n.d-0.03					Weigel *et al.*, 2004
			0.001-0.069				Wiegel *et al.*, 2004
	0.4	0.03					Khan and Ongerth, 2004[b]
	0.91-4.20	0.78-3.48					Clara *et al.*, 2005
	3.02 (7.1)	2.51 (4.7)	n.d.-1.030		n.d.-0.38	<0.010[tap]	Heberer *et al.*, 2002; 2002c[*]
		0.14-1.48					Paxeus, 2004
	0.4-1.3	0.3-1.7					Strenn *et al.*, 2004
	1.90	1.55					Fahlenkamp *et al.*, 2004

n.d.: not detected; [*]Mean (maximum) values; [a]Maximum concentrations; [b]Predicted values; [c]LOQ: 0.001 μg·L^{-1}.

Table 1.7. Occurrence of selected PPCPs in different aquatic compartments (μg·L^{-1}). *Cont.*

PPCP	Wastewater		Surface water		Ground water	Drinking water	Reference
	Influent	Effluent	River	Lake			
IPM		0.75 (11)	0.10 (0.91)			<LOQ (0.086)	Ternes, 2001a*; 2001b*[a]
	7.5	8.1 (11)	0.011-0.80		<LOQ (0.21)		Ternes and Hirsch, 2000*[b]
			1.6		<0.05	<0.05	Putschew *et al.,* 2000
	25-46	4-9					Steger-Hartmann *et al.,* 2002
SMX		0.40 (2)	0.03 (0.48)				Ternes, 2001a*
			0.48		0.47		Hirsch *et al.,* 1998
					0.41		Sacher *et al.,* 2001
		0.40 (2.0)	0.03 (0.48)		<0.02 (0.47)		Hirsch *et al.,* 1999*
			0.07-0.15 (0.5-1.9)				Kolpin *et al.,* 2002*
			<0.03-0.07				Wiegel *et al.,* 2004
			(<0.05-0.07)				Kolpin *et al.,* 2004*
		0.243 (0.871)					Miao *et al.,* 2004*
	1	0.9					Khan and Ongerth, 2004[c]
	1.75	1.3					Fahlenkamp *et al.,* 2004
		0.9					Heberer, 2002c
ROX		0.68 (1)	<LOQ (0.56)				Ternes, 2001a*[d]
			0.19				Hirsch *et al.,* 1998
		0.68 (1.0)	<0.02 (0.56)		<0.02 (<0.02)		Hirsch *et al.,* 1999*
			0.05 (0.18)				Kolpin *et al.,* 2002*
			<0.03-0.04				Wiegel *et al.,* 2004
			(<0.01)				Kolpin *et al.,* 2004*
		0.008 (0.018)					Miao *et al.,* 2004*
	1	1					Khan and Ongerth, 2004[c]
	0.5	0.4					Fahlenkamp *et al.,* 2004

*Mean (maximum) values; [a]LOQ: 0.01 μg·L^{-1}; [b]LOQ: 0.010 μg·L^{-1}; [c]Predicted values; [d]LOQ: 0.020 μg·L^{-1}.

Table 1.7. Occurrence of selected PPCPs in different aquatic compartments (μg·L^{-1}). *Cont.*

PPCP	Wastewater		Surface water		Ground water	Drinking water	Reference
	Influent	Effluent	River	Lake			
E1	0.027-0.040	0.003-0.009 (0.070)	<LOQ (0.0016)				Ternes *et al.*, 1999*; 2001a*[a]
	0.108	0.039					Snyder, 2002
	0.043	0.0054					Kobuke *et al.*, 2002
		0.0058					Larsson *et al.*, 1999
	0.055-0.077	<0.001					Andersen *et al.*, 2003[b]
	0.028-0.132	0.002-0.082					Johnson and Williams, 2004[c]
		<LOD					Boyd *et al.*, 2003[d]
			<LOD	<LOD		<LOD	Kolpin *et al.*, 2002*
	0.01-0.057	<LOD-0.18	0.027 (0.112)				Komori *et al.*, 2004[e]
							Bursch *et al.*, 2004*
	0.0096-0.018	0.0043-0.0072	0.0004 (0.005)		(0.0016)		Cargouët *et al.*, 2004
	0.025-0.132	0.003-0.082	0.0011-0.003				Baronti *et al.*, 2000
		0.002-0.047	0.0015				Belfroid *et al.*, 1999
		0.001-0.05	<0.0001-0.003				Desbrow *et al.*, 1998
	0.025-0.037	0.005-0.009					Joss *et al.*, 2004
	0.034-0.67	0.001-0.072					Clara *et al.*, 2005
		<0.002-0.035					Johnson *et al.*, 2005
		0.012-0.023					Fahlenkamp *et al.*, 2004

*Mean (maximum) values; [a]LOQ: 0.0005 μg·L^{-1}; [b]Total concentrations including fraction sorbed to suspended solids; [c]Review of concentrations in six Roman STPs; [d]LOD: 3 ng·L^{-1}; [e]LOD: 0.8 ng·L^{-1}.

Table 1.7. Occurrence of selected PPCPs in different aquatic compartments (μg·L^{-1}). *Cont.*

PPCP	Wastewater		Surface water		Ground water	Drinking water	Reference
	Influent	Effluent	River	Lake			
E2	0.015-0.021	<LOQ (0.064)	<LOQ (<LOQ)				Ternes *et al.*, 1999[*]; 2001a[*a]
	0.01	33					CSIC, 4/2001
			0.006-0.01				Duguet *et al.*, 2004
	0.050-0.081	<0.0005-0.01	<0.001-0.28				Kobuke *et al.*, 2002
		0.0011					Larsson *et al.*, 1999
	0.012-0.020	<0.001					Andersen *et al.*, 2003[b]
	0.004-0.025	0.0003-0.0025					Johnson and Williams, 2004[c]
		<LOD	<LOD	<LOD		<LOD	Boyd *et al.*, 2003[d]
			0.009 (0.093)				Kolpin *et al.*, 2002[*]
	<LOD-0.021	<LOD-0.011					Komori *et al.*, 2004[e]
			0.0001 (0.001)		0.0001 (0.0008)		Bursch *et al.*, 2004[*]
	0.011-0.017	0.0045-0.0086	0.0014-0.0032				Cargouët *et al.*, 2004
						(0.0021)[tap]	Kuch and Ballschmiter, 2001[*]
	0.004-0.025	0.0004-0.004	0.0001				Baronti *et al.*, 2000
		<0.0006-0.012	<0.0003-0.006				Belfroid *et al.*, 1999
		(0.021)					Stumpf *et al.*, 1996[*]
		0.002-0.05					Desbrow *et al.*, 1998
	0.003-0.010	<0.0005-0.001					Joss *et al.*, 2004
	0.014-0.125	0.003-0.030					Clara *et al.*, 2005
		<0.006-0.013					Johnson *et al.*, 2005
		0-0.010					Fahlenkamp *et al.*, 2004

[*]Mean (maximum) values; [a]LOQ: 0.001 μg·L^{-1} (effluent), 0.0005 μg·L^{-1} (river); [b]Total concentrations including fraction sorbed to suspended solids; [c]Review of concentrations in six Roman STPs; [d]LOD: 1 ng·L^{-1}; [e]LOD: 0.5 ng·L^{-1}.

Table 1.7. Occurrence of selected PPCPs in different aquatic compartments (μg·L^{-1}). *Cont.*

PPCP	Wastewater		Surface water		Ground water	Drinking water	Reference
	Influent	Effluent	River	Lake			
EE2		0.001-0.009 (0.042)	<LOQ (<LOQ)				Ternes *et al.*, 1999*; 2001a*[a]
			0.00004-0.005				Duguet *et al.*, 2004
		<0.007					Daugthon and Ternes, 1999
		0.017 (0.062)					Stumpf *et al.*, 1996*
		0.001-0.003					Heberer, 2002b
	0.0004-0.013	<0.0003-0.002	0.00004				Baronti *et al.*, 2000
					0.0024	0.0024[tap]	Adler *et al.*, 2001
		0.0045					Larsson *et al.*, 1999
	≈1	≈1					Tabak *et al.*, 1981
	0.006-0.010	<0.001					Andersen *et al.*, 2003[b]
	0.0005-0.013	0-0.0019					Johnson and Williams, 2004[c]
			0.073 (0.831)				Kolpin *et al.*, 2002*
	<LOD	<LOD					Komori *et al.*, 2004[d]
			(0.0003)		(0.0009)		Bursch *et al.*, 2004*
	0.005-0.007	0.0027-0.0045	0.0011-0.0029				Cargouët *et al.*, 2004
						(0.0005)[tap]	Kuch and Ballschmiter, 2001*
		<0.0002-0.008	<0.0001-0.004				Belfroid *et al.*, 1999
		(0.007)					Desbrow *et al.*, 1998*
	<0.0005-0.002	<0.0005-0.0005					Joss *et al.*, 2004
	0.003-0.070	0.002-0.106					Clara *et al.*, 2005
		0.0011-0.0028					Johnson *et al.*, 2005
		0.004					Fahlenkamp *et al.*, 2004

*Mean (maximum) values; [a]LOQ: 0.001 μg·L^{-1} (effluent), 0.0005 μg·L^{-1} (river); [b]Total concentrations including fraction sorbed to suspended solids; [c]Review of concentrations in six Roman STPs; [d]LOD: 1.2 ng·L^{-1}.

The presence of PPCPs in the STP effluents demonstrates that their elimination during STP treatment is not complete (Table 1.8). In addition, conjugates can be cleaved and reform the parent compound, thus increasing the concentrations.

Surface water

The concentrations of PPCPs in surface water are typically significantly lower than in STP effluents as a result of the dilution factor. As many PPCPs are degrade to some extent in STPs, it can be assumed that degradation takes also place in the aquatic environment. Consequently, the observed PPCP concentration in surface waters can be described as steady-state concentration, which is a function of its continuous input, dilution and degradation.

In surface waters, the concentrations of these compounds are rarely above 500 $ng \cdot L^{-1}$ and are frequently below 100 $ng \cdot L^{-1}$. The highest concentrations will occur at sites close to point sources, such as near STP discharges.

Groundwater

The occurrence of PPCPs in groundwater has also been reported (Ternes, 2001a; Heberer, 2002c; Hirsch *et al.,* 1999; Putschew *et al.,* 2000; Bursch *et al.,* 2004). Due to dilution and degradation during bank filtration or soil passage, fewer compounds and lower concentrations of PPCPs are found in groundwater than in surface water.

In groundwaters, the concentrations of PPCPs are in the $ng \cdot L^{-1}$-range (0-500 $ng \cdot L^{-1}$) and most of them are detected below the Limit of Quantification (LOQ).

Drinking water

Surface and groundwater are the principle water resources used for drinking water production. Therefore, the occurrence of PPCPs in these resources could have a negative impact on the purity of drinking water. Although up to date it is unclear whether the presence of PPCPs in drinking water at levels lower than 1 $\mu g \cdot L^{-1}$ can produce adverse health effects, based on precautionary principles, the concentration of these substances in drinking water should be as low as possible to minimize the risk of unpredictable long-term effects.

In drinking water, the concentrations of PPCPs are in the lower $ng \cdot L^{-1}$-range ($<100\ ng \cdot L^{-1}$) and most of them are detected below the LOQ.

Table 1.8. PPCPs removal efficiencies (%) in STPs.

PPCP	STP							Reference
	Location	*Treatment*[1]	*Flow* ($x10^{-3}$) ($m^3 \cdot d^{-1}$)	*Inhabit.* ($x10^{-3}$)	*Samp.*[*]	*Comments*[**]	*Removal*	
HHCB	Germany	-	-	-	-	-	34	Eschke *et al.*, 1994
	USA	-	-	-	-	-	87.4	Simonich *et al.*, 2000
	Netherlands	-	-	-	G	-	32-73	Artola-Gar. *et al.*, 2003a
	U.K.	RBC, SAF, OD, BFB, AS, TF	0.1-3.2	-	C	6 STPs	39-94	Kanda *et al.*, 2003
	Switzerland	P-EA , AS	-	0.2-17.2	C	16 STPs	75	Kupper *et al.*, 2004
	Netherlands	P-B	0.8-4.2	-	G	4 STPs	12-60	Artola-Gar. *et al.*, 2003b
	USA/EU	P-AS, TF, C, RBC/OD, La	1.4-100	-	C	17 STPs	64-99	Simonich *et al.*, 2002
						-	52-70	Bester, 2004
	Germany	P-B	200	350	C			
	Austria	P-AS	-	7	C	Rural, N,P	80	Clara *et al.*, 2004b
	Austria	P-AS	-	7-2,500	C	4 STPs, N,P	2-92	Kreuzinger *et al.*, 2004
	Germany	P-B	-	1,000	C	2 STPs, N,P	61-72	Fahlenkamp *et al.*, 2004
AHTN	Germany	-	-	-	-	-	60	Eschke *et al.*, 1994
	USA	-	-	-	-	-	86	Simonich *et al.*, 2000
	Netherlands	-	-	-	G	-	14-65	Artola-Gar. *et al.*, 2003a
	U.K.	RBC, SAF, OD, BFB, AS, TF	0.1-3.2	-	C	6 STPs	40-96	Kanda *et al.*, 2003
	USA/EU	P-AS, TF, C, RBC/OD, La	1.4-100	-	C	17 STPs	51-99	Simonich *et al.*, 2002
	Netherlands	P-B	0.8-4.2	-	G	4 STPs	12-60	Artola-Gar. *et al.*, 2003b
	Germany	P-B	200	350	C	-	54-70	Bester, 2004
	Austria	P-AS	-	7	C	Rural, N, P	80	Clara *et al.*, 2004b
	Austria	P-AS	-	7-2,500	C	4 STPs, N, P	0-92	Kreuzinger *et al.*, 2004
	Germany	P-B	-	1,000	C	2 STPs, N, P	71-82	Fahlenkamp *et al.*, 2004

[1]See foot of last table for abbreviations. [*]G: Grab sample; C: Composite sample; [**]N: nitrogen removal, P: phosphorus removal.

Table 1.8. PPCPs removal efficiencies (%) in STPs. *Cont.*

PPCP	STP							Reference
	Location	***Treatment*[1]**	***Flow* ($x10^{-3}$) ($m^3 \cdot d^{-1}$)**	***Inhabit.* ($x10^{-3}$)**	***Samp.*[*]**	***Comments*[**]**	***Removal***	
CBZ	Germany	P-B	53.3-64.7	312	C	P	7	Ternes, 1998; 2001a
	Las Vegas	P-AS-UV	303-322	-	G	-	85.4	Snyder, 2002
	Germany	-	-	-	-	-	<10	Heberer, 2002b
	Australia	P-B-D	6.5	23	-	-	6-39	Khan and Ongerth, 2004
	Austria	P-AS, TF	10-85	100-2,000	C	N, P	0	Clara *et al.*, 2004a
	Austria	P-AS/MBR	-	6-2,500	C	4 STPs, N, P	<20	Clara *et al.*, 2005
	Germany	-	-	-	C	-	8	Heberer *et al.*, 2002
	Austria	P-AS/MBR	-	7	C	Rural, N, P	0	Clara *et al.*, 2004b
	Austria	P-AS	-	7-2,500	C	4 STPs, N, P	0-35	Kreuzinger *et al.*, 2004
	5EU country	P-AS	1.4-328.8	6-900	C/G	N, P	<10-53	Paxeus, 2004
	Austria	P-AS	10-85	7-2,500	C	12 STPs, N, P	0	Strenn *et al.*, 2004
	Germany	P-B	-	1,000	C	2 STPs, N, P	0	Fahlenkamp *et al.*, 2004
DZP	Austria	P-AS	-	7-2,500	C	4 STPs, N, P	0-25	Kreuzinger *et al.*, 2004
ROX	Australia	P-B-D	6.5	23	-	-	4-14	Khan and Ongerth, 2004
	Austria	P- AS	-	7-2,500	C	4 STPs, N, P	0-75	Kreuzinger *et al.*, 2004
SMX	Australia	P-B-D	6.5	23	-	-	5-27	Khan and Ongerth, 2004
	Austria	P-AS	-	7-2,500	C	4 STPs, N, P	33-91	Kreuzinger *et al.*, 2004
IPM	Germany	P-B	-	312	C	P	0	Ternes and Hirsch, 2000
	Austria	P-AS	-	7-2,500	C	4 STPs, N, P	0-50	Kreuzinger *et al.*, 2004

[1]See foot of last table for abbreviations. [*]G: Grab sample; C: Composite sample; [**]N: nitrogen removal, P: phosphorus removal.

Table 1.8. PPCPs removal efficiencies (%) in STPs. *Cont.*

PPCP	STP							Reference
	Location	*Treatment*[1]	*Flow* ($x10^{-3}$) ($m^3 \cdot d^{-1}$)	*Inhabit.* ($x10^{-3}$)	*Samp.*[*]	*Comments*[**]	*Removal*	
IBP	Germany	P-B	53.3-64.7	312	C	P	90	Ternes, 1998; 2001a
	Brazil	AS+TF	120.1	624	C	-	22-75	Stumpf *et al.*, 1999
	Switzerland	P-B	-	8.5-26	C	3 STPs	96-99	Buser e*t al.*, 1999
	U.K.	RBC, SAF, OD, BFB, AS, TF	0.1-3.2	-	C	6 STPs	14-100	Kanda *et al.,* 2003
	Australia	P-B-D	6.5	23	-	-	4-52	Khan and Ongerth, 2004
	Austria	P- AS/MBR	-	6-2,500	C	4 STPs, N, P	<20-99	Clara *et al.,* 2005
	Austria	P- AS MBR	-	7	C	Rural, N, P	>95	Clara *et al.,* 2004b
	Austria	P-AS	-	7-2,500	C	4 STPs, N, P	0-99	Kreuzinger *et al.,* 2004
	5EU country	P-AS	1.3-328.8	6-900	C/G	N, P	52-99	Paxeus, 2004
	Austria	P-AS	10-85	7-2,500	C	12 STPs, N, P	>90	Strenn *et al.,* 2004
	Germany	P-B	-	1,000	C	2 STPs, N, P	>90	Fahlenkamp *et al.,* 2004
NPX	Germany	P-B	53.3-64.7	312	C	P	66	Ternes, 1998; 2001a
	Brazil	P-AS+TF	120.1	624	C	-	15–78	Stumpf *et al.*, 1999
	Australia	P-B-D	6.5	23	-	-	3-58	Khan and Ongerth, 2004
	5EU country	P-AS	1.3-328.8	6-900	C/G	N, P	42-93	Paxeus, 2004

[1]See foot of last table for abbreviations. [*]G: Grab sample; C: Composite sample; [**]N: nitrogen removal, P: phosphorus removal.

Table 1.8. PPCPs removal efficiencies (%) in STPs. *Cont.*

PPCP	STP							Reference
	Location	***Treatment*[1]**	***Flow* ($x10^{-3}$) ($m^3 \cdot d^{-1}$)**	***Inhabit.* ($x10^{-3}$)**	***Samp.****	***Comments*****	***Removal***	
DCF	Germany	P-B	53.3-64.7	312	C	P	69	Ternes, 1998; 2001a
	Switzerland	B	-	8.5-26	C	3 STPs	50	Buser e*t al.*, 1998
	Brazil	P-B	120.1	624	C	AS+TF	9-75	Stumpf *et al.*, 1999
	Germany	-	-	-	-	-	17	Heberer, 2002b
	Australia	P-B-D	6.5	23	-	-	7-31	Khan and Ongerth, 2004
	Austria	P-AS/MBR	-	6-2,500	C	4 STPs, N, P	<20-80	Clara *et al.*, 2005
	Germany	-	-	-	C	-	17	Heberer *et al.*, 2002
	Austria	P-AS/MBR	-	7	C	Rural, N, P	40-60	Clara *et al.*, 2004b
	Austria	P-AS	-	7-2,500	C	4 STPs, N, P	0-69	Kreuzinger *et al.*, 2004
	5EU country	P-AS	1.4-328.8	6-900	C/G	N, P	<10-80	Paxeus, 2004
	Austria	P-AS	10-85	7-2,500	C	12 STPs, N, P	39-74	Strenn *et al.*, 2004
EE2	Germany	P-B	53.3-64.7	312	C	P	0	Ternes, 2001a
	Italy	P-B	10-734	40-1,200	C	6 STPs	85	Baronti *et al.*, 2000
	Sweden	P-B	0.7	3.5	C	-	0	Larsson *et al.*, 1999
	Germany	P-B	66	300	C	P	90	Andersen *et al.*, 2003
	EU STP	-	-	-	-	Literature	85-90	Johnson and Williams, 2004
	France	P-AS, B	250-8,000	48.5-2,080	-	4 STPs	34-45	Cargouët *et al.*, 2004
	Germ/Brazil	P-AS, TF	41.2-120.1	312-624	C/G	2 STPs	0-78	Ternes *et al.*, 1999
	Switzerland	P-AS/MBR, FB	-	70-120	C	N, P	69-94	Joss *et al.* ,2004
	Austria	P-AS/MBR	-	6-2,500	C	4 STPs, N, P	<20-100	Clara *et al.*, 2005
	Austria	P-AS/MBR	-	7	C	Rural, N, P	60-70	Clara *et al.*, 2004b
	Austria	P-AS	-	7-2,500	C	4 STPs N, P	0-81	Kreuzinger *et al.*, 2004

[1]See foot of last table for abbreviations. *G: Grab sample; C: Composite sample; **N: nitrogen removal, P: phosphorus removal.

Table 1.8. PPCPs removal efficiencies (%) in STPs. *Cont.*

PPCP	STP							Reference
	Location	*Treatment*[1]	*Flow* ($x10^{-3}$) ($m^3 \cdot d^{-1}$)	*Inhabit.* ($x10^{-3}$)	*Samp.*[*]	*Comments*[**]	*Removal*	
E1	USA	P-AS-UV	303-322	-	G	-	64	Snyder, 2002
	Japan	-	-	-	-	47 STPs	87	Kobuke *et al.*, 2002
	Germany	P-B	53.3-64.7	312	C	P	0	Ternes, 2001a
	Italy	P-B	10-734	40-1,200	C	6 STPs	0-61	Baronti *et al.*, 2000
	Germany	P-B	66	300	C	P	98 (+E2)	Andersen *et al.*, 2003
	EU STP	-	-	-	-	Literature	61-98	Johnson *et al.*, 2004
	Japan	P-AS, A/O, A_2/O	12-680	-	G	20 STPs	45	Komori *t al.*, 2004
	France	P-AS, B	250-8,000	48.5-2,080	-	4STPs	44-59	Cargouët *et al.*, 2004
	Germ/Brazil	P-AS, TF	41.2-120.1	312-624	C/G	2 STPs	0-83	Ternes *et al.*, 1999
	Switzerland	P-AS, MBR, FB	-	70-120	C	N, P	49-99	Joss *et al.* ,2004
	Austria	P-AS/MBR	-	6-2,500	C	4 STPs, N, P	20-100	Clara *et al.*, 2005
	Austria	P-AS	-	7-2,500	C	4 STPs, N, P	6-99	Kreuzinger *et al.*, 2004
	USA/EU	P-AS, TF, OD, SAF	1.1-304.4	6.5-750	C	17 STPs, N, P	59-99	Johnson *et al.*, 2005
E2	Japan	-	-	-	-	47 STPs	92	Kobuke *et al.*, 2002
	Germany	P-B	53.3-64.7	312	C	P	64	Ternes, 2001a
	Italy	P-B	10-734	40-1,200	C	6 STPs	87	Baronti *et al.*, 2000
	EU STP	-	-	-	-	Literature	67-99	Johnson *et al.*, 2004
	Japan	P-AS, A/O, A_2/O	12-680	-	G	20 STPs	100	Komori *et al.*, 2004
	France	P-AS, B	250-8,000	48.5-2,080	-	4 STPs	43-60	Cargouët *et al.*, 2004
	Germ/Brazil	P-AS, TF	41.2-120.1	312-624	C/G	2 STPs	64-99	Ternes *et al.*, 1999
	Switzerland	P-AS, MBR, FB	-	70-120	C	N, P	88-98	Joss *et al.* ,2004

[*]G: Grab sample; C: Composite sample; [**]N: nitrogen removal, P: phosphorus removal.

[1]A/O: Anaerobic/Oxic; A_2/O: Anaerobic/Anoxic/Oxic; AS: Activated Sludge; B: Biological treatment; BFB: Biological Filter Bed; C: Carousel; D: Disinfection; EA: Extended Aeration; FB: Fixed Bed; La: Lagoon; MBR: Membrane Biological Reactor; OD: Oxidation Ditch; P: Primary treatment; RBC: Rotating Biological Contactor; SAF: Submerged Aerated Filter; UV: Ultraviolet.

Sewage sludge

The presence of PPCPs in the sludge will be determined by their partitioning to the solid phase during primary and secondary treatment and through active uptake into the biomass. It is likely that compounds that occur in the sludge are recalcitrant and not readily degraded. In contrast to liquid phase, the occurrence of PPCPs in sewage sludge has been reported in a less extent. A summary of the literature data available is shown in Table 1.9.

1.1.7. Environmental effects

PPCPs are potentially harmful environmental contaminants since they have been selected or designed to be biologically active against organisms. However, little information is available on the effects of active substances on organisms in the aquatic and terrestrial environment. High concentrations of some compounds ($mg \cdot L^{-1}$ range) have been found to produce effects in environmental organisms. However, effects on *Daphnia*, algae and bacteria have been also demonstrated using low concentrations in chronic tests. Most of these studies covered endocrine disruptors and antibiotics. For example, it was found that EE2 adversely affects the reproduction of the rainbow trout (*Oncorynchus mykiss*) at very low concentrations, below 1 $ng \cdot L^{-1}$ (Segner *et al.*, 2003; Metcalfe *et al.*, 2001). In the case of antibiotics, it is presumed that some of them lead to the formation of resistant bacterial strains in the environment (Ohlsen *et al.,* 2003).

The risk of adverse effects on humans through ingestion of pharmaceuticals contained in drinking water seems to be negligible. The maximum possible intake within a lifetime (assuming 2 L per day over 70 years) is far below a single therapeutic dose (Table 1.10). Thus, the risks posed to humans from pharmaceuticals seem to concern environmental hygiene rather than toxicology and pharmacology.

Furthermore, up to now risk assessments have been only undertaken for single substances and not for mixtures, and there are not procedures to assess risks connected with carcinogenic, mutagenic or reproductive toxic compounds. Besides toxicity, the element of persistence is of particular importance for the assessment of the environmental significance of PPCPs (Kummerer and Held, 1997). Persistent compounds increase the potential for long-term and hence varied effects.

Table 1.9. Occurrence of PPCPs in different solid compartments ($ng \cdot g^{-1}$).

PPCP	Sludge			SPM	Sediment	Reference
	Primary	*Secondary*	*Digested*			
	129±12	162±17				Llompart *et al.*, 2003
		4,500-8,500	15,000			Ternes *et al.*, 2005
				148-736		Winkler *et al.*, 1998
			6,030-11,450			Heberer, 2002a
			2,293-12,157			Herren and Berset, 2000
			27,000 (81,000)			Stevens *et al.*, 2003[*]
					54,000	Rimkus, 2003
					30-3,600	Fromme *et al.*, 2001
				13,000		Fooken *et al.*, 1997
HHCB		2,293-21,626	9,000-31,000	<50-13,722	<0.5-180	OSPAR , 2000
			4,300-13,400		80-21,800	EU, 2001
			7,400-36,000			Kupper *et al.*, 2004[a]
			(6,700-31,000)			
	39.3-257.7	29.5-234.6				Artola-Garicano *et al.*, 2003b[b]
	(1.1-1.6)	(1.4-1.8)				
			2,709-3,342			Bester, 2004[c]
			(2,500-29,000)			
	4,300-27,000	100-63,000	9,000-31,000	50-740	150-300	Balk and Ford, 1999

SPM: Suspended Particulate Matter; [*]Maximum values in brackets.
[a]Digested sludge either aerobically or anaerobically (literature values);[b]Total (Free) concentrations in $\mu g \cdot L^{-1}$;
[c]Mean value of STP studied (range of other STPs).

Table 1.9. Occurrence of PPCPs in different solid compartments (ng·g^{-1}). *Cont.*

PPCP	Sludge			SPM	Sediment	Reference
	Primary	*Secondary*	*Digested*			
	64±9	52±5				Llompart *et al.,* 2003
		1,400-4,300	6,600			Ternes *et al.,* 2005
				194-770		Winkler *et al.*, 1998
			2,520-5,070			Heberer, 2002a
			741-4,161			Herren and Berset, 2000
			4,700 (16,000)			Stevens *et al.*, 2003*
					3,900	Rimkus, 2003
					20-2,600	Fromme *et al.*, 2001
				13,000		Fooken *et al.*, 1997
AHTN		741-20,107	4,900-22,000	60-12,666	<0.5-104	OSPAR, 2000
			4,000-12,600		130-36,700	EU, 2001
			2,500-11,200			Kupper *et al.,* 2004[a]
			(5,800-21,000)			
	15.3-92.3	12.4-82.7				Artola-Garicano *et al.,* 2003b[b]
	(0.3-0.5)	(0.4-0.5)				
			1,343-1,746			Bester, 2004[c]
			(5,000-7,000)			
	3,300-14,000	100-34,000	900-22,000	60-1,200	150-300	Balk and Ford, 1999
CBZ	9 (0.2)-1,731 (3)	6 (0.1)	n.d.-0.01 (6)			Khan and Ongerth, 2002[d]
DZP					<LOD	Zuccato *et al.,* 2000[e]

SPM: Suspended Particulate Matter; *Maximum values in brackets.

[a]Digested sludge either aerobically or anaerobically (literature values);[b]Total (Free) concentrations in µg·L^{-1}; [c]Mean value of STP studied (range of other STPs); [d]Soluble concentration (µg·L^{-1}) in brackets. Predicted values – Measured values; [e]LOD: 0.009 ng·kg^{-1}.

Table 1.9. Occurrence of PPCPs in different solid compartments ($ng \cdot g^{-1}$). *Cont.*

PPCP	Sludge			SPM	Sediment	Reference
	Primary	***Secondary***	***Digested***			
IBP	1,240 (1)-3,988 (2)	243 (0.1)	0.006 (6)		<LOD-0.22	Zuccato *et al.*, 2000[a] Khan and Ongerth, 2002[b]
NPX	1,022 (2)-1,564 (5)	538 (2)	0.001 (0.1)			Khan and Ongerth, 2002[b]
DCF		200-450	220			Ternes *et al.*, 2005
IPM						-
SMX						-
ROX						-
E1		LOQ-37 7 <2	LOQ-16 22.8-27.8		LOQ-2	Ternes *et al.*, 2002[c] Andersen *et al.*, 2003 Joss *et al.* ,2004
E2		5-17 1.7 <2 -2.1	9-49 4.9-5.4		LOQ-1.5 500	Ternes *et al.*, 2002[c] CSIC, 2004 Andersen *et al.*, 2003 Joss *et al.* ,2004
EE2		LOQ-4 3 <2-2.4	2-17 <1.5		LOQ-0.9	Ternes *et al.*, 2002[d] Andersen *et al.*, 2003 Joss *et al.* ,2004

SPM: Suspended Particulate Matter [*]Maximum values in brackets.

[a]LOD: 0.04 $ng \cdot g^{-1}$; [b]Soluble concentration ($\mu g \cdot L^{-1}$) in brackets. Predicted values – Measured values;

[c]LOQ (sludge): 2 $ng \cdot g^{-1}$; LOQ (sediment): 0.2 $ng \cdot g^{-1}$; [d]LOQ (sludge): 4 $ng \cdot g^{-1}$; LOQ (sediment): 0.4 $ng \cdot g^{-1}$.

Table 1.10. Estimation of total PPCP intake with drinking water during lifetime.

PPCP	Concentration in drinking water (µg·L^{-1})	Total intake during lifetime (mg)	Therapeutic dose (mg)
CBZ	0.030	1.53	>100
DZP	0.024	1.23	6
IBP	0.003	0.15	>600
NPX	0.065	3.32	>25
DCF	0.010	0.51	>25
IPM	0.086	4.39	>20,000
ROX	0.190	9.71	>150
SMX	0.150	7.67	>400
EE2	0.002	0.12	0.03

From a legal point of view, the environmental risk assessment of human pharmaceuticals has been established with the EC Directive 93/39/EEC, which has been recently replaced by the new Directive 2001/83/EC. First drafts in the early nineties envisaged a consolidated guidance concept for environmental risk assessment of both veterinary and human medicinal products. Extensive discussions followed these procedures resulting finally in two individual proposals for each, veterinary and human pharmaceuticals. In 1996, substantial progress was achieved when the EMEA/CVMP guidance paper on the ERA of veterinary drugs (EMEA, 1997) was finalised and implemented in the EU.

In 2001, a discussion paper on the ERA of human drugs (EMEA 2001) was released for public consultation. As this draft was strongly opposed by several Member States, it was extensively reviewed and a much improved document CPMP/SWP/4447/00 draft (EMEA 2003) was released by the EMEA Committee for Proprietary Medicinal Products (CPMP) in July 2003 for a 6-months public consultation period. Last news indicated that the guideline was re-released for consultation by the end 2004 – beginning 2005. The general scheme of the procedure proposed is shown in Figure 1.2.

1.2. Sewage sludge

During the last twenty years, developments in municipal wastewater treatment strategies are characterised by two aspects (Rulkens, 2004). The first aspect is a continuous effort to improve the quality of the effluent by upgrading existing treatment plants and the designing and implementation of new more effective treatment plants.

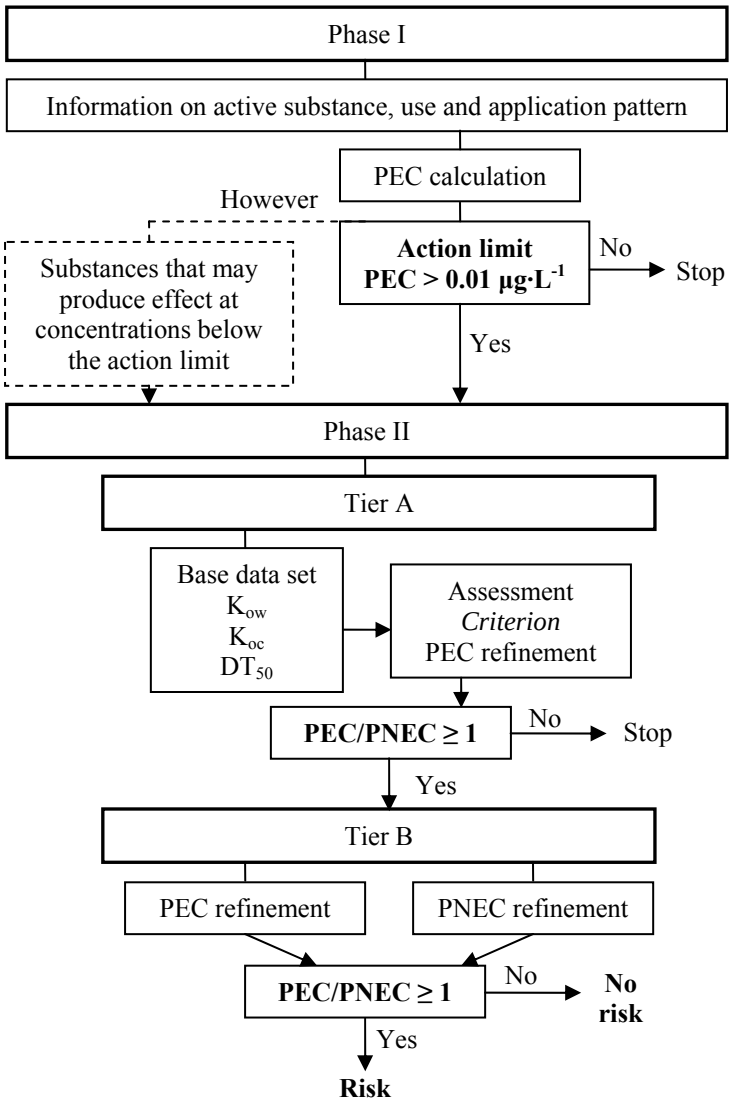


Figure 1.2. General scheme proposed for ERA of human pharmaceuticals.

The second aspect is an increasing awareness of the problems associated with the sewage sludge produced in the wastewater treatment process. These problems are a continuous increase in sludge production, the high costs of sludge treatment and the risks sewage sludge may have to the environment and human health. Due to this, the original application of the sludge as a fertiliser in agricultural systems has become increasingly under pressure (Campbell, 2000). Parallel to this

development, the government policy and regulations regarding the application of sludge in agriculture have changed considerably (Spinoza, 2001).

1.2.1. Definition and types of sludge

Sludge is a by-product of the wastewater treatment process. There are three main categories of sludge:

- Sludge originating from the treatment of urban wastewater.
- Sludge originating from the treatment of industrial wastewater.
- Sludge from drinking water treatment.

Sludge from conventional Sewage Treatment Plants (STPs) is derived from primary, secondary and tertiary treatment process (Figure 1.3). Frequently, the sludge contains between 1 and 2% by weight dry solids and is highly biodegradable. Each process has a different impact on the sludge characteristics.

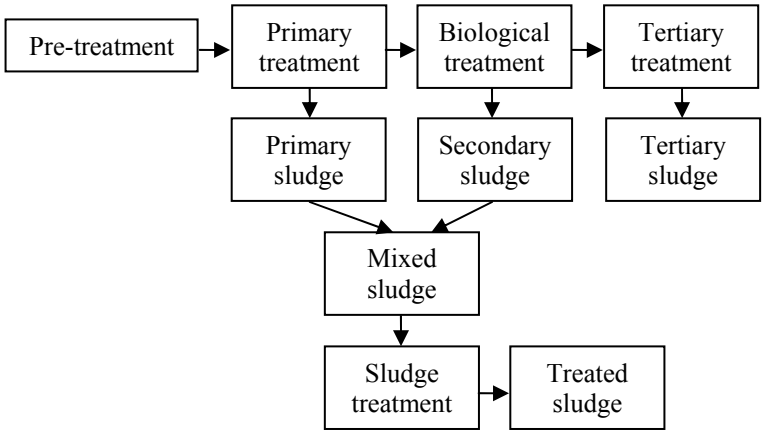


Figure 1.3. Scheme of wastewater treatment and sludge generation.

Pre-treatment

Pre-treatment consists of various physical and mechanical operations, such as screening, sieving, blast cleaning, oil separation and fat extraction. It allows the removal of voluminous items, sand and grease. The residues from pre-treatments are not considered to be sludge and they are disposed on in landfills.

Primary sludge

Primary sludge is produced following primary treatment, which consists of physical or chemical processes to remove matter in suspension (e.g. solids, grease and scum).

Sedimentation and flotation are the most common physical processes used in primary treatment. They remove 50-70% and 25-40% of the suspended solids and BOD, respectively.

Chemical treatments, i.e. coagulation and flocculation, are used to separate suspended solids when their normal sedimentation rates are too slow to provide effective clarification.

Secondary sludge

Secondary sludge is generated from the use of specially provided decomposers to break down the remaining organic materials in wastewater after primary treatment. The active agents in these systems are microorganisms, mostly bacteria, which need the available organic matter to grow. There are various techniques, such as lagooning, bacterial beds, activated sludge and biofiltration processes.

Mixed sludge

The primary and secondary sludge can be mixed together prior to sludge treatment generating a type of sludge referred to as mixed sludge.

Tertiary sludge

Tertiary sludge is generated when carrying out tertiary treatment, which is an additional process designed to remove remaining unwanted nutrients (mainly nitrogen and phosphorus) through high performance bacterial or chemical processes.

Treated sludge

After water purification, additional treatments need to be performed on sludge, in order to reduce its water content, pathogen load, volume and global mass, stabilise its organic matter and reduce the generation of odours.

Several treatments can be applied to achieve those purposes and the final sludge usually referred to as treated sludge.

1.2.2. Sludge generation and production

After the implementation of the Urban Waste Water Treatment Directive 91/271/EEC, the majority of the EU population will be served by STPs by the year 2005. As a result, the sludge quantities have increased in most countries during the 1990s. Figure 1.4 show the generation of sewage sludge in several EU countries during the period 1992-98, including projections for 2000 and 2005 (EEA, 2002).

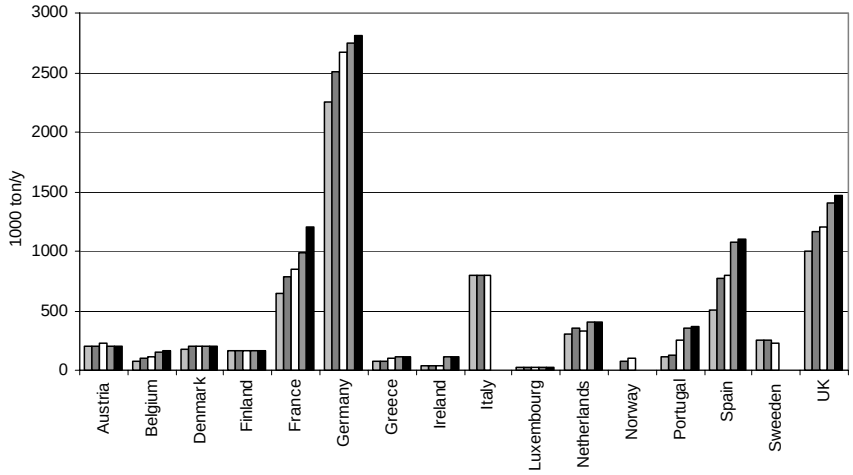


Figure 1.4. Sludge production in several EU countries. 1992 (■), 1995 (■), 1998 (□), 2000 (■) and 2005 (■).

The total amount of sludge produced is about 7 million tons of dry matter and it is expected to increase up to 11 million tons by the year 2005. Germany is the first sludge producer, followed by the United Kingdom, France, Spain and Italy, all producing more than 750,000 ton·y^{-1}. All other countries produce less than 250,000 ton·y^{-1}, except The Netherlands and Portugal, which are expected to generate almost 400,000 ton by the year 2005.

1.2.3. Sludge characteristics

The characteristics of sludge depend on the original pollution load of the treated wastewater and also on the technical characteristics of the treatment carried out. Conventional characterisation can be grouped in physical, chemical and biological parameters:

- Physical parameters give general information on sludge processability and handlability.
- Chemical parameters are relevant to the presence of nutrients and toxic compounds, thus being important for the final disposal.
- Biological parameters give information on microbial activity and organic matter presence, thus affecting its suitability for beneficial use.

Therefore, sewage sludge contains some compounds of agricultural value which may be usefully reused (organic matter, nutrients, potassium, calcium, etc.), whereas other substances are pollutants (heavy metals, pathogens and organic pollutants).

Organic matter

Sludge organic matter is mostly constituted of soluble matter, such as hydrocarbons, amino-acids, small proteins and lipids. Its content in urban sewage sludge is high (>50%), but varies according to the treatment and conditioning carried out on sludge.

Organic matter is mainly used for the improvement of the physical properties of the soil, such as structure or the retention capacity of minerals and water. Other benefits are the increase of soil population, activity and mineralization capacity.

Nitrogen and phosphorus content

The proportion of nitrogen and phosphorus in sewage sludge is comparable to that of animal manure, 20-80,000 and 10-90,000 $mg \cdot kg^{-1}$, respectively, and they are influenced by the operation of the STP and the sludge storage conditions.

Nitrogen is mostly found in the sludge under organic form and to a lesser extent as ammonia. The other mineral forms of nitrogen are found as traces. However, phosphorus is mostly present under mineral form (30-98% of total P).

They are used by the plants for its growth, the rigidity of its cell walls and for the development of its root system. However, as plants can only assimilate mineral forms, the agricultural value of the sludge is determined by the nitrogen and phosphorus availability, which is dependant on the sludge treatment as well as on external factors, such as temperature, humidity, pH and texture of the soil.

Other compounds of agricultural value

Other compounds present in the sludge such as calcium, potassium, sulphur, magnesium, sodium and oligo-elements (boron, cobalt, selenium) may be of interest due to their positive impacts on the pH, structure and permeability of the soil as well as in crop production. However, they may appear in sludge under various forms, thus being their efficiency dependant on their availability.

Heavy metals

Heavy metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn) are present in sludge between 0.3 and 2,000 $mg \cdot kg^{-1}$, approximately. There are three main origins for heavy metals in sewage sludge: domestic effluents, road runoff and industry. The proportion of each origin may be different for each compound.

Heavy metals may affect plant health and growth, soil properties and microorganisms, livestock and human health and accumulate in the environment. However, they can be beneficial on certain soils, correcting trace elements deficiency.

Pathogens

Sewage sludge contains various microorganisms, especially when biological treatments are carried out, and it can also contain plant pathogens. Only some of them have health-related impacts.

The presence of pathogens in the sludge is related to the sanitary level of the population and the type of industry in the region. The types of pathogens usually considered are viruses, bacteria, protozoa and helminths.

Organic pollutants

A wide variety of organic chemicals with diverse physical and chemical properties may be found in sludge. They also may affect soils, plants, animals and human health, and have impacts on the environment.

The most common considered compound are polynuclear aromatic hydrocarbons (PAH), polychlorinated biphenils (PCB), polychloro-dibenzodioxins/furans (PCDD/F), the sum of organohalogenous compounds (AOX), linear alkylbenzene sulfonates (LAS), nonylphenol and nonylphenol-ethoxylates (NPE) and di(2-ethylexyl)phthalate (DEHP). As they are not often mentioned in the national regulations, no survey has been regularly performed

describing the organic pollutant content in sewage sludge. However, recently most concern has been targeted on the presence of these ubiquitous organic pollutants and the Working Document on Sludge (EU, 2000) proposes limit values in the sludge for use on land.

1.2.4. Sludge treatment

Sludge produced by STPs is usually processed to reduce the water content of the sludge, its fermentation propensity and pathogens content. The different steps of the sludge treatment (Table 1.11) will depend on its further disposal or recycling.

Table 1.11. Steps of sludge treatment.

Steps	Types of process	Objectives
Conditioning	Chemical conditioning Thermal conditioning	- Sludge structure modification - Improvement of further treatment
Thickening	Gravity thickening Gravity belt thickener Dissolved air flotation	- Obtain sufficient density, strength and solids content to permit hauling for further disposal process - Reduce the water content of the sludge
Dewatering	Drying beds Centrifuging Filter belt Filter press	- Reduce the water content of the sludge
Stabilisation/ Disinfection	Biological processes: *Anaerobic digestion* *Aerobic digestion* *Long term liquid storage* *Composting* Chemical processes: *Lime treatment* *Nitrite treatment* Physical processes: *Thermal drying* *Pasteurisation*	- Reduce the organic matter of the sludge - Reduce the odour generation - Reduce the pathogen content of the sludge
Drying	Direct Indirect	- Highly reduce the water content

Conditioning

A preliminary phase of chemical or thermal conditioning may be conducted to improve further sludge thickening or dewatering.

Chemical conditioning is performed by using mineral agents (salts or lime), or organic compounds (polymers). Thermal conditioning consists of heating sludge to 150-200ºC for 30 to 60 minutes. The advantages and disadvantages of each of those possibilities are summarised in Table 1.12.

Table 1.12. Comparison of the different conditioning processes.

Conditioning	Advantages	Disadvantages
Chemical (mineral agents)	- Improvement of the cohesion and the density of the sludge	- Increase in sludge amount - Reduction of the organic matter content - Slow reaction
Chemical (organic agents)	- Reduction of the mass of sludge - No modification of the agricultural value - Lower quantities to be used - Easy to handle and transport	- Costs of the products
Thermal	- May be applied to all sludge - Efficient and stable process - Stabilisation and disinfection - Lower sludge amount	- Energy consumption - Odours - Increase in the pollution load of the filtrate

Thickening

Thickening is a first step to reduce sludge water content. Sludge reaches 10 to 30% dryness and it can still be pumped. There are several techniques which are compared in Table 1.13.

Dewatering

Dewatering is the following step after thickening and allows further reduction of the sludge water content (up to 30% dryness). There are several techniques which are compared in Table 1.14.

Drying

Drying is a thermal treatment which takes place at different temperatures, leading to dry matter content between 35 and 90%. It allows the elimination of the interstitial water, thus reducing the volume of sludge as well as allowing the

sludge stabilization and disinfection when the dry matter content exceeds 90%. It is also done to increase the calorific value of the sludge, to allow spreading using techniques similar those used for mineral fertilizers and to reduce the transportation costs.

Table 1.13. Comparison of the different thickening processes.

	Advantages	Disadvantages
Gravity thickening	- Easy to perform - Low energy consumption - Low investment costs	- Needs important room - Low performance on biological sludge
Gravity belt thickening	- Easy to perform - Compact	- Work force need - Cleaning water consumption - Polymer use compulsory
Dissolved air flotation	- Easy to perform - Little room needed - Little H_2S emission	- Not adapted to variable regimes - High energy consumption

Table 1.14. Comparison of the different dewatering processes.

	Advantages	Disadvantages
Drying beds	- Easy to operate - Adapted to small STPs - Low operation costs - High dryness reached	- Land requirement - Weather dependency - Risk o odours - Workforce requirements
Centrifuging	- Continuous operation - Compact - Possible automation	- Specialised maintenance - Sludge texture - Noise - High energy consumption - High investment costs
Filter belt	- Continuous operation - Easy to perform - Moderate investment costs	- Limited water content reduction - Cleaning water consumption - Supervision necessary
Filter press	- High water content reduction - Structure of the sludge - Possible automation	- Discontinuous operation - Low productivity - Consumption of mineral conditioner - Supervision necessary - High investment cost

Stabilisation and disinfection

The stabilisation aims at reducing the fermentation of the putrescible matter contained in the sludge and the emission of odours.

Disinfection consists of eliminating pathogens. Three types of processes can be used:

- Biological processes: anaerobic or aerobic digestion, composting and long term liquid storage.
- Chemical processes: lime and nitrite treatment.
- Physical processes: thermal drying and pasteurisation

Anaerobic digestion

Anaerobic digestion is applied to thickened sludge in order to reduce, stabilise and partially disinfect the treated volume of sludge. It is divided in three main phases:

- Hydrolysis of the macromolecules in smaller components.
- Production of acidic compounds from those smaller compounds.
- Gasification, generating carbon dioxide and methane.

Aerobic digestion

During aerobic digestion, sludge is placed in a vessel with aerobic miroorganisms. Heat is generated when these bacteria degrade organic matter, thus rising the temperature to over 70ºC. In these conditions, volatile matter is reduced by about 40% and some harmful organisms are destroyed.

Long term liquid storage

Storage of sludge has two essential purposes: regulating the flows of sludge to agriculture and homogenising its composition.

Long-term storage leads to an increase of the dry matter, a reduction of the organic matter and nitrogen and the destruction of some viruses and bacteria. In contrast, it can produce odours. Its efficiency depends on the duration of the storage.

Composting

Composting is an aerobic process consisting of aerating sludge mixed with a co-product such as sawdust or animal manure. It produces excess heat, which can be used to raise the temperature of the composting mass. Composted sludge presents higher agricultural value, a good level of disinfection and stabilisation,

reducing therefore the arising odours and the water content, making its handling easier.

Lime treatment

Lime treatment consists of the addition of lime to sludge in order to raise its pH to 12, thus destroying or inhibiting the biomass responsible for the degradation of the organic compounds. It also helps disinfecting sludge, increasing its dry matter content and making handling easier.

Nitrite treatment

Nitrite treatment consists of maintaining sludge in an acid environment (about pH 2 or 3) for 30 minutes where it undergoes the action of nitrite ion. It is an efficient stabilisation process, without generating odours and its impact on the sludge structure facilitates further dewatering.

Pasteurisation

Pasteurisation consists of heating the sludge to a temperature of 70 to 80ºC for a short period (about 30 minutes). It allows reduction of the amount of pathogens in the sludge, but it can not be considered as a stabilisation process.

1.2.5. Sludge reuse and disposal

Sewage sludge production is a continuous process and requires a flexible and secure range of outlets for its disposal to be economically and environmentally acceptable. The predominant disposal options available include landspreading, incineration and landfilling.

Landspreading

Landspreading is a way of recycling the compounds of agricultural value present in sludge to land. All types of sludge (liquid, semi-solid or dried sludge) can be spread on land. However, the use of each of them induces practical constraints on storage, transport and spreading.

This route may be cheaper than other disposal routes. However, the presence of pollutants in sludge implies that the practice should be carefully done and monitored. To this purpose, codes of practice and spreading schemes have been established in some countries, summarising the regulatory obligations.

Incineration

Incineration is a combustion reaction which produces a residual solid waste and a flue gas. According to the Waste Incineration Directive 2000/76/EC, different types of incineration may be considered: mono-incineration (sludge is incinerated in dedicated incineration plants), incineration (sludge is incinerated with other wastes, mainly household wastes) and co-incineration (sludge is used as fuel).

Several technologies also involving thermal oxidation, such as wet oxidation and pyrolysis, are being developed and introduced in the marked as an alternative to conventional combustion processes.

Landfilling

So far, landfilling has been the major route for sludge disposal. However, it should be a limited outlet in the future due to the implementation of the Landfill Directive 1999/31/EC, which states that this solution must be only chosen when no other ways exist.

There are two possibilities for landfilling sludge: mono-deposits (the landfill is only used for sludge) and mixed-deposits (the landfill is also used for municipal wastes). The conditions for disposal are set out in the regulations of each country.

Other routes

Other routes of sludge disposal are its use as a forest fertiliser, as a soil conditioner for the restoration of disturbed soils, as a soil forming material for reclaiming derelict land, and for producing soil for use on green areas in the urban environment.

Figure 1.5 shows the disposal routes for sewage sludge in several EU countries from 1996 to 1998 and Figure 1.6 the forecasts for the year 2005.

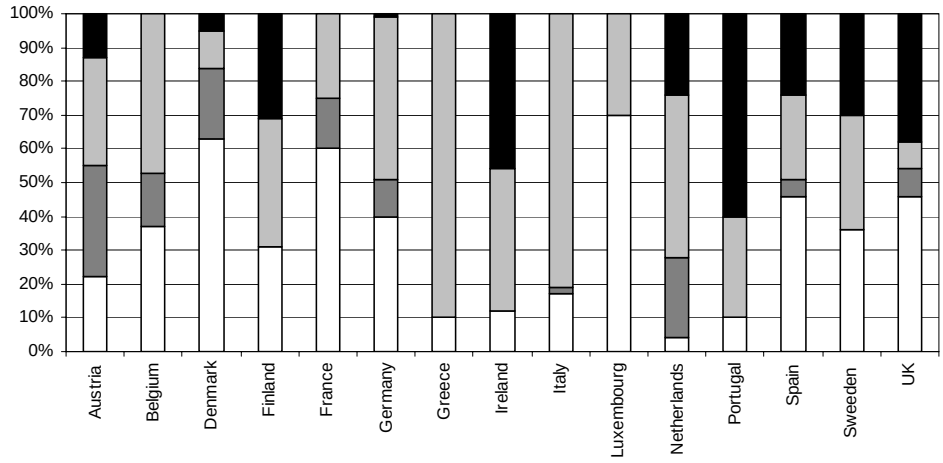


Figure 1.5. Sludge disposal routes in several EU countries during 1996-1998. (□) Agricultural; (■) Incineration; (■) Landfilling; (■) Others (Vegetalisation, disposal to sea, etc).

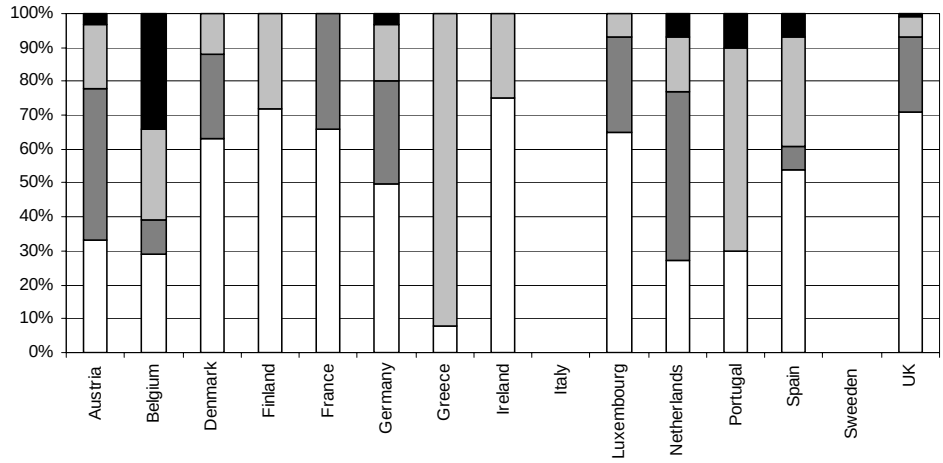


Figure 1.6. Forecasts for sludge disposal in several EU countries in 2005. (□) Agricultural; (■) Incineration; (■) Landfilling; (■) Others (Surface water, etc).

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Chapter 2

Materials and Methods

Summary

In this chapter, the analytical methods used in this work are described. It comprises the conventional parameters used for wastewater and sludge characterisation (alkalinity, biogas composition and production, organic matter, nitrogen, phosphate, sulphate, chloride, oil and grease, pH, temperature, solids, carbon and volatile fatty acids) and the PPCPs analysis. In the latter case, the methodology depends not only on the type of matrix, aqueous or solid phase, but also on PPCPs properties. In this way, the methods are divided for liquid and sludge samples. Besides, they are classified for *polycyclic musk fragrances* (Galaxolide and Tonalide), *neutral pharmaceuticals* (Carbamazepine and Diazepam), *acidic pharmaceuticals* (Ibuprofen, Naproxen and Diclofenac), *antibiotics* (Roxithromycin and Sulfamethoxazole), *X-Ray Contrast Media* (Iopromide) and *estrogens* (17β-estradiol, Estrone and 17α-ethinylestradiol).

The specific analytical methods used in a single part of the work are described in the corresponding chapter, as well as the experimental set-up.

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2.1. Physico-chemical properties

In this section, the methods used for the determination of the conventional parameters of wastewater and sludge are described. For soluble fraction analysis (COD_s, inorganic anions, nitrogen, carbon and VFA), the samples were previously centrifuged at 8,000 rpm for 10 minutes in order to remove suspended solids. All measurements have been performed in duplicate or in triplicate.

2.1.1. Alkalinity

Alkalinity of water is defined as the acid-neutralizing capacity. It comprises all the titratable bases, being mainly function of carbonate, bicarbonate and hydroxide content, although it may also include contributions from borates, phosphates, silicates or other bases if present.

Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end-point pH used. When alkalinity is due entirely to carbonate or bicarbonate content, the pH at the equivalence point of the titration is determined by the concentration of carbon dioxide (CO_2) at that stage. CO_2 concentration depends, in turn, on the total carbonate species originally present and any losses that may have occurred during titration. The pH values are suggested as the equivalence points for the corresponding alkalinity concentration as milligrams calcium carbonate ($CaCO_3$) per litre.

Alkalinity measurements are used in the interpretation and control of anaerobic processes, since the buffering capacity of the system should be enough to avoid the system destabilization caused by the possible accumulation of intermediate acid compounds which would lead to a pH drop and, consequently, the microorganisms death. For example, properly operating anaerobic digesters typically have supernatant alkalinities in the range of 1,000-3,000 mg $CaCO_3 \cdot L^{-1}$.

Total alkalinity (TA) can be considered as the sum of the alkalinity due to bicarbonate plus the Volatile Fatty Acids (VFA) and its end-point pH is 4.3. Partial alkalinity (PA), with an end-point pH of 5.75, corresponds to bicarbonate (Jenkins *et al.,* 1983) while the Intermediate alkalinity (IA), defined as the difference between the TA and the PA, corresponds approximately to the effect of VFA (Ripley *et al.,* 1986).

The ratio IA/TA is used as a control parameter in anaerobic digesters, recommending a value not higher than 0.3 (Switzembaum *et al.,* 1990; Soto *et al.,* 1993).

The alkalinity was determined following the method 2320 described in *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WPCF, 1999). It consists of a titration of a sample volume (normally 25 mL) at room temperature with standard acid (H_2SO_4 standardised against Na_2CO_3) to the desired pH, 5.75 for PA and 4.3 for TA.

The alkalinity, expressed as mg $CaCO_3 \cdot L^{-1}$, is then calculated from the following equation:

$$\text{Alkalinity} = \frac{A \times N \times 50{,}000}{V} \qquad \text{Eq. 2.1}$$

where:

A: mL of standard acid used until pH 5.75 (PA) or pH 4.3 (TA),

N: normality of standard acid, and

V: sample volume (mL).

2.1.2. Biogas composition

The biogas composition is an important parameter to determine the methanization potential of the anaerobic biomass. Besides, it is a good indicator of reactor performance, since an accumulation of acids in the system would lead to an increase of the CO_2 content in the biogas (produced during the neutralization of the acids by the bicarbonate).

Biogas composition (N_2, CH_4 and CO_2) is determined by gas chromatography (HP, 5890 Series II) equipped with a thermal conductivity detector (TCD). The stainless steel column is 2 m long with an external diameter of 1/8" and it is filled with Porapack Q (mesh 80/100). The temperatures of the injector, column and detector are 110, 35 and 110ºC, respectively. Helium is used as carrier gas with a flow of 15 $mL \cdot min^{-1}$. The sample volume (1 mL) is injected through a septum into the entrance of the instrument.

The calibration is performed with a standard mixture of gases (CH_4: 66%; CO_2: 30%; N_2: 2% and H_2S: 2%) by a response factor method, using the CO_2 as internal standard.

2.1.3. Biogas production

Biogas production has been measured using the flow meter designed by Veiga *et al.* (1990). It consists of two 20-cm-high glass columns of i.d. 3 cm whose lower ends are connected directly and whose central regions are connected by a hydraulic valve (a J-tube of i.d. 0.5 cm, the long arm emerging from Column I and the short arm from Column II). The columns contain liquid whose initial level is slightly below a level half-way between the two mouths of the J-tube, and which is displaced by gas entering the top of Column II from the digester. Two stainless-steel electrodes at different heights in Column I are connected in series with an electromechanical pulse counter (F.M. Mod. CI851) that clocks up one unit every time the liquid in Column I connects the two electrodes. A short time later, the level of the liquid in Column II falls below the lower mount of the J-tube, with the result that the gas in Column II is discharged to the environment via Column I, the level of liquid in Column I falls and the counter circuit is broken. The equipment was calibrated to measure 60 ± 0.5 mL per counter unit.

2.1.4. Biochemical Oxygen Demand (BOD)

BOD determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements for the biological oxidation of chemical compounds present in polluted waters. The test measures the oxygen consumed during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulphides and ferrous iron. It also may measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The seeding and dilution procedures proved an estimate of the BOD at pH 6.5 to 7.5.

BOD concentration in most wastewaters exceeds the concentration of dissolved oxygen (DO) available in an air-saturated sample. Therefore, it is necessary to dilute the sample before incubation to bring the oxygen demand and supply into appropriate balance. Because bacterial growth requires nutrients such as nitrogen, phosphorus and trace metals, these are added to the dilution water, which is buffered to ensure that the pH of the incubated sample remains in a suitable range for bacterial growth. The complete stabilization of the sample may require a period of incubation too long for practical purposes; therefore, 5 days has been accepted as the standard incubation period.

BOD was determined following the method 5210B described by *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WPCF, 1999). This method consists of filling with sample an airtight bottle of the specified size and incubating it at the specified temperature (20ºC) for 5 d, excluding light to prevent the possibility of photosynthetic production of DO. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO.

$$\text{BOD (mg } O_2 \cdot L^{-1}) = \frac{D_1 - D_2}{V} \qquad \text{Eq. 2.2}$$

where:

D_1: DO of diluted sample immediately after preparation ($mg \cdot L^{-1}$),

D_2: DO of diluted sample after 5 d incubation ($mg \cdot L^{-1}$), and

V: decimal volumetric fraction of sample used.

2.1.5. Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) is the amount of oxygen required to oxidise the organic matter present in the sample (wastewater or sludge) using a strong chemical oxidant (potassium dichromate) in an acid environment. A catalyst (silver sulphate) is used to improve the oxidation of some organic compounds. After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulphate to determine the amount of $K_2Cr_2O_7$ consumed, being the amount of oxidable matter calculated in terms of oxygen equivalents. Generally, the COD of a sample is higher than its BOD since there are more substances prone to be chemically than biologically oxidised.

The total and soluble Chemical Oxygen Demand (COD_t and COD_s) were determined following the method described by Soto *et al.* (1989), which is derived from method 5220C of the *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WPCF, 1999). The difference between total and soluble COD is that COD_t is determined using the raw sample, while for COD_s determination, the sample is previously centrifuged and then filtered through cellulose-fibre filters (Whatman, GFC) with a pore size of 0.45 μm.

Reagents

a. Standard potassium dichromate digestion solution: 10.216 g of $K_2Cr_2O_7$ and 33 g of $HgSO_4$ are dissolved in 500 mL of distilled water. Then, 167

mL of conc H_2SO_4 are added. The solution is cooled to room temperature and finally diluted to 1,000 mL.

b. Sulphuric acid reagent: 10.7 g of Ag_2SO_4 added to 1 L of conc H_2SO_4.

c. Ferroin indicator solution: 1.485 g of $C_{18}H_8N_2 \cdot H_2O$ (phenanthroline monohydrate) and 0.695 g of $SO_4Fe \cdot 7H_2O$ are dissolved in 100 mL of distilled water.

d. Standard potassium dichromate solution 0.05 N. 1.226 g of $K_2Cr_2O_7$, previously dried at 105ºC for 2 hours, are dissolved in 500 mL of distilled water.

e. Standard ferrous ammonium sulphate titrant (FAS) 0.035 N: 13.72 g of $Fe(NH)_4(SO)_2 \cdot 6H_2O$ are dissolved in distilled water. Then, 20 ml of conc H_2SO_4 are added and, finally, the solution is cooled and diluted to 1000 mL. Standardise the solution daily against standard $K_2Cr_2O_7$ digestion as follows: Put 5 mL of distilled water into a small beaker. Add 3.5 mL of sulphuric acid reagent. Cool to room temperature and add 5 mL of standard potassium dichromate solution (0.05 N). Add 1-2 drops of ferroin indicator and titrate with FAS titrant. The end-point is a sharp colour change from blue-green to reddish brown. Molarity of FAS solution is calculated with the following equation:

$$M_{fas} = \frac{5 \times 0.05}{V_{fas}} \qquad \text{Eq. 2.3}$$

where:

M_{fas}: molarity of FAS ($mol \cdot L^{-1}$), and

V_{fas}: volume of FAS consumed in the titration (mL).

This procedure is applicable to COD values between 90-900 $mg \cdot L^{-1}$, being higher values determined after dilution of the samples. To 2.5 mL of sample placed in 10-mL Pirex tubes, 1.5 mL of digestion solution and 3.5 mL of sulphuric acid reagent are added (the latter in such way that the mix is avoid until the digestion occurs in the block heater). Besides, a blank with distilled water is prepared in the same way. This blank acts as "reference", representing the COD of the distilled water. After being sealed with Teflon and tightly capped, the tubes are finally mixed completely and placed in the block digester (HACH 16500-100) preheated to 150ºC. The duration of the digestion is 2 h.

After digestion, the tubes are cooled to room temperature. Then, the content of the tubes is transferred to a beaker and, once added the ferroin indicator, the solution is titrated under rapid stirring with standard FAS. The end-point is the same as for the determination of FAS molarity. The COD is calculated with the following equation:

$$COD = \frac{(A - B) \times M_{fas} \times 8{,}000}{V} \qquad \text{Eq. 2.4}$$

where:

COD: Chemical Oxygen Demand (mg $O_2 \cdot L^{-1}$),

A: mL of FAS used for the blank,

B: mL of FAS used for the sample,

M_{fas}: molarity of FAS ($mol \cdot L^{-1}$), and

8,000: milliequivalent weight of oxygen x 1,000 $mL \cdot L^{-1}$.

2.1.6. Inorganic anions: NO_2^-, NO_3^-, Cl^-; PO_4^{3-} and SO_4^{2-}

Nitrite (NO_2^-), nitrate (NO_3^-), chloride (Cl^-), phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) are determined simultaneously by Waters Capillary Ion Analyzer (CIA). Sodium cromate (0.005 $mol \cdot L^{-1}$) is used as electrolyte (Vilas-Cruz *et al.*, 1994). Besides, an electro-osmotic modifier (50 $mL \cdot L^{-1}$) CIA-Pak™ OFM Anion BT Waters (Ewing *et al*., 1989; Heiger, 1992) is also added. The sample is forced to migrate through a capilar (melting silica covered with poliimida, 60 cm long and 45 μm of internal diameter) kept at 25°C by the application of an electric current. Depending on the ratio charge/mass of the ion, the migrating time is different. A hydrostatic injection (10 cm height for 30 seconds) and an indirect detection (UV, 254 nm, 240 kV, 16-22 μA) are used.

4-6 calibration points for each ion in the range of 3-100 $mg \cdot L^{-1}$ are daily used for the quantification of the samples. Previously to the analyses, the samples are filtrated through 0.45 μm membrane (Millipore).

2.1.7. Nitrogen

In waters and wastewaters, the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia and organic nitrogen. All these forms, as well as nitrogen gas (N_2), are biochemically interconvertible and they are the components of the nitrogen cycle.

Organic nitrogen is defined functionally as the organically bound nitrogen in the trinegative oxidation state, but it does no include all organic nitrogen compounds. Analytically, organic nitrogen and ammonia can be determined together and have been referred to as "Total Kjeldahl Nitrogen" (TKN), a term that reflects the technique used in their determination.

Total oxidised nitrogen is the sum of the nitrate and nitrite forms. Nitrate generally occurs in trace quantities in surface waters, but it may attain high levels in some groundwaters or effluents of nitrifying biological treatment plants (up to 30 mg $N\text{-}NO_3^-\cdot L^{-1}$). A limit of 10 mg $N\text{-}NO_3^-\cdot L^{-1}$ has been imposed on drinking water to prevent disorders. Nitrite is an intermediate oxidation state of nitrogen, either in the oxidation of ammonia or in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems and natural waters.

Total (TN), Inorganic (IN) and Total Kjeldhal Nitrogen (TKN)

TKN was determined in a total organic nitrogen analyzer (Rosemount-Dohrmann DN-1900) equipped with a quimioluminiscence detector with two channels. One channel determines the Total Nitrogen (TN) by oxidation at high temperature and the other determines the Inorganic Nitrogen (IN) by a chemical reduction. TKN is determined as the difference between TN and IN.

All the nitrogen present in the water can be catalytically oxidised to nitrous oxide (NO). The process for TN determination goes by in two steps. The first step is a catalytic oxidation (Cu as catalyst) in the combustion tube at 850°C and with pure oxygen (1 atm) as carrier gas. The second one is the chemical reduction of residual NO_2 with H_2SO_4 at 80ºC and catalyzed by $VaCl_3$. For the IN determination, only the second step (chemical reduction) is used. The NO obtained in the two steps is dried and forced to react with O_3 producing an unstable excited state NO_2^*. The change back of this oxide to its fundamental state emits a proton, from which the determination of TN and IN is carried out by quimioluminiscence using a multiplicator tube. The instrument is calibrated with a certified standard solution (KNO_3, 20 mg $N\cdot L^{-1}$) using a response factor method.

Ammonia nitrogen

Ammonia nitrogen is determined by a colorimetric method. It is based on the reaction of NH_3 with HClO and phenol, forming a strong-blue compound

(indophenol) which can be colourimetrically determined using a spectrophotometer (Shimadzu UV-1603, UV-Visible) at 635 nm.

Reagents

a. Solution 1: Phenol-nitroprusiate: 15 g of phenol and 0.05 g of sodium nitroprusiate are added to 250 mL of buffer solution (30 g $Na_3PO_4 \cdot 12H_2O$, 30 g $Na_3C_6H_5O_7 \cdot 2H_2O$ and 3 g EDTA per litre, adjusted to pH 12).

b. Solution 2: Hipochloride: 15 mL of commercial bleach are mixed with 200 mL of NaOH 1 N and filled up to 500 mL with distilled water.

To 2.5 mL of sample (diluted if necessary to get a maximum concentration of 1 mg $N\text{-}NH_4^+$/L), 1 and 1.5 mL of solution 1 and 2, respectively, are added. After waiting 45 min at room temperature, the concentration of $N\text{-}NH_4^+$ is measured in a spectrophotometer at 635 nm. The quantification is done with a 6-8 points calibration curve in the range of 0-1 mg $N\text{-}NH_4^+ \cdot L^{-1}$, using NH_4Cl as standard.

Nitrite

Nitrite concentration in wastewater is determined following the method 4500-NO_2^--B described in *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WPCF, 1999).

Nitrite is determined through the formation of a reddish purple azo dye produced at pH 2.0-2.5 by coupling diazotized sulphanilamide with N-(1-napththyl)-ethylenediamine dihydrochloride (NED dihydrochloride). The applicable range of the method for spectrophotometric measurements is from 10 to 100 μg $N\text{-}NO_2^- \cdot L^{-1}$.

Reagents

a. Sulphanilamide: 10 g of sulphanilamide are dissolved in 100 mL of conc HCl and 600 mL of distilled water. After cooling, the volume is filled up to 1 L with distilled water.

b. NED: 0.5 g of NED are dissolved in 500 mL of distilled water.

To 5 mL of sample (diluted if necessary to fit the concentration range of the method), 0.1 mL of each solution (sulphanilamide and NED) are added. After waiting 20 min for colour stabilisation, the sample is measured in a spectrophotometer (Shimadzu UV-1603) at 543 nm. The quantification is done

with 6-8 points calibration curve in the range of 0-0.24 mg $N\text{-}NO_2^-\cdot L^{-1}$, using commercial standards.

Nitrate

Nitrate concentration in wastewater is determined following the method 4500-NO_3^--B described in *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WPCF, 1999).

Measurement of UV absorption at 220 nm enables rapid determination of NO_3^-. Because dissolved organic matter also may absorb at 220 nm and NO_3^- does not absorb at 275 nm, a second measurement at 275 nm is used to correct the NO_3^- value.

To 5 mL of sample (diluted if necessary to get a maximum concentration of 4 mg $N\text{-}NO_3^-\cdot L^{-1}$), 0.1 mL of HCl 1N are added. Afterwards, the absorbance at 220 and 275 nm is measured in a spectrophotometer (Shimadzu UV-1603). The absorbance related to nitrate is obtained by subtracting two times the absorbance reading at 275 nm from the reading at 220 nm. The quantification is done with a 6-8 points calibration curve in the range of 0-4 mg $N\text{-}NO_3^-\cdot L^{-1}$, using KNO_3 as standard.

2.1.8. Oil and grease content

Oil and grease content in wastewater is determined following the method 5520B described in *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WPCF, 1999).

Dissolved or emulsified oil and grease is extracted from water by intimate contact with an extraction solvent. Some extractables, especially unsaturated fat and fatty acids, oxidize readily; hence, special precautions regarding temperature and solvent vapour displacement are include to minimize this effect.

Reagents:

a. HCl concentrated.

b. Petroleum ether 40-60°C (PA-ACS-ISO, 131315.1611).

100-200 mL of sample acidified to pH 2 with conc HCl is sequentially extracted with 30 mL of petroleum ether in a separatory funnel. After shaking vigorously and allowing the layers separate, the aqueous layer (placed in the bottom of the funnel) is drained into the original sample container. The solvent

layer is then drained through a funnel containing a filter paper (solvent-rinsed) into a clean tared distilling flask. Two more extractions are performed with 30 mL of solvent each, rinsing the sample container with each solvent portion. All the solvent extracts are combined in the clean tared flask. An additional 10 to 20 mL of solvent are added for the funnel final cleaning. The solvent is then evaporated in a water bath and the flask weighed once dried. The amount of oil and grease in the sample (Eq. 2.5) is the gain in weight of the tared distilling flask.

$$\text{mg oil and grease}\cdot\text{L}^{-1} = \frac{(A-B)\times 1{,}000}{V} \qquad \text{Eq. 2.5}$$

where:

A: tared distilling flask weight after extraction (mg),

B: clean tared distilling flask weight (mg), and

V: sample volume (mL).

2.1.9. pH

pH is one of the key parameters used in wastewater and sludge treatment, since its control is important to maintain the biological activity of the microorganisms involved in the treatment process.

pH measurements were performed with an electrode (Crison Instruments, S.A., 52-03) equipped with an automatic compensatory temperature device (Crison Instruments, S.A., 21-910-01) and connected to a measurement device (pH/mV). The sensibility of the instrument is ± 1 mV, corresponding to 0.01 pH units. The electrode is calibrated at room temperature with two standard buffer solutions of pH 7.02 and 4.00.

2.1.10. Temperature

Temperature is an important parameter in the anaerobic processes, since a temperature decrease would lead to a lower activity of the anaerobic biomass.

Temperature was determined by an electrode (Sentix 41.3, WTW) connected to a measurement device (Multiline P4 Universal Meter, WTW). The sensibility of this instrument is ± 1 mV, corresponding to 0.1°C of temperature.

2.1.11. Total and Suspended Solids

Solids present in water, either dissolved or in suspension, can be organic or inorganic. Total Solids (TS), Total Suspended Solids (TSS), Volatile Solids (VS) and Volatile Suspended Solids (VSS) are determined following the methods 2540B, 2540D and 2540E, respectively, described in *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WPCF, 1999).

TS are determined weighing a selected (in order to yield a residue between 2.5 and 200 mg) well-mixed sample volume in a previously clean (heated to 103-105ºC for 1 h) dish after being evaporated at 103-105ºC until constant weight. The increase in weight over that of the empty dish represents the total solids.

For the determination of TSS, a selected (in order to yield a residue between 2.5 and 200 mg) well-mixed sample volume is filtered through a weighed glass-fibre filter (Whatman, GF/C, 4.7 cm of diameter, 1.2 μm of pore size) and the residue retained on the filter is dried to a constant weight at 103-105ºC. The increase in weight of the filter represents the total suspended solids.

To determine the volatile solids (VS or VSS), the residue from method 2540B (TS) and 2540D (TSS) is ignited to constant weight at 550ºC. The weight lost on ignition corresponds to the volatile solids, since only a small amount of inorganic salts are decomposed and volatilised at that temperature. This determination is useful in the control of wastewater treatment plan operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge or industrial wastes.

2.1.12. Total Organic Carbon (TOC)

Organic carbon in water and wastewater may include a variety of organic compounds in different oxidation states. Total Organic Carbon (TOC) is a more convenient and direct expression of total organic content than either BOD or COD, but does not provide the same kind of information. Unlike BOD or COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganics that can contribute to the oxygen demand measured by BOD and COD (APHA-AWWA-WPCF, 1999).

To determine the quantity of organically bound carbon, the organic molecules must be broken down and converted to a single carbon molecular form that can be measured quantitatively.

TOC was determined by a Shimadzu analyzer (TOC-5000) as the difference between the Total Carbon (TC) and the Inorganic Carbon (IC). The instrument is connected to an automated sampler (Shimadzu, ASI-5000-S). TC is determined from the amount of CO_2 produced during the combustion of the sample at 680°C, using platinum immobilised over alumina spheres as catalyst. The IC is obtained from the CO_2 produced in the chemical decomposition of the sample with H_3PO_4 (25%) at room temperature. The CO_2 produced is optically measured with a nondispersive infrarred analyzer (NDIR) after being cooled and dried. High purity air is used as carrier gas with a flow of 150 $mL \cdot min^{-1}$. 4-point calibration curve in the range of 0-1 g $C \cdot L^{-1}$, using potassium phthalate as standard for TC and a mixture of sodium carbonate and bicarbonate ($Na_2CO_3/NaHCO_3$, 3:4 w/w) for IC, is used for the quantification.

2.1.13. Volatile Fatty Acids (VFA)

Volatile Fatty Acids (VFA), acetic, propionic, i-butiric, n-butiric, i-valeric and n-valeric, are intermediate products of the anaerobic digestion. A VFA accumulation reflects a kinetic disequilibrium between the acids producers and the acids consumers (Switzembaum *et al.*, 1990) and it is an indicator of process destabilization.

VFA are determined by gas chromatography (HP, 5890A) equipped with a Flame Ionization Detector (FID) and an automatic injector (HP, 7673A). The glass column (3 m long and 2 mm of internal diameter) is filled with Chromosorb WAW (mesh 100/120) impregnated with NPGA (25%) and H_3PO_4 (2%). The column, injector and detector temperatures are 105, 260 and 280°C, respectively. N_2, previously saturated with formic acid before entering into the injector, is used as carrier gas with a flow of 24 $mL \cdot min^{-1}$. Air and H_2 are used as auxiliary gases with flows of 400 and 30 $mL \cdot min^{-1}$, respectively. VFA, after being separated in the column according to their molecular weights, are burnt in a H_2-air flame and finally measured in the FID at 280°C. The quantification of the sample is made with a 6-8 point calibration curve for each acid in the range of 0-1 $g \cdot L^{-1}$ using pivalic acid as internal standard.

2.2. PPCPs analysis

2.2.1. Liquid samples

The steps for PPCPs analysis in aqueous samples comprise filtration, extraction and sample preparation, derivatisation (if needed) and detection. They are following described in more detail (Fink *et al.,* 2004).

Filtration

In order to avoid any kind of impurities and solid materials in the final extract, between 0.5 and 1 L of the raw sample was filtered over glass fibre filters (< 1µm).

Extraction and sample preparation

The analysis of PPCPs in environmental waters at the trace level requires a pre-concentration of these compounds prior to their quantitative determination. Two different techniques have been used: the Solid Phase Extraction (SPE) and the Solid Phase Micro Extraction (SPME).

Solid Phase Extraction

Solid Phase Extraction (SPE) is a widely used selective sample preparation technique which has replaced many classical methods, such as liquid-liquid extraction. In general, during SPE enrichment the analytes are sorbed when the liquid sample is passed through the solid phase material and desorbed by elution with an organic solvent. The adsorption mode of the analytes onto the SPE material depends on the characteristics of the applied materials and can be based on various interactions such as dispersion-, dipole/dipole-, ion/dipole-, hydrophobic interactions and ion exchange.

The diversity of SPE materials often allows for a specific selection of the SPE to the respective analytes (Baltusse *et al.*, 2002; Carson, 2000; Fritz and Macka, 2000; León-González and Pérez-Arribas, 2000).

Solid Phase MicroExtraction

For certain purposes, classical SPE was improved and miniaturized to the Solid Phase Micro Extraction (SPME). This technique uses small coated silica fibres, which are dipped into the aqueous sample solution for a pre-determined time. Analytes diffuse to and partition into the polymeric coating of the fibre and

are subsequently desorbed either thermally in the injection port of a GC or by solvents prior to HPLC or further sample preparation (Kataoka, 2002; Snow, 2000; Ulrich, 2000).

Derivatisation and detection

For some compounds, a derivatisation step prior to the final quantification is needed to assure the substance stability along the detector.

The final quantification implies two processes: the analytes separation and the analytes identification. Chromatography techniques are used for the first step and mass spectrometry (MS) for the second one.

Chromatography

Chromatography techniques are dynamic processes wherein a mobile phase transports the sample mixture across or through a stationary-phase medium. As the sample comes in contact with the stationary phase, interactions between the sample and the stationary phase molecules occur. A partitioning or separation of the components in the mixture results from the different affinity of each component with the stationary phase. As the separated components emerge or elute, a detector responds with a signal change that is plotted against time, thus producing a chromatogram.

Two main types of chromatography are widely used: gas (GC) and high-performance liquid (HPLC) chromatography.

In gas chromatography, the mobile phase is an inert carrier gas (e.g. He, Ar, N_2, H_2) and the stationary phase is often a high molecular weight liquid which is deposited either on the surface of finely divided particles or on the wall of a long capillary tubing. The GC column is coupled with a temperature controlled injection port and sample extracts are injected into the carrier gas stream at a temperature sufficient to insure vaporization of all components. The vaporized sample is transported through the column by the flow of the inert mobile phase to detector. The main parameters which can be altered to adopt a method to a certain separation problem are: temperature, gas flow, type and thickness of stationary phase, column length and diameter.

High performance liquid techniques are used to separate dissolved substances. Compounds are separated by injecting a plug of the sample mixture onto the column. The components in the mixture pass through the column at

different retention times, due to differences in their partitioning behaviour between the mobile liquid phase and the stationary solid phase. Two conditions can be used in HPLC determination: normal phase, which implies a very polar stationary phase and an unpolar mobile phase, and reverse phase, which is just the opposite. The latter technique is frequently applied in the trace analysis of pharmaceuticals and other organic pollutants.

Mass spectrometry

Chromatography techniques are very powerful for analytes separation, but they can not identify them. Mass spectrometry provides detailed structural information and high selectivity in the quantification of the compounds. This makes both techniques very compatible.

A mass spectrum is the plot of the relative abundance of the molecule ions and its fragments versus their mass-charge-ratio (m/z). Therefore, MS comprises three separate processes: ionization, mass separation and recording of the ions formed.

The ionization techniques depend on the chromatography used before. For GC, Electron Ionization (EI) and Chemical Ionization (CI) are the most common; however, ElectroSpray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) are applied in HPLC.

Mass spectrometers are classified according to the principle for separation of the ionic masses. Among the different detection systems available, ion trap and quadrupole mass spectrometers have achieved the widest use, due to their relative easy handling, maintenance and their reasonable price (Chapman, 1993; Settle, 1997).

Polycyclic musk fragrances (both in liquid and sludge samples) and neutral and acidic pharmaceuticals (in liquid samples) were detected by GC/MS/MS. The operating conditions are described in Table 2.1.

Carbamazepine and acidic pharmaceuticals (in sludge samples) and antibiotics and X-Ray contrast media (both in liquid and sludge samples) were detected by LC/MS/MS. The operating conditions are described in Table 2.2.

Some samples (liquid and sludge) of estrogens were analysed by GC/MS/MS and others by LC/MS/MS. The operating conditions are described in Table 2.1 and 2.2, respectively.

Table 2.1. Operating conditions of GC and MS/MS detection.

	Fragrances		
	Total load	***Soluble load***	***Sludge***
	Injector split-splitless		
Splitless time	1 min	1 min	-
Injector temperature	260ºC	250ºC	250ºC
Gas flow (He)	1 mL·min^{-1}	1 mL·min^{-1}	-
Pressure pulse	No	30 PSI (1 min)	-
Injector time/ volume	8 min	1 µL	3 µL
Solvent	Ethylacetate	Ethylacetate	-
	GC temperatures		
Initial temperature	60ºC	60ºC	50ºC
Initial time	2 min	2 min	0.75
1st ramp	10ºC·min^{-1}	10ºC·min^{-1}	20ºC·min^{-1}
Final temperature	250ºC	250ºC	160ºC
Isothermal time	0 min	0 min	0 min
2nd ramp	20ºC·min^{-1}	20ºC·min^{-1}	4ºC·min^{-1}
Final temperature	280ºC	280ºC	280ºC
Isothermal time	9.5 min	9.5 min	0 min
3rd ramp	-	-	20ºC·min^{-1}
Final temperature	-	-	300ºC
Isothermal time	-	-	10 min
	MS parameters		
Ionization mode	EI	EI	-
Filament current	20 µA	20 µA	-
Ion trap temperature	220ºC	220ºC	260ºC
Transfer line temperature	280ºC	280ºC	260ºC
Voltage	1700-1750 V	1700-1750 V	-
Scan velocity	0.76 s·scan^{-1}	0.76 s·scan^{-1}	-
Mass spectrum	45-400 m/z	45-400 m/z	-
m/z quantification	HHCB(243) AHTN (159)	HHCB (243) AHTN (159)	HHCB (243,187) AHTN (243,213)

Table 2.1. Operating conditions of GC and MS/MS detection. *Cont.*

	Neutral pharmaceuticals	**Acidic pharmaceuticals**	**Estrogens**
	Soluble load	***Soluble load***	***Soluble load/ Sludge***
		Inyector split-splitless	
Splitless time	1 min	1 min	-
Injector temperature	250ºC	280ºC	300ºC
Gas flow (He)	1 mL·min^{-1}	1 mL·min^{-1}	-
Pressure pulse	30 PSI (1 min)	No	-
Injector volume	1 µL	1 µL	5 µL
Solvent	Ethylacetate	Ethylacetate	-
		GC temperatures	
Initial temperature	60ºC	50ºC	50ºC
Initial time	2 min	1 min	3.5 min
1st ramp	10ºC·min^{-1}	10ºC·min^{-1}	20ºC·min^{-1}
Final temperature	250ºC	180ºC	240ºC
Isothermal time	0 min	7 min	0 min
2nd ramp	20ºC·min^{-1}	10ºC·min^{-1}	2ºC·min^{-1}
Final temperature	280ºC	230ºC	290ºC
Isothermal time	9.5 min	25 min	10 min
3rd ramp	-	20ºC·min^{-1}	-
Final temperature	-	250ºC	-
Isothermal time	-	5 min	-
		MS parameters	
Ionization mode	EI	EI	EI
Filament current	20 µA	10 µA	
Ion trap temperature	220ºC	220ºC	250ºC
Transfer line temperature	280ºC	280ºC	280ºC
Voltage	1700-1750 V	1700-1750 V	-
Scan velocity	0.76 s·scan^{-1}	1 s·scan^{-1}	-
Mass spectrum	45-400 m/z	100-330 m/z 140-420 m/z	150-450 m/z
m/z quantification	CBZ (193+236) DZP (256 + 283)	IBP (263) NPX (287) DCF (352+354+356)	E1 (342+257+244) E2 (416+326+285) EE2 (425+231+193)

Table 2.2. Operating conditions of LC and MS/MS detection.

	Acidic pharmaceuticals	**Antibiotics and Carbamazepine**
	HPLC conditions	
Column temperature	25ºC	25ºC
Flow rate ($\mu L \cdot min^{-1}$)	400	400
Injector volume (μL)	20	50
Time (min)-A-B-C-D (%)	0 – 40 – 0 – 60 - 0	0 – 0 - 100 – 0 - 0
[1]Eluents for acidic	6 – 95 – 0 – 5 - 0	15 – 0 - 74 – 0 - 26
[2]Eluents for antibiotics	15 – 40 – 0 – 60 - 0	17 – 0 - 62 – 0 - 38
		30 – 0 – 0 – 0 - 100
		36 – 0 – 100 – 0 - 0
Total duration (min)	20	50
	MS parameters	
Scan type	MRM	MRM
Polarity	Negative	Positive
Ionization mode	APCI	ESI
Filament emission current	30 μA	35 μA
Ion trap temperature	-	-
Transfer line temperature	700ºC	650ºC
Multiplicador voltage	-	-
Scan velocity	-	-
Mass spectrum	204-370 m/z	172-917 m/z (10-25 min)
m/z quantification	IBP (205.1+159.1+175.0)	SMX (254.2+156.0+108.1)
	NPX (229.0+170.0)	ROX (837.4+679.4+158.2)
	DCF (294.1+249.8+214.4)	CBZ (237.1+194.2+179.2)

[1]A: 100% acetonitrile picograde (pH 5.5); C: Formic acid, 10 mM (pH 3.0).
[2]B: 90% NH_4Ac (5 Mm) /10% Acetonitrile (pH 6); D: 20% of (90% NH_4Ac (5 Mm)/10% Acetonitrile) /80% Acetonitrile. Total pH: 7.5

Analytical procedures

Next, a detailed description of the analytical method for each group of compounds is presented. The retention times, absolute and relative recoveries, repeatability and detection and quantification limits of each method are indicated in Table 2.3.

Table 2.2. Operating conditions of LC and MS/MS detection. *Cont.*

	X-Ray contrast media	Estrogens
	HPLC conditions	
Column temperature	25ºC	25ºC
Flow rate ($\mu L \cdot min^{-1}$)	600	300-1,000
Injector volume (µL)	50	20
Time (min)-A-B-C-D (%)	0 – 0 – 100 – 0 - 0	0 – 10 - 0 – 90 – 0
[1]Eluents for X-Ray contrast media		8.5 – 30 – 0 – 70 – 0
[2]Eluents for Estrogens		22 – 0 – 30 – 0 – 70
		42 – 0 – 90 – 0 – 10
		61 – 10 – 0 – 90 - 0
Total duration (min)	15	70
	MS parameters	
Scan type	MRM	MRM
Polarity	Positive	Negative
Ionization mode	ESI	ESI
Filament emission current	30 µA	35
Ion trap temperature	-	-
Transfer line temperature	750ºC	750ºC
Multiplicador voltage	-	-
Scan velocity	-	-
Mass spectrum	143-1649 m/z	267-313 m/z
m/z quantification	IPM (791.8+572.8)	E1 (268.9+144.8+142.7) E2 (270.9+183.0+144.8) EE2 (294.9+144.8+142.8)

[1]B: 20% of (90% NH_4Ac (5 Mm)/10% Acetonitrile) (pH 6) /80% acetonitrile (pH 5.5).
[2]A: Methanol (pH 4); B: NH_3/Methanol (10 mM) (pH 9.8); C: Formic acid (10 mM) (pH 3.0); D: NH_3/H_2O (10 mM) (pH 9.2).

Polycyclic Musk Fragrances (PMF)

Two different methods have been used to determine polycyclic musk fragrances (Galaxolide and Tonalide) in liquid samples, depending on the objective: the SPME and the SPE.

The SPME method (Figure 2.1) allows the determination of the *total load* of PMF in the sample and it was only performed for musks (García-Jares *et al.*, 2002). 10 mL of sample were immersed in a bath at 100ºC for 5 min to equilibrate temperature. Then, the PDMS-DVB (65 µm polydimethylsiloxane-diviylbenzene, Supelco, USA) was exposed to the headspace over the sample (HS-SPME) for 25 min. Once finished the exposition, the fibre was immediately inserted into the GC injector and the chromatographic analysis was carried out. Desorption time was

set at 2 min, although an extra period of 5 min was considered to avoid carryover effect.

Table 2.3. Retention times, absolute and relative recoveries, repeatability, detection (LOD) and quantification (LOQ) limits for the analytical methods in liquid samples.

PPCP	*Retention time (min)*	*Recoveries (%)*		*Repeatability (%)*	*LOD* ($ng \cdot L^{-1}$)	*LOQ* ($ng \cdot L^{-1}$)
		Absolute	**Relative**			
HHCB	18.09	88	-	7.7 - 10.9	1.2	4
AHTN	18.16	90	-	9.9 - 15.1	1.8	6
CBZ	22.90	67	-	8.5	22.2	74
DZP	23.80	99	-	12.0	18.9	63
IBP	20.47	-	90	13.4	6.7	20
NPX	30.04	-	88	7.5	6.7	20
DCF	36.94	-	105	2.8	16.7	50
SMX	3.40	-	75 - 99	-	6.7	20
ROX	18.00	-	75 - 99	-	6.7	20
IPM	3.80	-	75 - 105	-	6.7	20
E1	22.19	-	84 - 96	-	0.5	1
E2	22.80	-	80 - 95	-	0.5	1
EE2	25.28	-	82 - 92	-	0.5	1

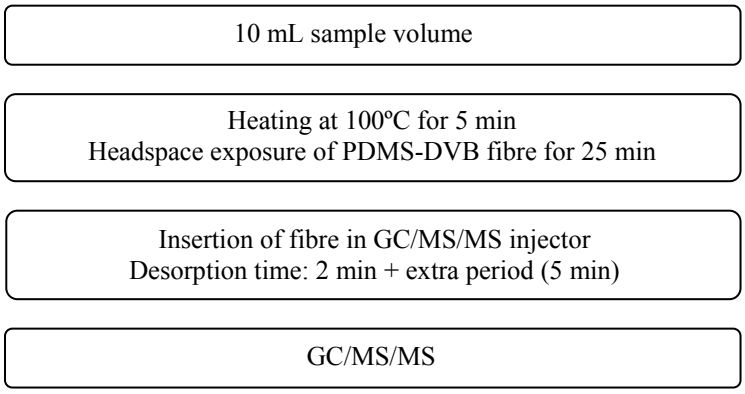

Figure 2.1: Scheme of the SPME method for polycyclic musks in liquid samples.

The SPE method (Figure 2.2) was used for the determination of the *soluble* load of PMF in liquid samples. 500 mL of wastewater was filtered through glass fibre filters (< 5µm pore size), adjusted to pH 2-2.5 and spiked with the surrogate standard (1.08 µg of meclofenamic acid). Afterwards, depending on sample contamination, 250 or 500 mL were used for the enrichment, which was performed in OASIS HLB 3cc cartridges (preconditioned by flushing 3 mL ethyl-

acetate, 3 mL methanol and 3 mL Milli-Q water adjusted to pH 2.5) with a flow rate of ~15 $mL \cdot min^{-1}$. Then, the cartridges were dried completely by a nitrogen stream for 30 min and the analytes eluted with 3 mL of ethyl-acetate. 40 ng of 2,4,6-trichlorobiphenyl (PCB 30) was added as internal standard to 200 µL of the final extract. Finally, the GC/MS/MS detection was carried out in a Hewlett Packard 5890 Series II coupled with Hewlett Packard 5971 Quadrupole Mass Selective Detector.

Due to their occurrence as ingredients in all kinds of cleansing products and cosmetics, the risk of sample contamination with musks when they are manipulated in the laboratory is significant, so extreme precautions to avoid sources of interference in the laboratory environment have been taken. Blank samples of the whole process have been analyzed every set of samples to discard potential contamination. In addition, spiked water samples have been analyzed periodically to evaluate the performance of the method.

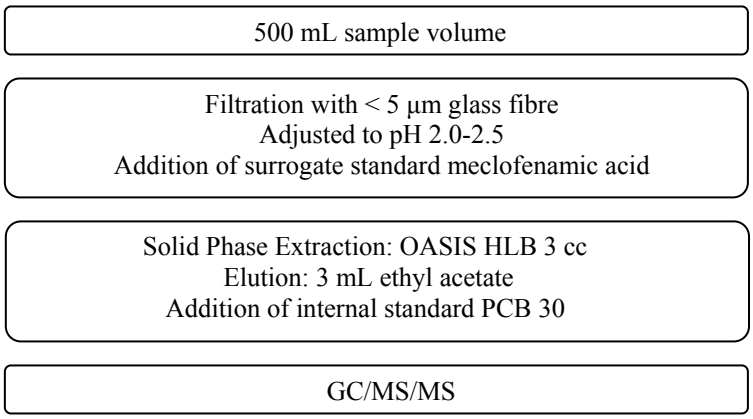

Figure 2.2: Scheme of the SPE method for musks and neutral pharmaceuticals in liquid samples.

Neutral pharmaceuticals

For the neutral pharmaceuticals (Carbamazepine and Diazepam), the analytical method used was the same as for determining the soluble load of musks (Figure 2.2).

Acidic pharmaceuticals

For the acidic pharmaceuticals (Ibuprofen, Naproxen and Diclofenac), the analytical method (Figure 2.3) described by Rodriguez *et al.* (2003) was used. 500 mL of wastewater was filtered through glass fibre or cellulose filters (< 5μm pore size), adjusted to pH 2-2.5 and spiked with the surrogate standard (1.08 μg of meclofenamic acid). Afterwards, depending on sample contamination, 250 or 500 mL were used for the enrichment, which was performed in OASIS HLB 3cc cartridges (preconditioned by flushing 3 mL ethyl-acetate, 3 mL methanol and 3 mL Milli-Q water adjusted to pH 2.5) with a flow rate of ~15 $mL \cdot min^{-1}$. Then, the cartridges were dried completely by a nitrogen stream for 30 min and the analytes eluted with 2 mL of ethyl-acetate. Afterwards, 800 μL of the analytes extract was derivatised with 200 μL of MTBSTFA (N-Methyl-N-(*tert.*-buthyldimethylsilyl) trifluoroacetamide at 60ºC for 1 hour. Finally, the GC/MS/MS detection was carried out in a Hewlett Packard 5890 Series II coupled with Hewlett Packard 5971 Quadrupole Mass Selective Detector. 200 ng of PCB 30 was added as internal standard in the final extract.

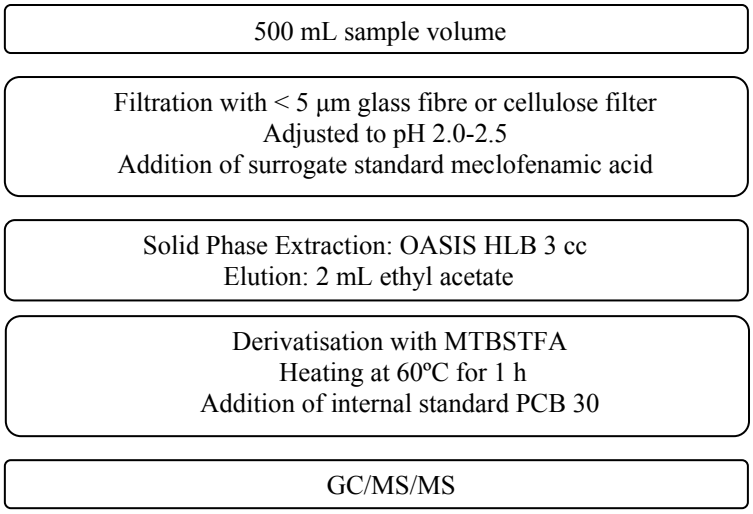

Figure 2.3: Scheme of the analytical method for acidic pharmaceuticals in liquid samples.

Antibiotics

For the antibiotics (Roxithromycin and Sulfamethoxazole), the analytical method (Figure 2.4) described by Hirsch *et al.* (1998) was used. 200 mL of

wastewater was filtered through glass fibre filters (< 1µm pore size), adjusted to pH 7-7.5 and spiked with the surrogate standards (500 ng of Oleandomycine and Sulfapirydine). Afterwards, the enrichment was performed in cartridges containing 0.1 g of LiChrolut ENV and 0.25 g of Isolute C_{18} (preconditioned by flushing 3 x 2 mL n-hexane, 1 x 2 mL acetone, 5 x 2 mL methanol and 5 x 2 mL water adjusted to pH 7.5) with a flow rate of ~20 $mL \cdot min^{-1}$. Then, the cartridges were dried completely by a nitrogen stream for 1 h and the analytes eluted four times with 1 mL of methanol. After evaporation to dryness, the residue was solved in 50 µL methanol and 450 µL phosphate buffer (500 mL of Na_2HPO_4 0.02 $mol \cdot L^{-1}$ and 400 mL of KH_2PO_4 0.02 $mol \cdot L^{-1}$). Finally, the detection was carried out by LC electrospray Tandem MS (API 365), previous filtration of the final extract.

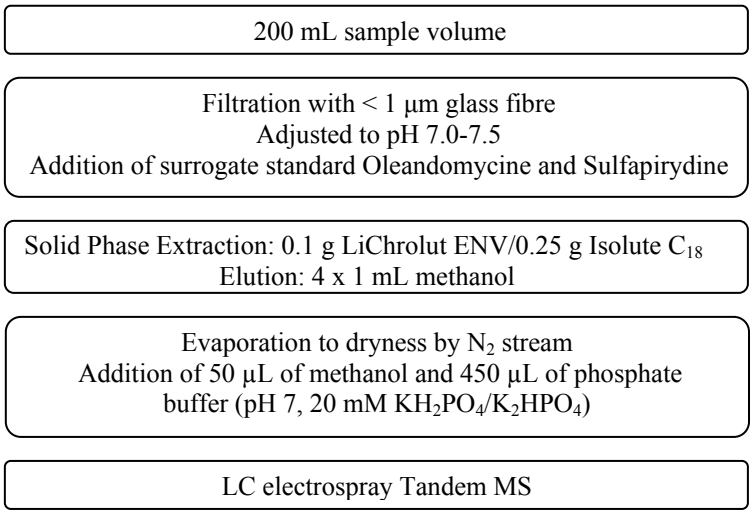

Figure 2.4: Scheme of the analytical method for antibiotics in liquid samples.

X-Ray Contrast Media

For the X-Ray contrast medium (Iopromide), the analytical method (Figure 2.5) described by Ternes (2001) was used. 200 mL of wastewater was filtered through glass fibre filters (< 1µm pore size), adjusted to pH 2.8 and spiked with the surrogate standard (500 ng of desmethoxy-iopromide, DMI). Afterwards, the enrichment was performed in cartridges containing 0.2 g of LiChrolut ENV and 0.15 g of RP-C_{18} ec (previously preconditioned by flushing 3 x 2 mL n-hexane, 1 x 2 mL acetone, 5 x 2 mL methanol and 5 x 2 mL water adjusted to pH 2.8) with

a flow rate of ~10 mL·min^{-1}. Then, the cartridges were dried completely by a nitrogen stream for 1 h and, once the RP-C_{18}ec material was removed, the analytes were eluted four times with 1 mL of methanol. After evaporation to dryness, the residue was solved in 50 µL methanol and filled up with 450 µL phosphate buffer (pH 7, 20 mM KH_2PO_4/K_2HPO_4). Finally, the detection was carried out by LC electrospray Tandem MS (API 365), previous filtration of the final extract.

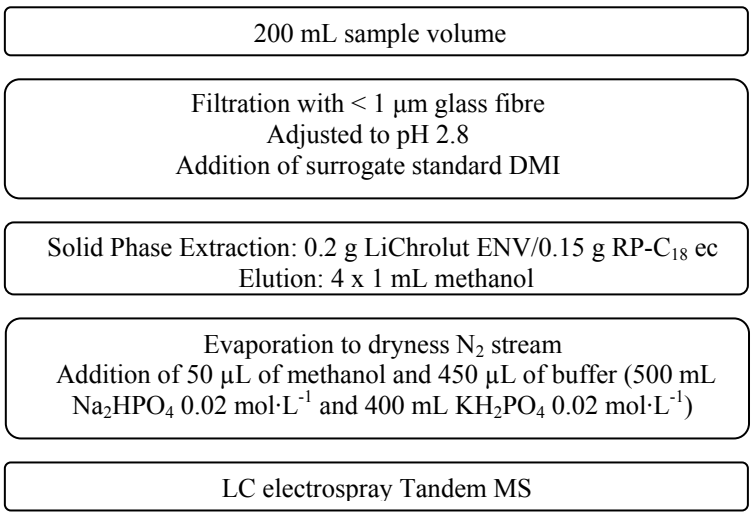

Figure 2.5: Scheme of the analytical method for X-Ray contrast media in liquid samples.

Estrogens

For the estrogens (17β-estradiol, Estrone and 17α-ethinylestradiol), the analytical method (Figure 2.6) described by Ternes (2001) was used. 500 mL of wastewater was filtered through glass fibre filters (< 1µm pore size), adjusted to pH 3.0 and spiked with the surrogate standard (100 ng of 17β-estradiol-17β-acetate). Afterwards, the enrichment was performed in cartridges containing 0.5 g of RP-C_{18} ec (preconditioned by flushing 1 x 2 mL n-hexane, 1 x 2 mL acetone, 3 x 2 mL methanol and 5 x 2 mL water adjusted to pH 3.0) with a flow rate of ~20 mL·min^{-1}. Then, the cartridges were dried completely by a nitrogen stream for 1 h and the analytes eluted four times with 1 mL of acetone. After evaporation to 200 µL, the silica-gel clean-up is performed. 1 g of silica gel 60 (heated over night at 150ºC and deactivated with 1.5% Milli-Q water) was dissolved in 4 mL of solvent

(n-hexane/acetone, 65:35 v/v). 200 µL of extract were transferred into the cartridge and rinsed 5 times with 1 mL of solvent. The extract was evaporated to dryness by a gentle nitrogen stream, rinsed with 200 µL of acetone and derivatised with 50 µL of MSTFA (N-methyl-N-(trimethylsilyl)-trifluoroacetamide) / TMSI (trimethylsilylimidazole) / DTE (dithioerytrol) (1000:2:2, v/v/s) for 1h at 60ºC. After evaporation to dryness, the residue was solved in 200 µL of n-hexane. Finally, the detection was carried out by GC ion trap MS. 100 ng of Mirex (internal standard) was added to the final extract.

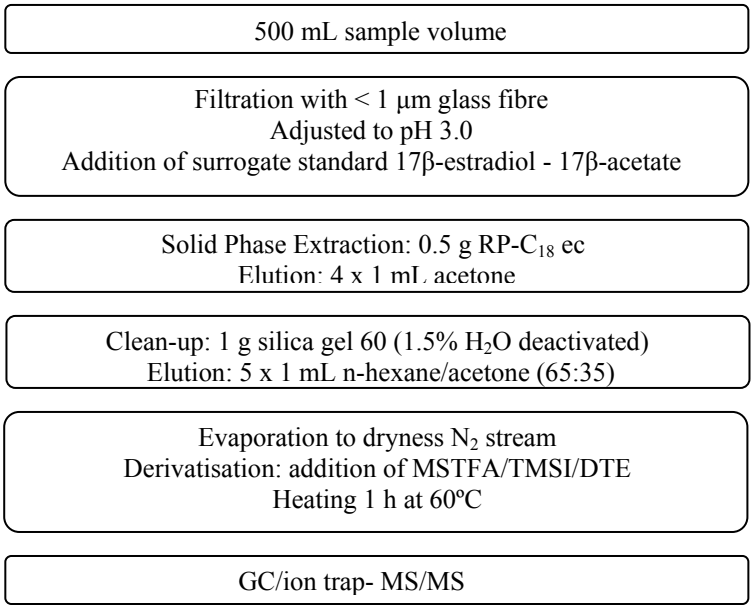

Figure 2.6: Scheme of the analytical method for estrogens in liquid samples.

The method for estrogens by LC/MS/MS was the same as described before, but neither silica-gel clean-up nor derivatisation step were performed. The extract from the SPE was evaporated to dryness by a gentle nitrogen stream and finally dissolved with 50 µL of methanol and 450 µL of buffer (methanol/water, 10:90, v/v) before LC/MS/MS detection.

2.2.2. Sludge samples

The first step common for all PPCPs being analysed in sludge samples was the extraction (Ternes *et al.*, 2002; Ternes *et al.*, 2005; Löffler and Ternes, 2003). Ultrasonic Solvent Extraction (USE) is used. 0.5 g (0.2 g for musks) of sludge

was sequentially extracted with 4 and 2 mL (3 mL for estrogens) of methanol and 2 times (3 for musks) with 3 mL of acetone. The surrogate standards (Tonalid-D_3 for musks; Dihydro-carbamazepine for Carbamazepine; Fenotrop and Cl,Br-Diclofenac for acidics; DMI for X-Ray contrast media; Oleandomycine, Sulfapirydine and Sulfamethoxazole-D4 for antibiotics and 17β-estradiol-17β-acetate for estrogens) were spiked in the first extraction slurry. For each extraction step, the slurry was ultrasonicated for 5-10 min. Then, it was centrifuged at 3000 rpm for 5 min and the supernatant was collected. The 4 solvent fractions were finally combined and the resulting volume was reduced in a BUCHI device.

Analytical procedures

Next, a detailed description of the analytical method for each group of compounds is presented. The retention times, absolute and relative recoveries, repeatability and detection and quantification limits of each method are indicated in Table 2.4.

Table 2.4. Retention times, absolute and relative recoveries, repeatability, detection (LOD) and quantification (LOQ) limits for the analytical methods in sludge samples.

PPCP	*Retention time (min)*	*Recoveries (%)*		*Repeatability (%)*	*LOD ($ng \cdot g^{-1}$)*	*LOQ ($ng \cdot g^{-1}$)*
		Absolute	**Relative**			
HHCB	15.05	52 - 76	-	10 - 12	-	250
AHTN	15.30	54 - 94	-	15 - 20	-	250
CBZ	19.40	40 - 46	74 - 96	3 - 11	-	20
DZP	23.90	22 - 28	38 - 58	-	-	20
IBP	16.00	35 - 55	64 - 86	4 - 12	-	20
NPX	10.80	131 - 134	95 - 97	2 - 17	-	20
DCF	15.60	34 - 56	63 - 89	7 - 13	-	20
SMX	3.40	65 - 75	99 - 113	8 - 93	-	20
ROX	18.00	36 - 41	62 - 71	9 - 24	-	20
IPM	8.10	108 - 132	189 - 223	12 - 55	-	50
E1	22.19	77 - 104	117 - 119	3 - 11	-	2
E2	22.80	66 - 73	83 - 117	10 - 18	-	2
EE2	25.28	57 - 99	94 - 113	15 - 25	-	4

Polycyclic Musk Fragrances

The method for musk fragrances in sludge (Figure 2.7) has been described by Ternes *et al.* (2005). The extract from USE is combined with 250 mL of groundwater at pH 7.0-7.5 and enriched by SPE using 0.5 g of RP-C_{18} Bulk

Sorbent (preconditioned with 1 x 2 mL hexane, 1 x 2 mL acetone, 3 x 2 mL methanol and 3 x 2 mL water adjusted to pH 7.0-7.5) at a flow rate of ~20 $mL \cdot min^{-1}$. Then, the cartridges were dried completely by a nitrogen stream for 1 h and the analytes eluted four times with 1 mL of methanol. The extract was then evaporated down to 200 µL by a gentle nitrogen stream. Subsequently, the silica-gel clean-up was performed. 1 g of silica gel 60 (heated over night at 150ºC and deactivated with 1.5% Milli-Q water) was dissolved in 6 mL of solvent (hexane/ethylacetate, 85:15 v/v). 200 µL of extract were transferred into the cartridge and rinsed 5 times with 1 mL of solvent. After evaporation to dryness, the extracts were dissolved in 200 µL of n-hexane. The detection was carried out by GC/MS/MS (Hewlett Packard 5890 Series II coupled with Hewlett Packard 5971 Mass Selective Detector). 500 ng of PCB 30 was added as internal standard in the final extract.

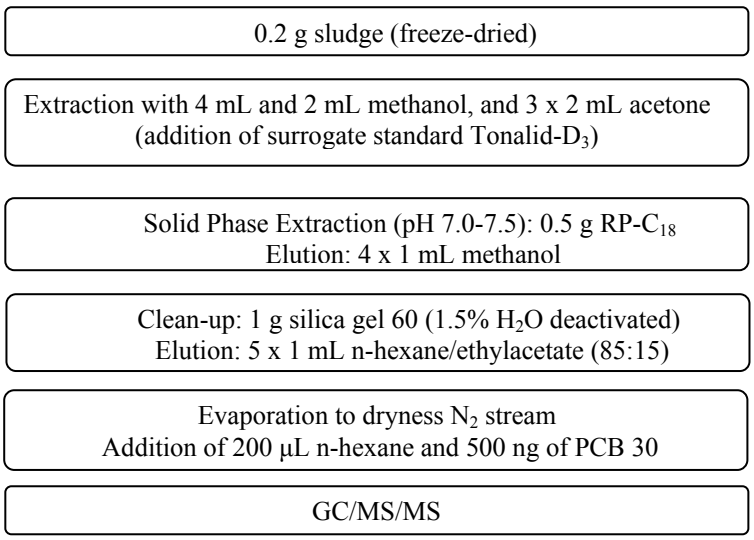

Figure 2.7: Scheme of the analytical method for polycyclic musks in sludge samples.

Acidic pharmaceuticals

The method for acidic pharmaceuticals in sludge (Figure 2.8) has been described by Ternes *et al.* (2005) The extract from USE was combined with 250 mL of groundwater at pH 2.0 and enriched by SPE using OASIS MCX 3 cc cartridges (preconditioned with 3 x 2 mL hexane, 1 x 2 mL acetone, 5 x 2 mL methanol and 5 x 2 mL water adjusted to pH 2.0) at a flow rate of ~20 $mL \cdot min^{-1}$. Then, the cartridges were dried completely by a nitrogen stream for 20 min and

the analytes eluted four times with 1 mL of acetone. The eluates were reduced to approximately 100 µL and mixed with 200 µL of methanol. After evaporation to about 200 µL, the residue was filled up to 500 µL with buffer (formic acid, 0.01 M). Finally, the detection was carried out by LC electrospray Tandem MS (API 365), previous filtration of the final extract.

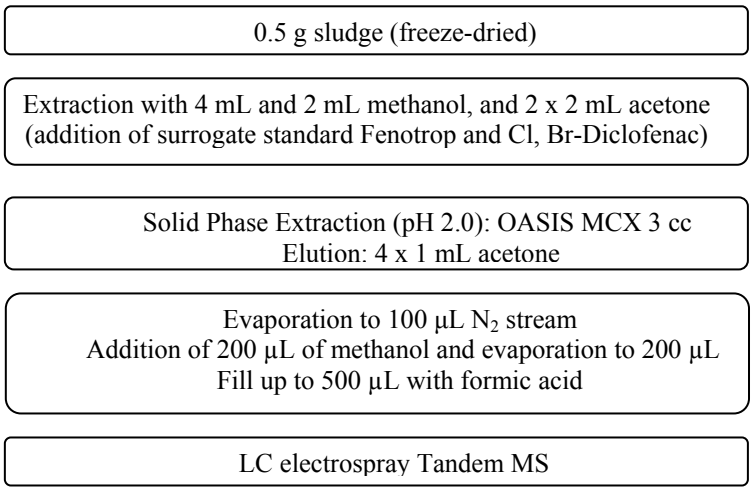

Figure 2.8: Scheme of the analytical method for acidic pharmaceuticals in sludge samples.

Carbamazepine and antibiotics

The extract from USE (Figure 2.9) was combined with 250 mL of groundwater at pH 7.5 and enriched by SPE using OASIS HLB 6 cc cartridges (preconditioned with 3 x 2 mL hexane, 1 x 2 mL acetone, 5 x 2 mL methanol and 5 x 2 mL water adjusted to pH 7.5) at a flow rate of ~20 $mL \cdot min^{-1}$. Then, the cartridges were dried completely by a nitrogen stream for 1 h and the analytes eluted four times with 1 mL of methanol. After evaporation to dryness, the residue was dissolved with 50 µL of methanol and 450 µL of buffer (500 mL Na_2HPO_4 0.02 $mol \cdot L^{-1}$ and 400 mL KH_2PO_4 0.02 $mol \cdot L^{-1}$). Finally, the detection was carried out by LC electrospray Tandem MS (API 365), previous filtration of the final extract.

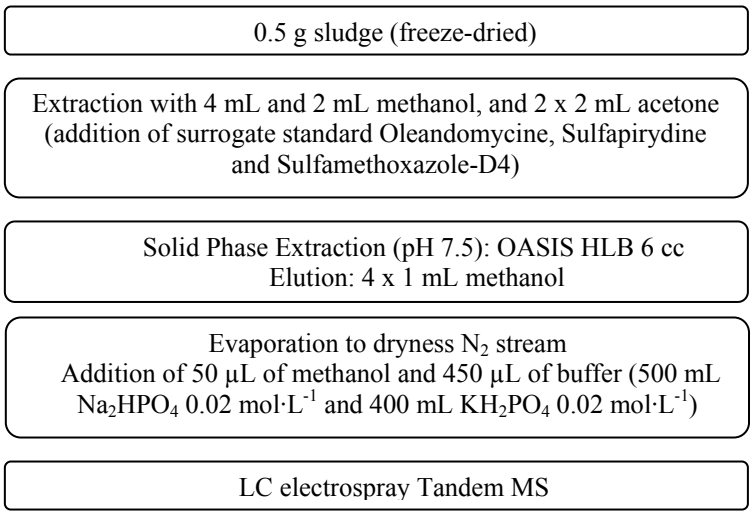

Figure 2.9: Scheme of the analytical method for Carbamazepine and antibiotics in sludge samples.

X-Ray Contrast Media

The method for X-Ray contrast media in sludge (Figure 2.10) has been described by Ternes *et al.* (2005). The extract from USE was combined with 250 mL of groundwater at pH 2.8 and enriched by SPE using 0.2 g Isolute ENV and 0.15 g RP-C_{18} (preconditioned with 3 x 2 mL hexane, 1 x 2 mL acetone, 5 x 2 mL methanol and 5 x 2 mL water adjusted to pH 2.8) at a flow rate of ~10 mL·min^{-1}. Then, the cartridges were dried completely by a nitrogen stream for 1 h and, once the RP-C_{18} material was removed, the analytes were eluted four times with 1 mL of methanol. After evaporation to dryness, the residue was dissolved with 20 µL of methanol and filled up to 500 µL with phosphate buffer (pH 7, 20 mM KH_2PO_4/K_2HPO_4). Finally, the detection was carried out by LC electrospray Tandem MS (API 365), previous filtration of the final extract.

Estrogens

The method for estrogens in sludge (Figure 2.11) has been described by Ternes *et al.* (2002). The extract from USE was first cleaned by Gel Permeation Chromatography (GPC) and then with silica-gel. 4.5 mL of the extract dissolved in 5 mL acetone/cyclohexane (1:3, v/v) and filtered over PTFE filter was injected in the GPC column (Autotrep 1000, Columbia). The fraction between 14 and 27

min, containing the estrogens, was collected and reduced to ~1 mL with a rotatory evaporator at 20 kPa and 40ºC.

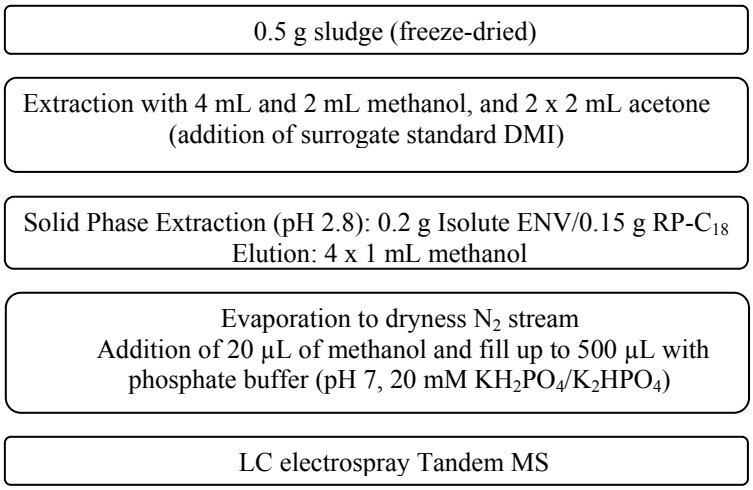

Figure 2.10: Scheme of the analytical method for X-Ray contrast media in sludge samples.

After evaporation to dryness, the residue was dissolved in 200 µL of hexane/acetone (65:35, v/v). Subsequently, the silica-gel clean-up was performed. 1 g of silica gel 60 (heated over night at 150ºC and deactivated with 1.5% Milli-Q water) was dissolved in 5 mL of solvent (hexane/acetone, 65:35 v/v). 200 µL of extract were transferred into the cartridge and rinsed 5 times with 1 mL of solvent. The eluates were evaporated to ~300 µL and combined with 250 mL of groundwater at pH 3.0 for enrichment by SPE using 0.5 g of RP-C_{18} (preconditioned with 3 x 2 mL hexane, 1 x 2 mL acetone, 5 x 2 mL methanol and 5 x 2 mL water adjusted to pH 3.0) at a flow rate of ~20 $mL \cdot min^{-1}$. Then, the cartridges were dried completely by a nitrogen stream for 1 h and the analytes eluted four times with 1 mL of acetone. After evaporation to 200 µL, the extracts were derivatised with 50 µL of MSTFA/TMSI/DTE (1000:2:2, v/v/s) for 1h at 60ºC. After evaporation to dryness, the residue was solved in 200 µL of n-hexane. Finally, the detection was carried out by GC ion trap MS. 100 ng of Mirex was added as internal standard in the final extract.

The method for estrogens in sludge samples by LC/MS/MS was the same as described before, but neither GPC nor derivatisation were performed. The extract

from the SPE was evaporated to dryness by a gentle nitrogen stream and finally dissolved with 50 µL of methanol and 450 µL of buffer (methanol/water, 10:90, v/v) before LC/MS/MS detection.

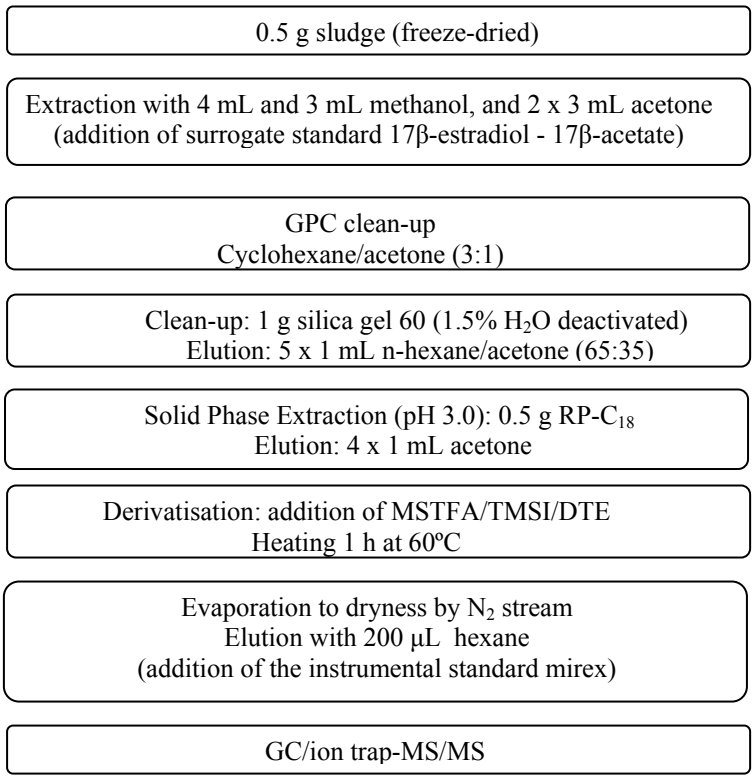

Figure 2.11: Scheme of the analytical method for estrogens in sludge samples.

2.3. References

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Chapter 3

Behaviour of pharmaceuticals, cosmetics and hormones in a sewage treatment plant[1,2]

Summary

Two cosmetics (Galaxolide and Tonalide), eight pharmaceuticals (Carbamazepine, Diazepam, Ibuprofen, Naproxen, Diclofenac, Roxithromycin, Sulfamethoxazole and Iopromide) and three hormones (Estrone, 17β-estradiol and 17α-ethinylestradiol) have been surveyed along the different units of a municipal Sewage Treatment Plant (STP). Among all the substances considered, significant concentrations in the influent were only found for the two musks (Galaxolide and Tonalide), two anti-inflammatories (Ibuprofen and Naproxen), the natural estrogens (Estrone and 17β-estradiol), one antibiotic (Sulfamethoxazole) and the X-ray contrast medium (Iopromide), being the other compounds considered found below the limit of quantification. During primary treatment, only the fragrances (30-50%) and 17β-estradiol (20%) are partially removed. In contrast, the biological treatment (conventional activated sludge) causes an important reduction (35-75%) in all compounds detected, with the exception of Iopromide, which remains in the water phase. The overall removal efficiencies within the STP range between 70-90% for the fragrances, 40-65% for the anti-inflammatories, around 65% for 17β-estradiol and 60% for Sulfamethoxazole. On the other hand, the concentration of Estrone increases along the treatment due to two main factors: i) the cleavage of glucuronides during the first steps of the treatment, and ii) the partial oxidation of 17β-estradiol in the aeration tank.

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[1] **Carballa, M., Omil, F., Lema, J.M., Llompart, M., García-Jares, C., Rodríguez, I., Gómez, M. and Ternes, T.** (2004). Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Research,* 38: 2918-2926.

[2] **Carballa, M., Omil, F., Lema, J.M., Llompart, M., García-Jares, C., Rodríguez, I., Gómez, M. and Ternes, T.** (2005). Behaviour of pharmaceuticals and personal care products in a sewage treatment plant of northwest Spain. *Water Science and Technology,* 52 (8): 29-35.

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3.1. Introduction

3.1.1. Pharmaceutical and Personal Care Products in sewage

Earlier investigations of drug residues in STP effluents were focused on clofibric acid, the major metabolite of the three lipid regulators (etofibrate, etofyllinclofibrate and clofibrate). Garrison *et al.* (1976) conducted the first study to detect drugs in sewage effluent, detecting clofibric acid at concentrations up to 2 $\mu g \cdot L^{-1}$ in raw and treated wastewater in Kansas City, USA. Hignite and Azarnoff (1977) verified those results and measured up to 10 $\mu g \cdot L^{-1}$ of clofibric acid and 95.6 $\mu g \cdot L^{-1}$ of salicylic acid in the sewage effluent at the same STP. Waggott (1981) found clofibric acid in the River Lee (Great Britain) at concentration levels below 0.01 $\mu g \cdot L^{-1}$ and in Spain clofibric acid was detected in groundwater samples (Galceran *et al*., 1989). In Germany, this lipid regulator was even found in drinking water. Heberer and Stan (1996) quantified concentrations up to 0.27 $\mu g \cdot L^{-1}$ in Berlin tap water.

Systematic studies on the occurrence and fate of PPCPs in the environment have been carried out world-wide (Ternes, 1998; Heberer *et al.,* 1999; Baronti *et al.,* 2000; Kolpin *et al.,* 2002; Kanda *et al.,* 2003; Boyd *et al.,* 2003; Weigel *et al.,* 2004). Many of these samples have been taken from wastewater (Buser *et al.*, 1999; Huppert *et al.*, 1998; Ternes, 1998), but also from surface (Kolpin *et al.,* 2004; Buerge *et al.,* 2003; Bursch *et al.,* 2004; Wiegel *et al.,* 2004) and groundwaters (Buser *et al.*, 1998; Halling-Sorensen *et al.*, 1998). Some substances have been also detected at the entrance of drinking water facilities (Putschew *et al.,* 2000; Ternes, 2001; Zuccato *et al.,* 2000; Boyd *et al.,* 2003) and in tap water (Adler *et al.,* 2001; Kuch and Ballschmiter, 2001; Heberer *et al.*, 2002). The authors stated that PPCPs residues can be expected in the aquatic environment under the following scenarios: high application and the excretion of unmetabolized drugs at high percentage levels.

Some of the most representative PPCPs found in STPs are antibiotics, lipid regulators, anti-inflammatories, antiepileptics, tranquillizers, contrast media and contraceptives, with different chemical structures. Because of that, a considerable effort is being made in order to develop the analytical techniques necessary to quantify their occurrence in effluents, but also to assess their chemical properties, their biodegradability potential, etc.

In developed countries, the primary route of these substances into the environment is through the use of consumer products and human medicines that are discharged down-the-drain to municipal sewage treatment. Other sources are hospital or industrial discharges.

Significant differences in the concentrations detected can be observed even between different geographical areas as mentioned by Heberer (2002a) for the consumption of fragrances and their occurrence in the environment. So far, most of the studies focused on PPCPs have been carried out in USA and central and northern countries of EU, both areas with moderate climates. However, data from treatment plants located in Southern Europe are scarce, a lack of information that should be dealt with in the near future to have a complete picture of the occurrence and fate of these compounds in the whole EU.

3.1.2. Fate in Sewage Treatment Plants

Modern STPs play a key role in the entrance of PPCPs into the environment. They were designed to handle human waste of mainly natural origin, thus they can effectively accomplish carbon and nitrogen removal, as well as microbial pollution control.

The dramatic increase in the production and emission of synthetic organic chemicals for industrial and domestic use has obliged STPs to improve their efficiency, since conventional treatment technologies had not been specifically designed for their elimination. At present, most STPs consist of two treatment steps: a physical treatment, in which the removal of the chemical is mostly due to sorption to solids, thus being the effectiveness directly related to the size and density of the particles; and a biological treatment, where the removal is achieved by bacterial biodegradation, which mainly occurs via oxidation.

Most PPCPs introduced along with the raw wastewaters suffer unknown fates during the treatment. The main mechanisms for pollutants removal from the incoming waste stream are:

a) sorption to filterable solids, which are later removed with the sludge; and

b) microbial degradation to lower molecular weight products, leading sometimes to complete mineralization (CO_2 and H_2O).

Although the microbiota of the STPs may have been exposed to many micropollutants for a number of years, two factors affect the effective microbial

removal of these substances. First, the extremely low concentrations of most drugs limit their biodegradative fate, since for most compounds it is governed by nongrowth-limiting (enzyme-saturating) substrate concentrations (copiotrophic metabolism). However, PPCPs are present at concentrations at enzyme-subsaturating levels, which necessitate an oligotrophic metabolism. Second, many new drugs are introduced to the market each year, most of them with a low biodegradability.

The removal efficiencies of PPCPs in STPs are largely unknown. Most studies reports removal of the parent compounds from the aqueous phase by comparing influent and effluent concentrations. However, it is not possible to distinguish between the three major fates of a substance: a) degradation to lower molecular weight compounds, b) physical sequestration by solids (and subsequent removal as sludge), and c) formation of conjugates that can be later hydrolysed back to yield the parent compound. Therefore, by simply following disappearance of a substance from the liquid phase, it is not possible to conclude if it was structurally altered or mineralised.

Apart from the physico-chemical properties of specific compounds, several parameters influence removal efficiencies through STPs. Ternes (1998) found that wet weather runoff dramatically reduced the removal rates (from over 60% to below 5%) for certain drugs in a facility located close to Frankfurt/Main. Clearly, even for drugs efficiently removed, the operational state (microbial activity and environmental conditions) of the STP can exert a dramatic effect on the removal efficiencies (Tyler and Routledge, 1998; Johnson et al., 2000; Johnson and Sumpter, 2001). Other factors include transitions between seasons and sporadic influxes of toxicants from various sources. Overflows from STP failures or overcapacity events lead to direct, untreated introduction of sewage into the environment.

The overall removal rates published in literature vary strongly, depending on the country, the type of STP and the considered compounds. In Germany, reported efficiencies range from 10 to 90% depending on the nature of the compound (Ternes, 1998). In Brazil, removal efficiencies corresponding to pharmaceutical polar compounds vary from 12 to 90%, with higher efficiencies being obtained in activated sludge processes than in biofilters (Ternes et al., 1999a). Another study carried out in USA (US EPA, 2003), concluded that many PPCPs were removed around 80%. Kreuzinger *et al.* (2004) investigated several

Austrian STPs reporting removal efficiencies from 0 to 99%, depending on the compound. Paxeus (2004) surveyed STPs from 5 EU countries (France, Greece, Italy, Sweden and Denmark) in which the removal efficiencies ranged between 10 and 99%. Fahlenkamp *et al.* (2004) reported PPCPs eliminations of 20-85% in two German STPs.

In most studies, removal includes both degradation and sorption and the difference between both mechanisms has not been assessed yet. In the case of polar compounds, such as carboxylic acids, for which the adsorption effects are expected to be very low, the main mechanism of elimination is attributed to biodegradation. However, the studies carried out by Schäfer and Waite (2002) indicate that less than 10% are effectively biodegraded. In contrast, for substances with high sorption properties, their removal must be governed by sorption onto sludge.

3.1.3. Objective

The aim of this study was to investigate the occurrence and fate of the 13 compounds considered in this work along the different units of a municipal STP located in Galicia (NW Spain). The removal efficiency from the water phase of each substance in each particular treatment unit has been determined.

3.2. Materials and Methods

3.2.1. Sewage Treatment Plant

The sewage treatment plant studied in this work, located in Galicia (NW Spain), has been designed to treat waters of 100,000 population equivalents. It treats mainly household discharges, but also has important hospital discharges (>1,200 beds) and a moderate number of industries.

The plant includes three main sections: pre-treatment, primary treatment and secondary treatment (Figure 3.1). After the reception and pumping of the inlet wastewaters, the pre-treatment section comprises units for coarse screening (bar racks), fine screening and aerated chambers for grit and fat removal. The primary treatment is carried out in circular sedimentation tanks. Finally, the secondary treatment consists of biological reactors using the conventional activated sludge process (mixed reactors followed by a sedimentation tank). The supernatant of the secondary sedimentation unit constitutes the final effluent of the plant.

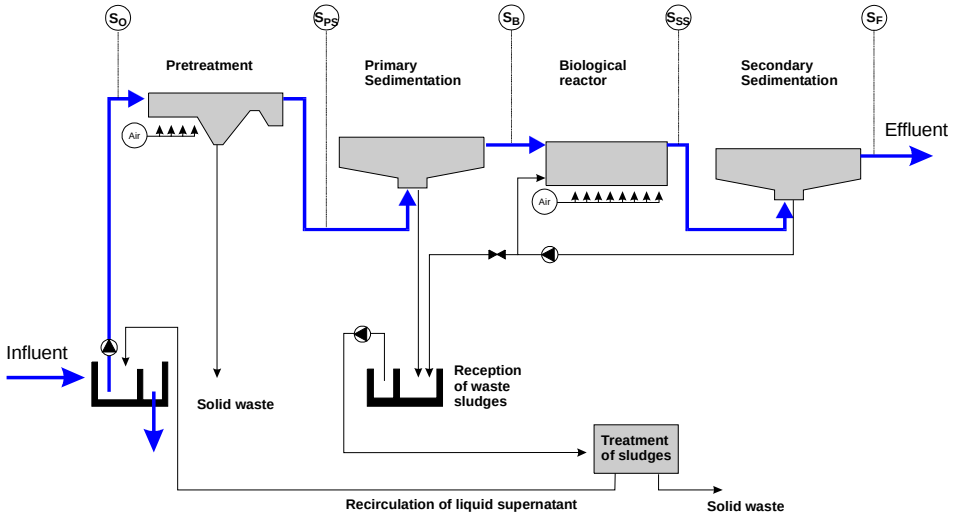

Figure 3.1. Diagram of the municipal sewage treatment plant and location of the sampling points.

The excess of secondary sludge, together with the solids obtained from the primary sedimentation, are treated in a specific unit from which a solid waste and a liquid stream, recycled to the inlet of the plant, are obtained (Fig. 3.1).

The sampling points for analysis were the following (Fig. 3.1): i) Inlet to the grit removal unit (So); ii) Inlet to the primary sedimentation tank (Sps); iii) Inlet to the biological reactor (Sb); iv) Inlet to the secondary sedimentation tank (Sss), and; v) Outlet of the secondary sedimentation tank (Sf).

In four sampling periods (October 2001, January, April and June 2002), daily composite samples were taken at each sampling point by an automatic device. Taking into account that the operating Hydraulic Retention Time (HRT) in the STP is 24 h approximately, the effluent sample was taken time related to the influent. All compounds were measured during the four integrated campaigns, with the exception of estrogens, antibiotics and X-Ray contrast media, which have been only analyzed for the sampling period of April 2002.

The main operation parameters of the plant and the average flow rates during the sampling periods are indicated in Table 3.1.

Table 3.1. Details of the Sewage Treatment Plant investigated.

Treatment	Conventional Activated Sludge			
Treatment comments	BOD removal			
People served (equivalents)	100,000			
HRT total (h)	24			
HRT biological step (h)	6-8			
SRT (d)	1-3			
Sampling period	***October 2001***	***January 2002***	***April 2002***	***June-July 2002***
Flow ($m^3 \cdot d^{-1}$)	51,890	51,506	51,721	51,134
L/d person	519	515	517	511
Effluent pH	7.3	7.2	7.3	7.2

3.2.2. Analytical methods

Biochemical Oxygen Demand (BOD), total and soluble Chemical Oxygen Demand (COD_t and COD_s), pH, temperature and solids (TS, VS, TSS and VSS) were determined by Standard Methods (APHA-AWWA-WPCF, 1999) as described in Chapter 2 (sections 2.1.4, 2.1.5, 2.1.9, 2.1.10 and 2.1.11, respectively).

Nitrogen (TN, IN, TKN and N-NH_4^+) and carbon (TC, IC and TOC) content were determined according to sections 2.1.7 and 2.1.12 of Chapter 2, respectively.

The inorganic anions (NO_2^-, NO_3^-, Cl^-, PO_4^{3-} and SO_4^{2-}) were analyzed by Capillary Electrophoresis as described in section 2.1.6 of Chapter 2.

The soluble content of PPCPs was determined according to section 2.2.1 of Chapter 2. In the case of Galaxolide and Tonalide, complementary methodology was used to determine the total load in each sample (Figure 2.1 of Chapter 2). Values given for the different samples of the STP considered in this work correspond to the average value of two aliquots of each composite sample.

3.2.3. Calculations

Removal efficiencies for all PPCPs were calculated taking into account the concentration at the inlet of the plant (So), at the inlet of the biological reactor (Sb) and in the final effluent (Sf). The elimination during primary (R_P) and biological (R_B) treatment and the overall removal (R_O) in the plant was calculated using equations 3.1, 3.2 and 3.3, respectively. In all cases, S_0 was used as the reference in order to be able to compare and to add the partial removals and obtain the overall one.

$$R_P = \frac{So - Sb}{So} \times 100 \qquad \text{Eq. 3.1}$$

$$R_B = \frac{Sb - Sf}{So} \times 100 \qquad \text{Eq. 3.2}$$

$$R_O = \frac{So - Sf}{So} \times 100 \qquad \text{Eq. 3.3}$$

3.3. Results and Discussion

3.3.1. Conventional parameters in a sewage treatment plant

Table 3.2 shows the values obtained in the different integrated campaigns for the main characteristics of the wastewaters, such as solids content (total and suspended), chemical and biochemical oxygen demand, total organic carbon, nitrogen, chloride, sulphate and phosphate. The overall efficiencies achieved for COD and TSS along the entire STP were 77-94% and 92-94%, respectively.

Table 3.2. Characterisation of the wastewaters along the different units of the STP (in $mg \cdot L^{-1}$).

Month	Sample*	TS	VS	TSS	VSS	Cl^-	SO_4^{2-}	HPO_4^{2-}
October 2001	So	581	330	258	191	42.6	-	-
	Sps	553	308	223	175	56.3	42.4	4.0
	Sb	368	195	65	55	54.5	32.5	-
	Sss	2,573	1,843	2,234	1,787	59.0	43.6	12.6
	Sf	323	105	20	18	51.6	40.8	-
January 2002	So	863	500	298	235	51.3	-	-
	Sps	835	418	268	220	85.6	92.7	1.9
	Sb	500	240	85	78	53.0	59.8	4.0
	Sss	2,510	1,878	2,123	1,718	57.0	63.9	24.1
	Sf	335	118	23	18	50.3	44.9	-
April 2002	So	530	305	258	207	45.1	-	-
	Sps	515	295	243	197	41.9	48.2	3.9
	Sb	500	195	170	88	51.1	76.0	1.7
	Sss	1,110	695	860	697	50.5	76.2	5.9
	Sf	345	110	15	15	48.4	73.3	1.1
June-July 2002	So	540	273	227	173	43.6	46.8	3.5
	Sps	602	312	259	210	59.2	61.9	7.9
	Sb	484	193	73	65	33.2	58.3	2.8
	Sss	2,520	1,841	2,275	1,800	48.6	53.3	18.9
	Sf	208	47	18	13	50.6	60.3	3.7

*See Figure 3.1 for sampling points location.

Table 3.2. Characterisation of the wastewaters along the different units of the STP (in $mg \cdot L^{-1}$). *Cont.*

Month	Sample*	BOD	COD_t	COD_s	TC	TOC	TN
October 2001	So	137	331	137	51.2	22.6	16.5
	Sps	143	299	134	70.2	34.3	21.7
	Sb	47.5	107	99	52.6	20.5	19.5
	Sss	-	1,432	436	79.2	55.6	9.1
	Sf	7.5	49	40	28.1	13.0	11.3
January 2002	So	217	503	149	81.1	40.8	12.7
	Sps	460	497	136	72.6	36.2	26.0
	Sb	75	242	84	66.8	37.4	21.2
	Sss	-	3,196	259	103.0	74.3	19.7
	Sf	18.5	30	14	41.2	17.2	13.9
April 2002	So	156	-	265	53.8	23.0	18.1
	Sps	345	275	172	45.5	17.5	15.9
	Sb	53	272	145	61.3	30.0	15.6
	Sss	-	2,017	811	234.7	218.0	6.2
	Sf	9.0	56	52	28.5	11.8	9.8
June-July 2002	So	155	386	130	59.5	24.9	15.1
	Sps	154	385	200	59.1	23.1	15.3
	Sb	48	173	102	42.8	20.8	12.8
	Sss	-	2,476	147	22.5	9.2	5.8
	Sf	7.0	76	52	20.5	8.9	8.6

*See Figure 3.1 for sampling points location.

Johnson *et al.* (2005) performed a survey along several European STPs (Belgium, France, The Netherlands, Germany, Switzerland, Finland, Sweden and Norway) with different treatment and management practices. A summary is shown in Table 3.3. It can be observed that both the concentrations of BOD and COD in the influent and effluent and their removals in the STP studied are in the range of those obtained in other European STPs.

3.3.2. Occurrence of PPCPs in raw wastewaters

Table 3.4 shows the influent concentrations of the PPCPs detected in the STP during the four sampling campaigns.

Among all PPCPs considered in this work, the following have been quantified in the wastewaters investigated: HHCB, AHTN, IBP, NPX, SMX, IPM, E1 and E2. On the contrary, CBZ, DZP, DCF, ROX and EE2 were found below the limit of quantification.

Table 3.3. Comparison of the main characteristics of the STP studied with other European STPs.

STP	COD influent ($mg \cdot L^{-1}$)	BOD influent ($mg \cdot L^{-1}$)	COD effluent ($mg \cdot L^{-1}$)	BOD effluent ($mg \cdot L^{-1}$)	COD removal (%)	BOD removal (%)
This study	*331 - 503*	*137 - 217*	*30 - 76*	*7 - 19*	*77 - 94*	*86 - 97*
1	372	85	35	4	91	95
2	233	55	45	<4	81	>95
3	-	-	45 – 73	10 – 21	-	-
4	-	-	27 – 38	<8	-	-
5	-	-	35	<2	-	-
6	-	-	24	5	-	-
7	187	54	22	4.7	88	91
8	176	142	34	9	81	94
9	293	135	25	1.6	92	99
10	490	240	51	6	90	98
11	330	150	<30	<3	>90	>98

Table 3.4. PPCPs concentrations ($\mu g \cdot L^{-1}$) in the influent of the STP investigated during the four sampling campaigns.

Substance	October 2001	January 2002	April 2002	June/July 2002
Galaxolide	2.1	3.4	3.2	1.4
Tonalide	0.9	1.7	1.5	0.7
Carbamazepine	<0.07*	<0.07*	<0.07*	<0.07*
Diazepam	<0.06*	<0.06*	<0.06*	<0.06*
Ibuprofen	2.8	5.7	2.6	2.8
Naproxen	3.5	4.6	1.8	2.2
Diclofenac	<0.05*	<0.05*	<0.05*	<0.05*
Sulfamethoxazole	-	-	0.6	-
Roxithromycin	-	-	<0.02*	-
Iopromide	-	-	6.6	-
Estrone	-	-	0.0024	-
17β-estradiol	-	-	0.0016	-
17α-ethinylestradiol	-	-	<0.001*	-

*Limit of Quantification (LOQ).

The main source of these compounds in sewage is their application in human medicine. Only few of them, such as Naproxen or Sulfamethoxazole are additionally used in veterinary medicine.

Apart from the seasonal (weather, pattern use) variation between samples at the inlet of the STP (point So), it can be seen that all these compounds, except the

natural estrogens, are present in the range of 0.6-6.6 $\mu g \cdot L^{-1}$, which leads to initial loads up to 350 $g \cdot d^{-1}$.

The measured concentrations may be compared with the per capita daily release into the STP estimated from the consumption. The latter value would represent the maximum concentration expected if no losses (metabolism, elimination processes, dilution) occur from the consumption point to the STP. For that purpose, it is assumed that the entire volume of PPCP used is disposed of into the collector system and treated in the STP. This implies that the substances do not volatilise to air or remain on skin and surfaces, in the case of musks, and that all the pharmaceuticals purchased are either consumed or thrown to the toilets. Table 3.5 shows the results obtained.

Table 3.5. Maximum PPCPs concentrations expected in the STP influent estimated from the consumption rates.

PPCP	Spain		Santiago de Compostela	
	Consumption ($ton \cdot y^{-1}$)	Per capita use ($\mu g \cdot capita^{-1} \cdot d^{-1}$)	Consumption ($kg \cdot y^{-1}$)	Influent conc. ($\mu g \cdot L^{-1}$)
HHCB	163.5	10,370	349.4	18.6
AHTN	39.3	2,493	84.0	4.5
CBZ	19.9	1,262	42.5	2.3
DZP	0.9	57	1.9	0.1
IBP	276.1	17,511	589.9	31.3
NPX	-	-	-	-
DCF	32.3	2,049	69.0	3.7
IPM	-	-	-	-
SMX	12.7	806	27.1	1.4
ROX	0.25	16	0.5	0.03
E1	0.18	12	0.4	0.02
E2	0.12	8	0.3	0.01
EE2	0.012	0.8	0.03	0.001

In general, the values estimated were higher than the measured levels (Table 3.4), indicating that the release to the sewer may be far less than 100%. The differences may be caused by: i) overestimated use volumes, ii) incomplete release to the sewer system, or iii) loss in the sewer system (degradation, dilution, sedimentation). These calculations illustrate that predicted environmental concentrations should not be based on estimations but on measured data when available and reliable.

In the following sections, the concentrations of each group of PPCPs are discussed and compared with other values found in literature. Some observed patterns are differing from findings in other countries because of many reasons, such as specific usage profiles.

Polycyclic musk fragrances

The two polycyclic musk fragrances, Galaxolide and Tonalide, were detected in the range of 1.4-3.4 and 0.7-1.7 $\mu g \cdot L^{-1}$, respectively, which indicates not significant fluctuations in their concentrations during the year. Kupper *et al.* (2004) stated that the release of these compounds into the wastewater is relatively constant, with the private households as main source.

These values are in the range of those reported in literature (Table 1.7), between 0.8-19.2 $\mu g \cdot L^{-1}$ for Galaxolide and 0.2-12.5 $\mu g \cdot L^{-1}$ for Tonalide.

Kupper *et al.* (2004) also found lower loads in the STP influent than those estimated from the consumption which can be due to either overestimation of the production or degradation processes in the sewer.

Carbamazepine and Diazepam

During all the sampling periods, Carbamazepine was found below the limit of quantification. However, in further campaigns, Carbamazepine was detected (above LOD), but it could not be quantified (below LOQ).

In comparison with literature (Table 1.7), this result is quite strange, since Carbamazepine was widely found in STP influents and effluents, ground-, surface and drinking water, with maximum concentrations of 3.8 $\mu g \cdot L^{-1}$ (Snyder, 2002), 6.3 $\mu g \cdot L^{-1}$ (Ternes, 1998), 1.1 $\mu g \cdot L^{-1}$ (Ternes, 2001), 7.1 $\mu g \cdot L^{-1}$ (Wiegel *et al.*, 2004) and 0.03 $\mu g \cdot L^{-1}$ (Ternes, 2001), respectively.

As revealed by pharmacokinetical data (Table 1.5) only 1-2% of Carbamazepine is excreted unmetabolized. The major metabolite in humans is 10,11 epoxy-carbamazepine, which is hydrolyzed further and excreted principally as glucuronides. Additionally, Carbamazepine is inactivated by hydroxylation of the aromatic ring or N-glucuronidation at the carbamoyl moiety. These glucuronide-conjugates can presumably be cleaved either in the sewer or later in the STP, thus increasing the environmental concentrations. However, it could not be quantified in the wastewaters analysed.

Diazepam is less commonly detected in sewage as occurred in this study (below LOD). This result is concordant to the low concentration expected from the consumption. One reason may be the fact that Diazepam is mostly metabolised in the human body yielding to three main metabolites: N-desmethyldiazepam, oxazepam and temazepam.

Ternes (1998) reported maximum concentrations in STP effluents of 0.04 $\mu g \cdot L^{-1}$; Zuccato *et al.* (2000) reported levels up to 0.0012 $\mu g \cdot L^{-1}$ in surface waters; Daughton and Ternes (1999) reported groundwater concentrations ranging from 10 to 40 $\mu g \cdot L^{-1}$ and it was also found in drinking water up to 0.024 $\mu g \cdot L^{-1}$ (Zuccato *et al.,* 2000).

Anti-inflammatories

Ibuprofen was detected between 2.6 and 5.7 $\mu g \cdot L^{-1}$. These values are similar to those reported by Buser *et al.,* (1999) in Swiss STPs, but they are significantly higher than the ones indicated by Stumpf *et al.* (1999) in Brazilian STPs. The minimum and maximum concentrations reported in literature (Table 1.7) are 0.3 $\mu g \cdot L^{-1}$ (Stumpf *et al.,* 1999) and 11 $\mu g \cdot L^{-1}$ (Kanda *et al.,* 2003), respectively.

Kanda *et al.* (2003) found that concentrations of Ibuprofen were higher during the middle of the day, but it could not be checked in this work since 24 h-composite samples were used.

Taking into account that only 15% of Ibuprofen is excreted unmetabolized (Table 1.5), it is more likely to find the Ibuprofen main metabolites (hydroxyl- and carboxyl-ibuprofen) in higher concentrations. It cannot be dilucidated from this work since no metabolites were measured, but levels ranging between 1.3-6.7 $\mu g \cdot L^{-1}$ and 1.6-23.0 $\mu g \cdot L^{-1}$ have been detected for hydroxyl- and carboxyl-ibuprofen, respectively (Weigel *et al.*, 2004; Stump *et al.,* 1998).

Naproxen was detected in the range of 1.8-4.6 $\mu g \cdot L^{-1}$. These values are higher than those reported by Ternes (2001), but lower in comparison with the 8 $\mu g \cdot L^{-1}$ reported by Khan and Ongerth (2004).

Diclofenac was not detected in this study, but its occurrence in several aquatic compartments is widely described in literature (Table 1.7). The minimum and maximum concentrations found in raw waters are 0.4 $\mu g \cdot L^{-1}$ (Khan and Ongerth, 2004) and 7.1 $\mu g \cdot L^{-1}$ (Heberer *et al.,* 2002), respectively.

In this case, there are important seasonal differences between the concentrations of anti-inflammatories along the year, being the highest ones measured in January. This was also found in other studies (Heberer *et al.,* 2002) and it is probably due to the more extensive application of such drugs during winter period because the cold and humid weather causes an increase of rheumatic diseases.

Antibiotics

In the case of selected antibiotics, Sulfamethoxazole was quantified with concentrations of 0.6 $\mu g \cdot L^{-1}$, being Roxithromycin found below the LOQ.

The concentration of Sulfamethoxazole is slightly lower than the range reported in literature, from 1 $\mu g \cdot L^{-1}$ (Khan and Ongerth, 2004) to 1.75 $\mu g \cdot L^{-1}$ (Fahlenkamp *et al.,* 2004). The same authors reported levels of Roxithromycin in raw waters ranging between 0.5 and 1 $\mu g \cdot L^{-1}$.

X-ray contrast media

Iopromide was found in the range of 6-7 $\mu g \cdot L^{-1}$, similar to the values found by Ternes and Hirsch (2000), but much lower that the range reported (Table 1.7) by Steger-Hartmann *et al.* (2002).

Iodinated X-ray contrast media, applied at high amounts mostly in hospitals, but also in practical surgeries, have been identified by Gartiser *et al.* (1996) as the main contributors to the loads of total adsorbable organic halogens (AOX) in clinical wastewaters. A direct correlation between input of sewage derived from hospitals and contamination by X-ray contrast media cannot be drawn, but X-ray contrast media excreted from households appears to be at least on par with that coming from hospitals (Ternes and Hirsch, 2000).

Generally, the loads of the X-ray contrast media are significantly increased on weekdays, since X-ray examinations are performed in hospitals and radiological practices predominantly from Monday to Friday (Ternes and Hirsch, 2000). However, it can not be concluded from this study, because all the sampling campaigns were performed during the week.

Steroid estrogens

Estrogens are excreted mainly as conjugates of sulphuric and glucuronic acids. Although steroid conjugates do not possess a direct biological activity, they can act as precursor hormone reservoirs able to be converted to free steroids by

bacteria in the environment. However, steroid estrogens are mainly present in the environment in their unconjugated form, thus suggesting that deconjugation (activation) occurs within the sewage system, and/or that conjugates are more rapidly degraded. It is widely reported in literature that deconjugation occurs mainly in sewers than into STPs (Baronti *et al.,* 2000; Johnson and Sumpter, 2001; Johnson and Williams, 2004).

Johnson and Williams (2004) indicate that sulphate conjugates are the dominant on reaching the sewage treatment works with very little glucuronide conjugates left since they are cleavage either before excretion or in the sewer transit. These sulphate conjugates are more persistent, being even able to survive sewage treatment.

In this study, only the unconjugated form was measured. Higher concentrations of E1 (2.4 $ng \cdot L^{-1}$) than E2 (1.6 $ng \cdot L^{-1}$) were detected, which is related to the fact that humans excrete more E1 than E2 in their urine (Johnson *et al.,* 2000). Although strong daily variations of natural estrogens in the influent of STPs are widely reported in literature (Andersen *et al.*, 2003; Joss *et al.,* 2004), the values detected in this work are very low compared to the range reported in literature (Table 1.7), 9.6-670 $ng \cdot L^{-1}$ (Cargouet *et al.,* 2004; Clara *et al.,* 2005) and 3-125 $ng \cdot L^{-1}$ (Joss *et al.,* 2004; Clara *et al.,* 2005) for E1 and E2, respectively.

From the consumption data and considering that the prodrug mestranol is converted after administering into EE2 by demethylation (Ternes *et al.,* 1999b), this synthetic hormone should have been detected in the raw waters. However, it was found below the LOQ.

In literature, the concentrations of EE2 are lower than those of the natural estrogens, ranging from 0.4 $ng \cdot L^{-1}$ (Baronti *et al.,* 2000) to 70 $ng \cdot L^{-1}$ (Clara *et al.,* 2005).

3.3.3. Removal during primary treatment

Table 3.6 shows the concentration profiles of the PPCPs detected along the different units of the STP for each sampling campaign and the average values of the 4 campaigns with the standard deviation are represented in Figure 3.2.

An increase in the concentrations at the inlet of primary and biological treatment compared to the raw influent of the STP was observed for some compounds, which suggests either contribution of the supernatants from the sludge treatment processes or cleavage of glucuronides during the first steps of

the treatment. The analytical deviation of the methodology used must be also taken into account.

Table 3.6. Profiles of PPCPs detected along the different units of the STP during the four sampling campaigns (μg·L^{-1}, except estrogens in ng·L^{-1}).

Sampling campaign	Sample point*	HHCB	AHTN	IBP	NPX	SMX	IPM	E1	E2
October 2001	So	2.10	0.90	2.75	3.45	-	-	-	-
	Sps	4.40	1.50	2.83	3.75	-	-	-	-
	Sb	1.40	0.60	2.84	3.48	-	-	-	-
	Sss	45.40	3.25	0.20	1.40	-	-	-	-
	Sf	0.60	0.20	0.91	1.85	-	-	-	-
January 2002	So	3.40	1.69	5.70	4.60	-	-	-	-
	Sps	3.10	1.63	5.80	4.10	-	-	-	-
	Sb	1.60	0.97	5.80	4.80	-	-	-	-
	Sss	28.70	14.78	0.60	2.10	-	-	-	-
	Sf	0.50	0.15	2.10	2.60	-	-	-	-
April 2002	So	3.18	1.53	2.64	1.79	0.58	6.60	2.40	1.60
	Sps	2.30	1.14	2.81	1.78	0.47	7.50	2.40	3.00
	Sb	1.82	0.94	2.95	1.59	0.64	7.20	3.40	2.40
	Sss	17.72	7.82	0.52	0.65	0.25	8.80	-	<1**
	Sf	0.49	0.16	0.97	0.80	0.25	9.30	4.40	<1**
June/July 2002	So	1.35	0.67	2.75	2.18	-	-	-	-
	Sps	0.94	0.53	2.92	2.27	-	-	-	-
	Sb	1.29	0.69	1.02	0.83	-	-	-	-
	Sss	17.72	7.82	0.23	0.13	-	-	-	-
	Sf	0.46	0.15	0.44	0.16	-	-	-	-

*See Figure 3.1 for sampling points. **Limit of quantification (LOQ).

Typically, sorption and settling of solids play a major role in the removal of chemicals from primary treatment, although some degradation can also occur. Therefore, only those substances with higher sorption coefficients (Table 1.6) are expected to be eliminated during primary treatment. Moreover, sorption on solids also depends on solids concentration, thus being expected more elimination at higher solids loads.

In the following sections, the fate of each group of compounds is discussed and compared with literature values. For the calculation of the average removal efficiencies, the data from the last campaign (June/July 2002) were not included

since the results obtained were different from the previous ones probably due to matrix problems in the chemical analysis.

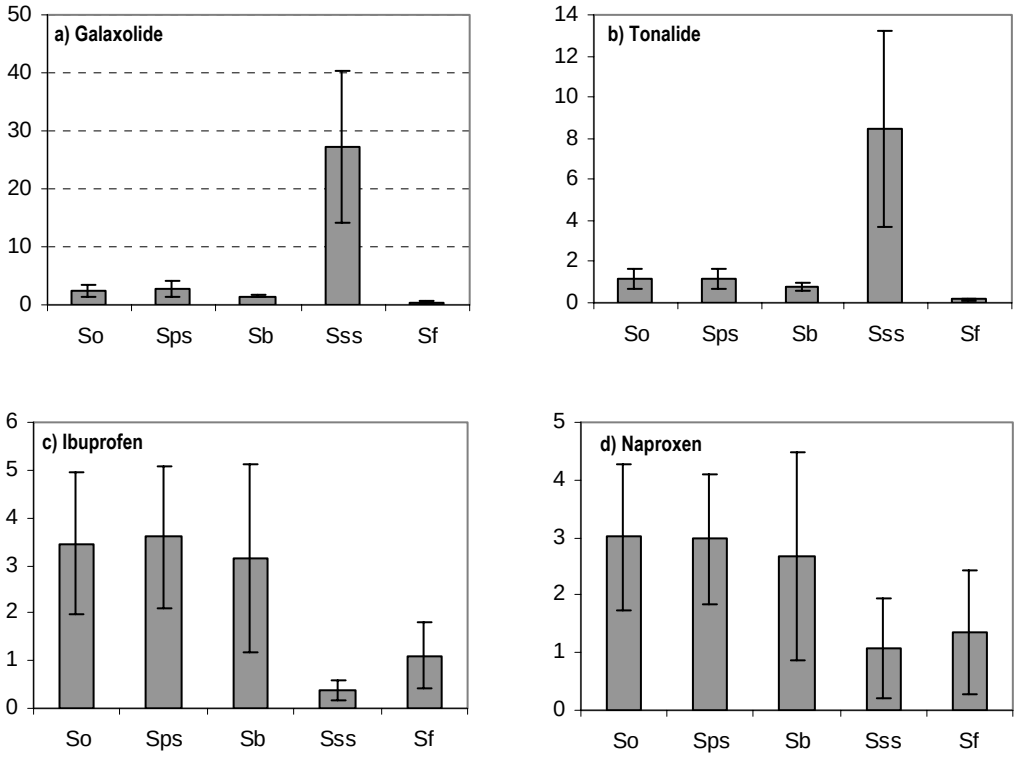

Figure 3.2. Concentration profiles ($\mu g \cdot L^{-1}$) of PPCPs detected along the different units of the STP. See Figure 3.1 for sampling points.

Polycyclic musk fragrances

Polycyclic musk fragrances were well removed in the primary settler, around 43% of Galaxolide and 38% of Tonalide (Fig. 3.2a and 3.2b). These efficiencies are closely related to suspended solids elimination (60-65%), which indicates that sorption onto solid particles is the key mechanism involved. In fact, among all the substances considered in this work, musks possess the highest distribution coefficients between the solid and liquid phase.

Simonich *et al.* (2002) reported removal efficiencies of HHCB and AHTN during primary treatment in STPs of United States and Europe of 29.9 and 28.9%, respectively.

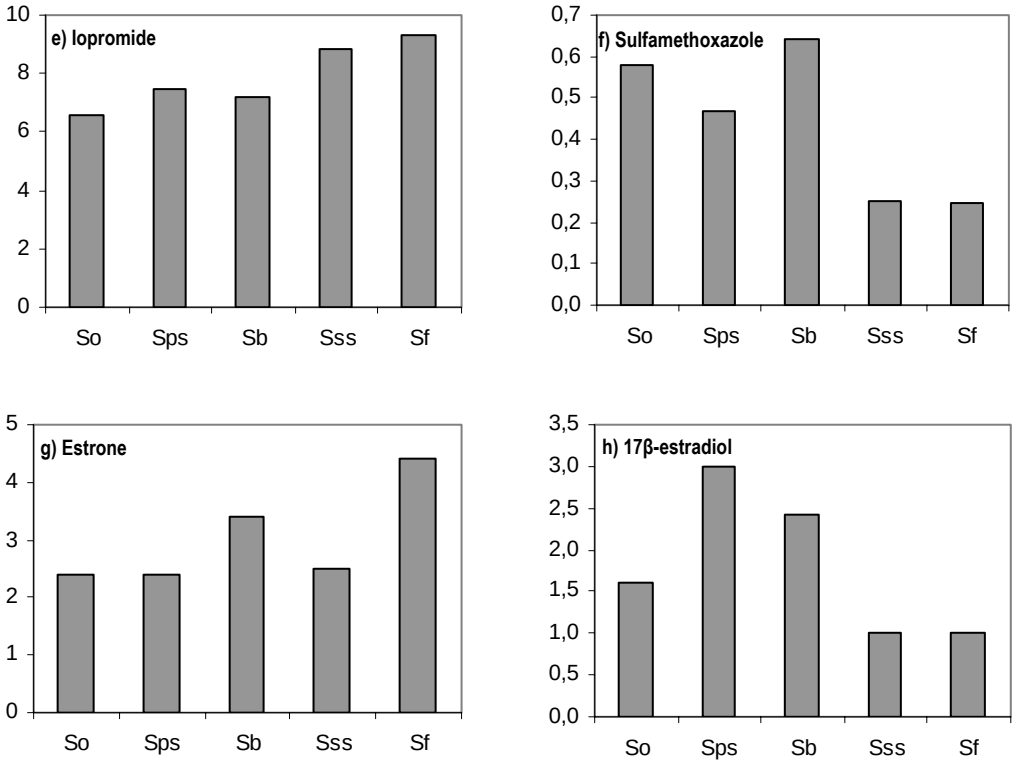

Figure 3.2. Concentration profiles ($\mu g \cdot L^{-1}$, except estrogens in $ng \cdot L^{-1}$) of PPCPs detected along the different units of the STP. *Cont.*

Steroid estrogens

The concentrations of E1 and E2 increased during primary treatment. As already stated, this fact can be explained by cleavage of conjugated steroid estrogens in the first steps of the STP. This assumption is consistent with the findings of Adler *et al.* (2001), which show that on average 58% of total E1, 50% of total E2 and 26% of total EE2 were conjugated in raw sewage from different parts of Germany. Matsui *et al.* (2000), probably the first to quantify a complete concentration profile through the treatment process using an immunoassay for measuring E2, observed an increase in immunoreactivity toward the end of the primary treatment, which may be caused by conjugated estrogens being cleaved to free estrogens.

In the case of E1 (Figure 3.2g), the increase occurred after primary settling; however, for E2 (Fig. 3.2h), it happened in the first unit of the plant (grit and fat separator), to be afterwards slightly reduced (around 20%) in the primary settler.

Other PPCPs

No significant reduction was observed for anti-inflammatories (Figure 3.2c and 3.2d), Sulfamethoxazole (Figure 3.2f) and Iopromide (Figure 3.2e) during the pre-treatment and sedimentation steps. This is concordant with their hidrophilic natures, with very low solid-liquid distribution coefficients, which leads to them being mainly present in the liquid phase.

3.3.4. Removal during biological treatment

All the PPCPs detected, except Iopromide, were removed during biological treatment with efficiencies between 30% and 75%. In general, for those substances eliminated during primary treatment, an additional reduction was achieved in the biological process.

In this case, it can not be distinguish between the two main removal mechanisms: sorption and biodegradation. While, for the most lipophilic substances, the elimination may be governed by sorption; biodegradation is expected to be the key mechanism for the polar compounds.

Although not all substances are better degraded at higher SRT, in general, the biological degradation of micropollutants is improved with increasing SRT (Kreuzinger et al., 2004; Clara et al., 2004b). This is also valid for substances being degraded in co-metabolism as a co-substrate, because in this case the SRT necessary for the degradation of the primary substrate is the relevant parameter.

Polycyclic musk fragrances

Galaxolide and Tonalide are eliminated during biological treatment with removal efficiencies around 30-40% and 45-50%, respectively. It is important to stand out the high concentrations of these compounds in the samples with high solids content, such as the outlet of the biological reactor (point Sss, Table 3.6, Figure 3.2a and 3.2b). When these samples were filtered, the soluble concentrations were extremely low (around 1.0 $\mu g \cdot L^{-1}$ and 0.5 $\mu g \cdot L^{-1}$ for Galaxolide and Tonalide, respectively). This fact clear indicates the high ability of these substances to bind to solid particles.

Artola-Garicano *et al.* (2003b) assumed that only the chemical that is freely dissolved in the aqueous phase is available for degradation. This implies that the chemical adsorbed to solids needs to desorb in order to be degradable. The same author (Artola-Garicano *et al*., 2003c) stated that low microbial degradation rate constants together with substantial binding of the substrates to the solids in the aeration tank lead to low biodegradation rates. Moreover, the common practice of partly recycling the waste sludge does not contribute directly to increase the elimination of a chemical if it is both hydrophobic and hardly degradable. Nevertheless, sludge recycling increases indirectly the chances of adaptation of the microorganisms to these chemicals.

The removal efficiencies reported in literature (Table 1.8) for musks vary strongly, from 2% (Kreuzinger *et al.,* 2004) to 99.9% (Simonich *et al.,* 2002), being the main influencing factors the technology employed and the SRT. For example, Kanda *et al.* (2003) reported that the removal of musks is higher at plants using activated sludge treatment or an oxidation ditch compared to biological filters or reed beds. It is probably related with an increase in the retention time (filter bed < non nitrifying AS < nitrifying AS < oxidation ditch).

Anti-inflammatories

Both anti-inflammatories detected were significantly reduced during the biological treatment (Table 3.6), with removal efficiencies of 60-70% for Ibuprofen (Figure 3.2c) and 40-55% for Naproxen (Figure 3.2d).

As previously described, Ibuprofen is mainly excreted as two metabolites: hydroxyl (IBP-OH) and carboxyl (IBP-CX). It is widely reported in literature (Weigel *et al,* 2004; Ternes, 2001; Buser *et al.,* 1999) that while IBP and IBP-CX were almost quantitatively eliminated (>95%) during biological treatment, IBP-OH was hardly affected (less than 20%) and thus is the dominant compound in STP effluents and rivers (average values of 0.34 $\mu g \cdot L^{-1}$ were reported by Ternes, 2001). It could not be confirmed in this study, since only the parent compound was measured.

In literature (Table 1.8), the removal efficiencies of Ibuprofen range from 0% (Kreuzinger *et al.,* 2004) to 99.9% (Buser *et al.,* 1999), depending on the type of treatment used and the SRT of the plant. Similarly to fragrances, Kanda *et al.* (2003) reported that the removal of Ibuprofen is higher at plants using activated

sludge treatment or an oxidation ditch compared to biological filters or reed beds; again related to the SRT.

Concerning Naproxen, the efficiencies obtained in this work are in the upper limit of the range reported in literature (Table 1.8), from 15% (Stumpf *et al.*, 1999) to 93% (Paxeus, 2004).

Antibiotics

Sulfamethoxazole was removed, around 67%, during the biological step. This is an intermediate value compared to the range reported in literature, 33-91% (Kreuzinger *et al.*, 2004).

X-ray contrast media

Iopromide is the only compound detected which was not removed during biological treatment. Due to the high hidrophilicity of the substituted benzene derivated, it passes unaltered waste water treatment plants and thus, it is expected to be found in rivers, lakes and raw drinking water (Putschew *et al.*, 2000).

Similar results were obtained by Ternes and Hirsch (2000); however, Kreuzinger *et al.* (2004) reported removal efficiencies up to 50% in different Austrian STPs.

Steroid estrogens

17β-estradiol was removed during the biological treatment (47%), resulting in concentrations below the LOQ in both the effluent of this unit and in the final effluent of the plant. In contrast, Estrone concentrations were higher after the biological step, illustrating the fact that under oxidizing conditions, 17β-estradiol is quickly converted into Estrone, which is much more slowly degraded (Ternes *et al.*, 1999b). Taking into account the initial concentration of the 17β-estradiol (3 $ng \cdot L^{-1}$) and the limit of quantification (LOQ) of 1 $ng \cdot L^{-1}$, it can be assumed that at least 2 $ng \cdot L^{-1}$ were removed, which agrees with the increase in the Estrone concentration.

Andersen *et al.* (2003) found that more nonconjugated E1 and E2 were discharge from the denitrification tank than the maximum quantity which had entered it (approximately the double), while the input and output of EE2 were approximately equal. This fact indicates that cleavage of conjugates still takes place in the biological units. It could be another reason to explain the higher

concentrations of E1 in the final effluent. In the same study, high removal of natural estrogens (>98%) and EE2 (90%) is reported, which occurs mainly under denitrifying conditions and in the aerobic compartment, respectively. EE2 elimination was only found in STPs operated at higher SRT (11-13 d); high loaded plants (only BOD removal) did not exhibit more than minor EE2 reduction. But it could not be confirmed in this study since EE2 was found below the LOQ.

These compounds possess quite high sorption coefficients, which mean that sorption can play an important role. Holbrook *et al.* (2004) reported a substantial sorption (up to 60%) of E2 and EE2 onto colloidal material from the activated sludge system, being the amount associated to solids dependant on the organic carbon content. Microbial degradation may also influence the distribution of substances between the colloidal and dissolved phases.

3.3.5. Concentrations in STP effluents

While the concentration in the influent is primarily function of population pattern use (volume use and per capita water use), the concentration in the final effluent is a function of pattern use as well as of plant design and operation. Therefore, although influent concentrations may vary within a wide range (up to a factor of 2) due to daily fluctuations and specific use profiles, the effluent concentrations are quite constant, due to the high hydraulic retention time.

From effluent concentrations, the levels in the surface waters where the final effluent is discharged can be calculated following the recommendation from the Food and Drug Administration in the United States of a 10-fold dilution factor.

Clara *et al.* (2005) indicates that effluent concentrations of PPCPs are not related to the influent but depend only on the operated SRT, whereas the treatment efficiencies depend on inflow concentrations. In literature, it is stated that facilities employing longer sludge retention times during treatment (nitrifying and denitrifying plants) show significant lower effluent concentrations for some PPCPs as compared to trickling filter or activated sludge facilities applying shorter times. Drewes *et al.* (2002) reported that concentrations of anti-inflammatory drugs varied three orders of magnitude between samples of not nitrified (HRT<10 h) and samples of nitrified/denitrified effluents (HRT>10 h). However, other compounds, such as Carbamazepine, showed no dependency on the treatment applied.

Table 3.7 shows the average concentrations and loads of detected substances in the influent and effluent of the STP investigated as well as the overall removal.

Table 3.7. Average concentrations ($\mu g \cdot L^{-1}$) and loads ($g \cdot d^{-1}$) of detected PPCPs in the influent and effluent of the STP investigated as well as the overall removal in the plant (%).

Substance	Mean conc. Influent	Mean conc. Effluent	Load influent	Load effluent	Removal
Galaxolide	2.51	0.51	129.4	26.3	79.7
Tonalide	1.20	0.17	61.9	8.8	85.8
Ibuprofen	3.46	1.11	178.4	57.2	67.9
Naproxen	3.01	1.35	155.2	69.6	55.2
Sulfamethoxazole	0.58	0.25	29.9	12.9	56.9
Iopromide	6.60	9.30	340.3	479.5	No removal
Estrone	0.0024	0.0044	0.124	0.227	-83.1
17β-estradiol	0.0016	0.0010	0.083	0.052	37.3

Polycyclic musk fragrances

The concentrations of Galaxolide and Tonalide in the final effluent range from 0.46 and 0.15 $\mu g \cdot L^{-1}$ to 0.60 and 0.20 $\mu g \cdot L^{-1}$, respectively. These values are in the lower limit reported in literature: 0.030-13.3 $\mu g \cdot L^{-1}$ for Galaxolide (Ricking *et al.,* 2003; Fromme *et al.,* 2001) and 0.02-6.8 $\mu g \cdot L^{-1}$ for Tonalide (Osemwengie and Gestenberger, 2004; Simonich *et al.,* 2002).

The amounts of musks discharged into the environment are relatively low compared to the amounts used (Tables 1.4 and 1.5). Buerge *et al.* (2003) estimated that between 6 and 10% of the amount used is discharged from STPs. Hence, a significant proportion has been lost during use (volatilisation), the major fraction ends up in sewage sludge and these compounds are also degraded to some extent.

Anti-inflammatories

Ibuprofen and Naproxen levels in the final effluent range from 0.44 and 0.16 $\mu g \cdot L^{-1}$ to 2.10 and 2.60 $\mu g \cdot L^{-1}$, respectively. These values are in the lower limit reported in literature: 0.002-85 $\mu g \cdot L^{-1}$ for Ibuprofen (Buser *et al.,* 1999; Farré *et al.,* 2001) and 0.02-6.28 $\mu g \cdot L^{-1}$ for Naproxen (Metcalfe *et al.*, 2003; Drewes *et al.,* 2002).

In the case of IBP, Paxeus (2004) stated that whereas IBP and IBP-CX are the dominating species in raw sewage, in treated wastewaters, IBP-OH is more prominent.

Antibiotics

The concentration of Sulfamethoxazole in the final effluent is 0.25 $\mu g \cdot L^{-1}$. This value is the lower limit reported in literature, from 0.24 $\mu g \cdot L^{-1}$ (Miao *et al.,* 2004; Fromme *et al.,* 2001) to 2.0 $\mu g \cdot L^{-1}$ (Hirsch *et al.,* 1999).

X-ray contrast media

The concentration of Iopromide in the final effluent is 9.30 $\mu g \cdot L^{-1}$. This value is in the upper limit reported in literature, from 0.8 $\mu g \cdot L^{-1}$ to 11.0 $\mu g \cdot L^{-1}$ (Ternes, 2001).

Steroid estrogens

The concentrations of Estrone and 17β-estradiol in the final effluent are 4.4 and below 1 $ng \cdot L^{-1}$, respectively. These values are in the lower limit reported in literature: 1-180 $ng \cdot L^{-1}$ for Estrone (Clara *et al.,* 2005; Komori *et al.,* 2004) and 0.3-60 $ng \cdot L^{-1}$ for 17β-estradiol (Johnson and Willians, 2004; Ternes *et al.,* 1999a).

The predominant presence of Estrone in STPs effluents is due to three factors: a) its high stability during treatment, b) the cleavage of glucuronide conjugates, and c) the oxidation of E2 to E1. E1 is excreted in urine preferentially as sulphate instead of glucuronide (Baronti *et al.,* 2000). Under this hypothesis, remarkable amounts of E1 enter STPs still as sulphate conjugated, being the liberation of E1 done by various bacterial strains in the activated sludge. This could explain the low removal rates of E1 in STP. Besides, arylsulfatase enzyme is likely to be less common, explaining the persistence of E1 (Johnson and Sumpter, 2001).

Although, it can not be confirmed in this work, since no conjugates were measured, Komori *et al.* (2004), opposite to the findings of other studies, reported higher concentrations (even higher that the free compound) in both influent (up to 3.6 $\mu g \cdot L^{-1}$) and effluent (up to 1.8 $\mu g \cdot L^{-1}$), which indicates that conjugated estrogens still remain at high concentrations after STP treatment.

3.3.6. Overall removal in the STP

Since volatilisation should play a minor role in the elimination of these substances in STPs (Struijs *et al,* 1991), the two mechanisms involved in the elimination of these substances are sorption and biodegradation. In general, overall removal of biodegradable-nonsorptive substances is positively correlated with plant BOD removal and overall removal of nonbiodegradable-sorptive compounds is positively correlated with plant TSS removal. This is consistent because BOD removal is dependent on the efficiency of biodegradation in the plant and TSS removal is dependent on the efficiency of solids settling in the plant.

Removal efficiencies are negatively affected by disturbances in the activated sludge process (Paxeus, 2004), but positively influenced by higher sludge age (Kreuzinger *et al.,* 2004). Moreover, the treatment efficiencies depend mainly on inflow concentrations (Clara *et al.,* 2005). This assumption is also supported by Blok (2001), who showed that there is no reason to expect the same removal efficiency in STPs when influent concentrations of chemicals are different. Higher concentrations of certain chemicals will favour the growth of certain microbes leading to higher biodegradation rates for these chemicals. Adaptation does simply lead to similar effluent concentrations, which are in accordance with the current study and others in literature (Artola-Garicano *et al.,* 2003c; Simonich *et al.,* 2002).

Figures 3.3 and 3.4 show the average removal efficiencies during primary and biological treatment as well as the overall removal in the plant.

All the PPCPs detected, except Iopromide, are removed in the STP with efficiencies between 40 and 90%.

Polycyclic musk fragrances

The overall removal efficiencies in the STP were 70-85% for Galaxolide and 75-90% for Tonalide.

In contrast to the other PPCPs considered in this work, the total concentration of musk fragrances has been additionally determined by SPME. Comparing the dissolved and the total values, it was observed that whereas the dissolved concentrations remain virtually constant throughout all compartments, the total concentrations are highly dependant on the VSS content in the individual unit. This result was also observed by Artola-Garicano *et al.* (2003a).

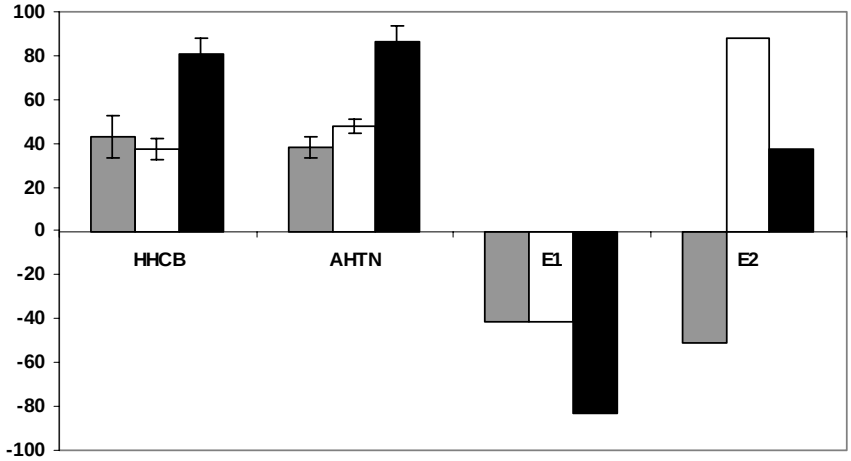

Figure 3.3. Removal efficiencies (%) of fragrances and estrogens during primary (■), biological (□) and overall treatment (■).

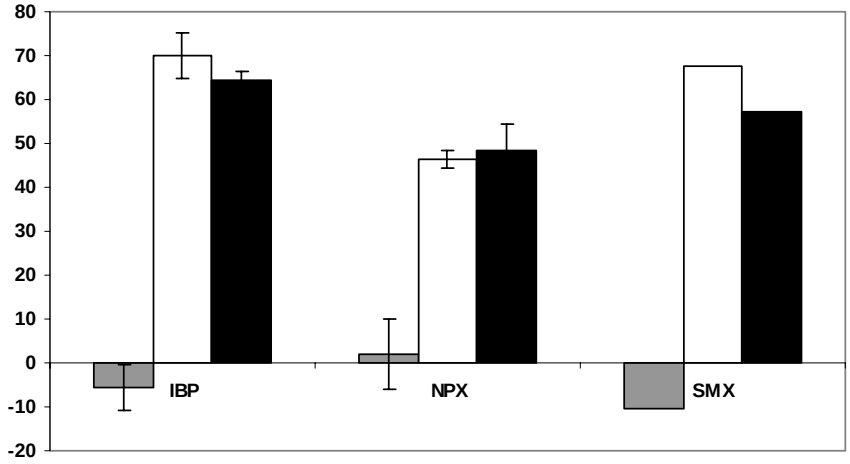

Figure 3.4. Removal efficiencies (%) of anti-inflammatories and Sulfamethoxazole during primary (■), biological (□) and overall treatment (■).

They concluded from their investigations that the free concentrations of AHTN and HHCB in the different compartments of STPs are mostly mediated by biological degradation, whereas the total concentrations are mediated by the content of solids. Thus, higher removal efficiencies may be caused by better solids elimination in the plant. Besides, low biodegradation or volatilization rates

would lead to a small decrease of the free concentrations, and this decrease would be compensated by desorption from the solids to maintain steady state. Substantial biodegradation would decrease the total concentrations of these substances and, accordingly, would reduce the free concentration.

Therefore, for compounds with high solid-liquid distribution coefficient, results from unfiltered samples can give a more complete information about the real presence of these substances in the environment (Jensen and Schäfer, 2001; Kolpin *et al.*, 2002). These works have concluded that not only does the existence of a high solid-liquid phase distribution coefficient have to be considered, but that also so do other factors, such as the relative concentration PPCP-particulates. Therefore, for these substances, only the freely dissolved concentration is available for passive uptake into organisms (microbial degradation).

According to Simonich *et al.* (2002), HHCB and AHTN are assumed not to degrade but to adsorb mainly to sludge during wastewater treatment. They reported removal ranging from 87.8 to 88.8% for activated sludge plants, 50.6-83.4% for carousel plants, 86.1-93.0% for oxidation ditch plants, 78.1-81.0% for trickling filter plants, 80.8-81.7% for a rotating biological contactor plant and 98.7-99.9% for lagoons. The simplest form of treatment, lagoon, resulted in the most effective for musks removal. This is likely due to the long retention times (90-120 d), with sufficient time for biodegradation, photodegradation, sorption and settling and/or volatilization from the lagoon.

In the case of Galaxolide, an oxidation product (Galaxolidone or HHCB-lactone, produced from Galaxolide under aerobic conditions) has been detected not only in rivers (Franke *et al.,* 1999), but also in laboratory experiments (Itrich *et al.,* 1998) in samples of activated sewage sludge, indicating somehow that the degradation of Galaxolide in STPs would yield to this metabolite, although it could not be confirmed so far. The question whether this compound is originated from metabolic processes in fish or river sediments or from sewage treatment processes is answered by Bester (2004), who reported that HHCB is transformed (7%) to HHCB-lactone during the sewage treatment process. He also detected HHCB-lactone in the influent of a German STP, which is explained since this metabolite is sometimes included in the technical Galaxolide product (about 10% of Galaxolide). He observed overall removals from the liquid phase for HHCB and AHTN of 60 and 80%, respectively, pointing to sorption onto sludge as the main responsible process.

Carbamazepine and Diazepam

In general, poor removal efficiencies (7-20%) are reported in literature for Carbamazepine (Ternes, 1998; Heberer, 2002b; Stamatelatou *et al.,* 2003; Clara *et al.,* 2004a). However, other authors reported higher values, up to 53% (Paxeus, 2004) or to 85% (Snyder, 2002), which indicates the great variability in literature data (Table 1.8). It could not be checked in this study because Carbamazepine was found below LOQ in the raw wastewaters.

For Diazepam, the data available are really scarce. Only, Kreuzinger *et al.* (2004) reported elimination up to 25% of this compound.

Anti-inflammatories

In the case of the anti-inflammatories detected, significant overall removal efficiencies were achieved for both compounds: 60-70% for Ibuprofen and 40-55% for Naproxen. As previously stated, this reduction only took place during the biological treatment.

Stumpf *et al.* (1999) reported similar elimination of IBP (75%) in conventional activated sludge system, but slightly higher for NPX (78%). In this study, higher removal of these substances was obtained in the conventional activated sludge system as compared to the biological filter.

In general, the removal efficiencies obtained in this study are in the same range as those indicated in literature (Table 1.8).

Antibiotics

The only antibiotic detected, Sulfamethoxazole, is overall removed around 57%, mainly during the biological step. This value is in the range of those reported by Kreuzinger *et al.* (2004), from 33 to 91%.

In the elimination of polar antibiotics, sorption also plays an important role, since a large part of elimination is achieved by absorption on activated sludge which is partly mediated through hydrophobic interactions (Hirsch *et al.*, 1999).

X-ray contrast media

The results obtained for the contrast medium considered, Iopromide, indicate that there is no significant removal of this compound along the plant.

Ternes and Hirsch (2000) also reported that Iopromide is neither sorbed nor degraded in appreciable amounts when passing through the STP close to

Frankfurt/Mainz. It can be due to the fact that X-ray contrast media are designed to exhibit extremely high chemical and biological stability to maintain their efficiency within the X-ray examination and to prevent undesired toxicological effects caused by degradation products, thus they are not readily biodegradable.

Steroid estrogens

While E2 is removed around 67% (20 and 47% in primary and biological treatment, respectively), E1 is produced along the STP (-80%). This fact was already explained, being the main responsible factors the cleavage of conjugates and the oxidation of E2.

In comparison with literature, the elimination of E2 found in this work falls in the lower range reported, from 43% (Cargouet *et al.*, 2004) to 99% (Johnson and Williams, 2004; Joss *et al.,* 2004). Concerning E1, the elimination of this compound occurs when higher SRT are applied. Johnson *et al.* (2005) reported higher elimination of E1 when applying longer HRT and SRT in the plant and longer HRT in the biological part, while no influence of temperature was observed. It can be due to the higher biomass concentration in the activated sludge tank, i.e. more bacteria available for degradation. Besides, perhaps some of the slower growing microorganisms associated with nitrifying sludge (long SRT) have a greater capacity to remove steroid estrogens as has been shown for 17α-ethinylestradiol (Vader *et al.,* 2000).

Due to their physico-chemical properties (Table 1.6), steroid estrogens should be adsorbed onto sludge. However, Andersen *et al*. (2003) carried out a mass balance of estrogens in a German municipal sewage treatment plant and they concluded that only 5% of the estrogens are sorbed onto digested sewage sludge. They also stated that E1 and E2 show slow sorption kinetics and no equilibrium between the sorbed and dissolved estrogens is established.

Concerning the synthetic hormone EE2, although it can not be dilucidated from this study, its relatively high levels in the effluent may indicate that a significant portion of this substance is passing through the sewage system undegraded. The removal efficiencies reported in literature vary strongly, from no reduction (Ternes, 2001; Larsson *et al.,* 1999) to 90% (Baronti *et al.,* 2000; Andersen *et al.,* 2003; Joss *et al.,* 2004). Therefore, EE2 seems to be more resistant to biodegradation in STPs and thus accounting for 35-50% of the estimated estrogenic activity in rivers (Cargouët *et al,* 2004). Moreover, the ratio

between EE2 and natural estrogens in the water is higher than the theoretical ratio based on human excretion rates, indicating a faster degradation of the natural estrogens, which is supported by the reported efficiencies in literature (Table 1.8).

3.4. Conclusions

A group of 13 PPCPs corresponding to different kinds of substances (musks, pharmaceuticals and hormones) has been used as an indicator of the presence of this type of pollution in the municipal wastewaters generated by a city of around 100,000 inhabitants in Galicia (NW Spain). The occurrence of 8 out of 13 substances considered has been detected up to $\mu g \cdot L^{-1}$-level in both influent and effluent of the STP. Increase in the concentrations in the influent of primary treatment than influent of the STP was observed for some compounds, which suggests either contribution of supernatant from sludge treatment processes or cleavage of glucuronide forms.

Most substances detected were not eliminated completely in the STP and thus, being discharged as contaminants into the receiving waters. The final fate in the environment strongly depends on the environmental conditions (temperature, salinity, pH, biological activity). For instance, while winter temperatures may reduce biodegradation rates, the large increase in dilution due to winter rains may ensure that PPCPs concentrations would remain below the no effect level.

A higher elimination of the substances was achieved in the secondary treatment than in primary treatment, although the degree of reduction depends on each single substance. It is not known yet as to how much of the removal was due to adsorption onto sludge or was a result of degradation within the STP.

HHCB and AHTN have been identified as the two most important synthetic musk compounds due to their use volumes and their detection frequencies and concentrations in environmental samples. They were removed in both primary and secondary treatment mainly due to sorption processes, leading to overall efficiencies up to 90%. They are considered potential environmental pollutants due to their low rates of biological and chemical degradation and their high lipophilicity, which leads to most part of them being discharged associated to the sludge.

Both anti-inflammatories were very well reduced during biological treatment with efficiencies ranging between 40 and 65%. Due to their hydrophilic nature, this elimination is mainly due to biological degradation. It is reported in literature

(Zwiener *et al.,* 2000; Zwiener *et al.,* 2002) that the degradation of Ibuprofen leads to two main metabolites, hydroxyl-Ibuprofen and carboxy-Ibuprofen, but more work is required to study their occurrence and fate under different operating conditions.

The antibiotic detected, Sulfamethoxazole, is also noticeable removed (around 60%) during biological treatment, being its reduction once again mainly due to biological degradation. Its concentration in the final effluent did not exceed 1 $\mu g \cdot L^{-1}$, level that is unlikely to affect the growth and survival of aquatic organisms (Miao *et al.,* 2004).

Great persistence of Iopromide is reported in literature (Ternes and Hirsch, 2000), as it was observed in this work. No elimination was obtained for this substance along the STP. Therefore, this compound is expected to be ubiquitously distributed in the aquatic environment because it is used in large amounts, more than 99% of the dosage is excreted in nonmetabolised form and it is highly persistent under STP treatment and, probably, under environmental conditions.

For the natural estrogens, a significant removal was achieved for E2 (around 65%), whereas the concentrations of E1 increased along the treatment. This fact was already explained by the cleavage of glucuronides and the oxidation of E2. Therefore, considering the impact on estrogenicity, any current assessment would highlight E1, considering its concentration, relative persistence in treatment and potency. In spite of its greater estrogenicity potency (Johnson and Sumpter, 2001), the impact of E2 should be much lower due to the higher removal obtained in STPs. EE2 would also be highlighted for its even greater persistence in treatment and potency, although its concentration is often too low.

Despite their extensive application in human medicine, around 20 $ton \cdot y^{-1}$ in our country (Table 1.4), Carbamazepine could not be detected in the raw wastewaters. Great persistence of this substance is widely reported in literature (Andreozzi *et al.,* 2002; Clara *et al.,* 2004a; Strenn *et al.,* 2004) since it is neither subjected to degradation nor to adsorption processes during wastewater treatment, as well as the increase caused by glucuronides cleavage. These characteristics qualify Carbamazepine as a suitable marker for anthropogenic influences on the aquatic environment (Clara *et al.,* 2004a).

Diazepam and Roxithromycin were also not detected in the raw wastewaters in this study. In contrast to Carbamazepine, these compounds are used in fewer amounts (Table 1.4), approximately 900 and 250 $kg \cdot y^{-1}$, respectively, which

would yield to estimated influent concentrations of about 150 $ng \cdot L^{-1}$ for Diazepam and 40 $ng \cdot L^{-1}$ for Roxithromycin.

The synthetic hormone, EE2, despite being used in quite important amounts (around 12 $kg \cdot y^{-1}$, which would lead to 1 $ng \cdot L^{-1}$ in the influent), it could not be detected in the raw wastewaters. This fact can be due to either an overestimation of the consumption or to some problems in the analytical determination.

In general, high loaded activated sludge plants with a SRT around 1 d show low removal of selected micropollutants (Kreuzinger *et al.,* 2004). For these systems, adsorption to the activated sludge is the most important way of removal from the liquid phase. But when the hydraulic retention time is lower than the time needed for adjustment of the adsorption equilibrium the maximal possible adsorption may not be reached. In contrast, low loaded STPs with a SRT higher than 10 d yield to higher reduction of the emissions. Therefore, from the point of protecting the aquatic environment and process stability at STPs, tertiary treatment with at least nitrification is recommend for all wastewater treatment plants. Further reduction of the sludge loading rate for denitrification or aerobic sludge stabilisation does not lead to a significant additional increase of the removal rate, but can be beneficial for recovery of aeration energy invested for nitrification.

Thus, if removing PPCPs from wastewaters became a requirement, sewage treatment tanks across Europe could be doubled or trebled in size in order to permit an extensive biodegradation of these compounds. However, in many cases this is likely to prove impractical given the limited land available for many urban STP sites and then, another measures may need to be considered, such as the incorporation of tertiary treatments (ultrafiltration, ozonation, UV treatment, etc), since they have been shown effective at removing some PPCPs, or a modification of sludge treatment as well as the possibility of source control. However, much research is needed to determine the cost-effectiveness of these processes.

Finally, a hazard-assessment is needed to determine the toxicological relevance of exposure to trace quantities of pharmaceuticals and endocrine disruptors. Even though the sanitary risk is limited in the case of drinking water plant equipped with technological processes used to eliminate other micropollutants like pesticides, the precautionary principles require that drinking water should be free of such anthropogenic contaminants.

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Chapter 4

Fate of PPCPs in a Sewage Treatment Plant: mass balance calculations[1]

Summary

The fate of two musks (Galaxolide and Tonalide), 4 pharmaceuticals (Ibuprofen, Naproxen, Sulfamethoxazole and Iopromide) and the two natural estrogens (Estrone and 17β-estradiol) has been investigated along the different water and sludge treatment units in a Sewage Treatment Plant (STP). Measurements of these substances have been carried out both in water and sludge phase in order to perform the mass balances through the different units of the plant.

In this chapter, two different methods for performing the mass balance calculations are presented. The first method uses the measured data in both liquid and sludge phase and the second one uses the solid-water distribution coefficient (K_d) to calculate the concentrations in the solid phase from those measured in the liquid phase. Both methods are compared in order to evaluate the suitability of the second one since the concentrations in the sludge phase are not always available.

The main outcome of this study is the mechanism involved in the PPCPs elimination in STPs. In that way, Ibuprofen, Naproxen and Sulfamethoxazole are biologically degraded in the aeration tank, while musks are mainly sorbed onto the sludge. For pharmaceuticals, about 40% of the initial load passes through the plant unaltered and it is discharged into the river, being the amount associated to sludge lower than 0.5%. In contrast, between 20 and 40% of the initial load of musks leaves the plant sorbed to sludge, being less than 10% present in the final effluent.

[1]**Carballa, M., Omil, F. and Lema, J.M.** (2006). Mass balance of Pharmaceutical and Personal Care Products (PPCPs) in a Sewage Treatment Plant. *Environ. Sci. Technol.*,(submitted).

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4.1. Introduction

The dramatic increase in the production and emission of synthetic organic chemicals for industrial and domestic use has forced Sewage Treatment Plants (STPs) to improve their efficiency. Since the sources of this kind of pollution are difficult to be eliminated, specific treatment processes in STPs must be optimized, because the profile of these compounds in the final effluent is dependant on the design and operation of the STP (Simonich *et al.,* 2002).

To date, it is known that municipal STPs are able to partially remove some PPCPs, but so far it is unknown if the resulting levels in the discharges are low enough to avoid adverse effects in the receiving environment. In general, it should be taken into account that if no sorption or degradation occurs, the inlet load of PPCPs will be present in the STP effluent. Ozonation, UV-radiation, membrane filtration and activated carbon are potential treatments that might improve the effectiveness of PPCPs removal in a STP (Ternes *et al.,* 2002b; Huber *et al.,* 2003; Khan *et al.,* 2004). However, implementation of these techniques would increase the cost of wastewater treatment. Alternatively, understanding the fate of these substances within the STPs might yield removal methods based on a better management or minor modifications of existing STPs.

To evaluate the efficiency of STPs, several studies have been carried out in which the difference between the concentration of the chemical in the influent and that in the effluent has been compared (Simonich *et al.,* 2002; Kupper *et al.,* 2004; Paxeus, 2004; Strenn *et al.,* 2004; Johnson *et al.,* 2005). However, to understand the fate of PPCPs along STPs, it is essential to obtain information on the distribution of these substances between the aqueous and the solid phases, to get insight in the contribution of each process (sorption, volatilisation or biodegradation) in the overall removal.

In literature, only few studies deal with the fate of PPCPs along the different units of the STP treatment. Andersen *et al.* (2003) studied the fate of estrogens in a German municipal sewage treatment plant. They reported removal efficiencies of the natural estrogens (E1 and E2) and the synthetic hormone (EE2) of 98% and 90%, respectively. The natural estrogens were degraded biologically in the denitrifying and aerated nitrifying tanks, whereas EE2 was only degraded in the nitrifying tank. Only about 5% of the estrogens were sorbed onto digested sewage sludge. Matsui *et al.* (2000) also performed a profile of estrogen removal along the different steps of a Japanese STP using a immunoassay for E2 in combination

with the yeast estrogen screening (YES) assay for measuring estrogen activity. The estrogenicity measured by YES tended to decline during the treatment train, but the major reduction was found in the denitrification step.

Simonich *et al.* (2002) studied the removal of fragrances during primary and secondary treatment in several U.S. and European wastewater treatment plants with different treatment technologies. They found that the concentration of musks was reduced by 15-50% in the primary effluent, whereas the overall plant removal ranged from 59% to 99%, depending on the design of the plant. They stated that the removal of sorptive and non-sorptive musks is correlated with the reduction of TSS and BOD in the plant, respectively. Artola-Garicano *et al.* (2003a) studied the removal of HHCB and AHTN by measuring the dissolved and total concentrations. They found that while the dissolved concentrations remained virtually constant throughout all the compartments of the STPs, the total concentrations were dependant on the volatile solids content in a given compartment, resulting in much more variation. Bester (2004) performed a mass balance of HHCB and AHTN in a typical German sewage treatment plant from measurements in the influent, effluent and digested sludge. They found that about 35% of both compounds passed through the plant unaltered, more AHTN (around 80%) than HHCB (around 50%) was sorbed to the sludge and the degradation of HHCB to HHCB-lactone (Galaxolidone) only accounted to 5-10%.

Khan and Ongerth (2004) modelled the fate of 50 pharmaceutical residues in Australian sewage by quantities of use and fugacity calculations. With two exceptions (Irbesartan and Simvastatin), the sorption of the substances to sludge was lower than 10%, whereas biodegradation (varying from 10 to 80%) was highly dependant on the substance.

4.1.1. Objective

The objective of this work is to carry out a mass balance of each PPCP through the different treatment units of a selected STP in order to study the mechanism of elimination involved in their removal. For that purpose, two different methods of mass balance calculations, one based on *measured* data and the other on *estimated/calculated* data for the solid phase, are proposed and compared.

4.2. Materials and Methods

4.2.1. Sewage Treatment Plant

The sewage treatment plant considered was already described in Chapter 3 (section 3.2.1). A basic flow-scheme of the plant with the location of the liquid and sludge sampling points is shown in Figure 4.1.

The PPCPs concentrations in the aqueous phase were obtained from the four sampling campaigns carried out during 2001 and 2002 (Chapter 3). However, the PPCPs concentrations in the sludge were measured during one sampling period (April 2002) and only for some PPCPs (Galaxolide, Tonalide, Ibuprofen and the natural estrogens).

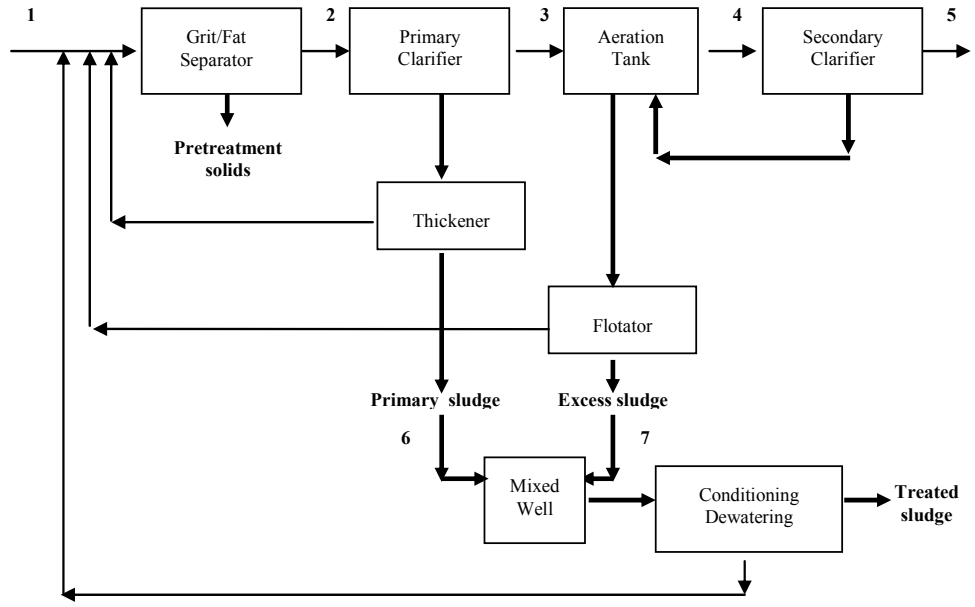

Figure 4.1. Diagram of the municipal sewage treatment plant and location of the sampling points.

4.2.2. Analytical methods

Wastewaters and sludge conventional parameters were determined by Standard Methods (APHA-AWWA-WPCF, 1999) as described in Chapter 2.

The content of PPCPs in the liquid and sludge phase was determined according to section 2.2.1 and 2.2.2 of Chapter 2, respectively.

4.2.3. Mass balance calculations

As natural estrogens are subjected to transformations between themselves (Ternes *et al.*, 1999; Joss *et al.,* 2004), the combined concentrations of both substances were used for the mass balance calculations.

Two approaches have been used to carry out the mass balance of each compound along the different units of the STP. The difference lies in the calculation of the fraction sorbed onto sludge.

- Method I (Fig. 4.2): it uses *measured* data, thus being only possible for those PPCPs determined in both liquid and sludge phase, i.e. musks (Galaxolide and Tonalide), Ibuprofen and the natural estrogens (Estrone and 17β-estradiol).

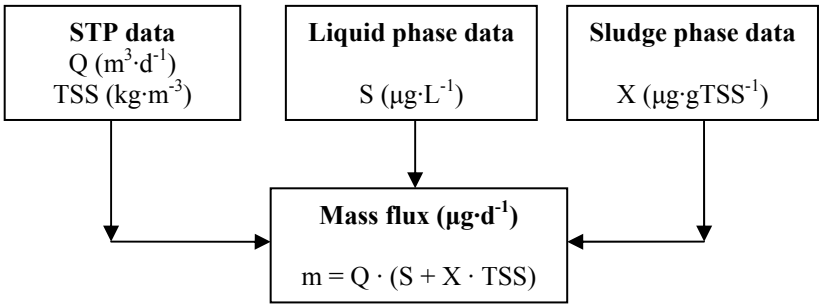

Figure 4.2. Scheme of mass balance calculations by Method I.

- Method II (Fig. 4.3): it is based on the measured soluble concentrations of all substances detected in the STP (HHCB, AHTN, IBP, NPX, SMX, IPM, E1 and E2) and, by means of the K_d values (Ternes *et al.,* 2004), the concentrations in the sludge were *calculated/estimated*. For IBP and IPM, the K_d values used for primary sludge were 20 and 5 $L \cdot kg^{-1}$, respectively. The K_d values for Naproxen and the natural estrogens were assumed the same as for Ibuprofen and EE2, respectively.

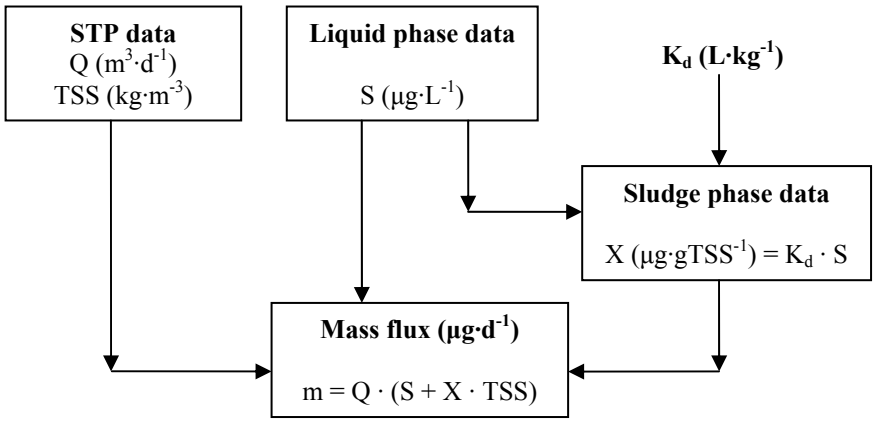

Figure 4.3. Scheme of mass balance calculations by Method II.

4.2.4. Removal efficiencies calculation

The removal efficiency of a substance in a specific unit was calculated as the difference between the total mass flux entering and that leaving the unit, divided by the total mass flux of the substance at the inlet of the STP (Equation 4.1). This means that the total mass flux at the inlet of the STP was considered as a reference in order to compare the different eliminations along the STP treatment.

$$\text{Removal}\,(\%) = \frac{m_i - m_{out}}{M_i} \qquad \text{Eq. 4.1}$$

where:

m_i: mass flux at the inlet of the unit ($\mu g\ PPCP \cdot d^{-1}$),

m_{out}: mass flux at the outlet of the unit ($\mu g\ PPCP \cdot d^{-1}$), and

M_i: mass flux at the inlet of the STP ($\mu g\ PPCP \cdot d^{-1}$).

4.3. Results and Discussion

4.3.1. Sewage Treatment Plant operation

A complete characterisation of the wastewaters treated in the STP was carried out for each sampling campaign (Table 3.2 in Chapter 3). Only the Total Suspended Solids (TSS) content in the wastewaters was used in the mass balance calculations. The average values considered for each sampling point were: 0.25 $kg \cdot m^{-3}$ (point 1), 0.25 $kg \cdot m^{-3}$ (point 2), 0.12 $kg \cdot m^{-3}$ (point 3), 2 $kg \cdot m^{-3}$ (point 4) and 0.02 $kg \cdot m^{-3}$ (point 5).

The minimum and maximum flows during the four sampling periods were 49,070 $m^3 \cdot d^{-1}$ and 56,488 $m^3 \cdot d^{-1}$, respectively. An average value of 53,000 $m^3 \cdot d^{-1}$ was used in the mass balances.

The average TSS concentrations in the sludge purge from the thickener and the flotator are 70 and 20 $kg \cdot m^{-3}$, respectively. After dewatering and conditioning, approximately 9 t of sludge is produced daily with a concentration of 450 $kg \cdot m^{-3}$.

4.3.2. PPCPs concentrations in the liquid and sludge phase

The PPCPs concentrations in the aqueous and sludge phase in the different sampling points are summarised in Tables 4.1 and 4.2, respectively.

Table 4.1. PPCPs liquid concentrations ($\mu g \cdot L^{-1}$) in the sampling points of the STP.

		1	2	3	4	5
HHCB	**Min**	2.1	2.3	1.3	1.0	0.46
	Max	3.4	3.4	1.8	1.0	0.60
	Average	*2.8*	*2.9*	*1.6*	*1.0*	*0.5*
AHTN	**Min**	0.7	1.1	0.6	0.5	0.15
	Max	1.7	1.6	1.0	0.5	0.20
	Average	*1.2*	*1.4*	*0.8*	*0.5*	*0.2*
IBP	**Min**	2.6	2.8	2.8	0.2	0.4
	Max	5.7	5.8	5.8	0.6	2.1
	Average	*4.2*	*4.3*	*4.3*	*0.4*	*1.3*
NPX	**Min**	1.8	1.8	1.6	0.1	0.2
	Max	4.6	4.1	4.8	2.1	2.6
	Average	*3.2*	*3.0*	*3.2*	*1.1*	*1.4*
IPM		6.6	7.5	7.2	8.8	9.3
SMX		0.6	0.5	0.6	0.3	0.3
E1		0.0024	0.0024	0.0034	0.0025	0.0044
E2		0.0016	0.0030	0.0024	<LOQ*	<LOQ*
E1 + E2		0.0040	0.0054	0.0058	0.0035	0.0054

*LOQ: 0.001 $\mu g \cdot L^{-1}$.

Table 4.2. PPCPs sludge concentrations ($\mu g \cdot g^{-1}$) in the sampling points of the STP.

	1	4	6	7
HHCB	37	25	31	30
AHTN	19	9	4	9
IBP	0.14	0.07	-	0.11
E1*	<LOQ	<LOQ	<LOQ	<LOQ
E2	0.021	0.004	0.025	0.035
E1+E2	0.023	0.006	0.027	0.037

*LOQ: 0.002 $\mu g \cdot g^{-1}$.

4.3.3. Mass balance results

The input data and the results of the mass balance calculations for the considered compounds are shown in Tables 4.3 and 4.4 for Method I and in Tables 4.5 and 4.6 for Method II, respectively.

Figure 4.4 shows a summary of the mass loads in the influent, final effluent and treated sludge and Table 4.7 indicates the percentage of the initial load at the STP being discharged in the final effluent and associated to solids, as well as the fraction degraded. Finally, a summary of the removal efficiencies in the pre-treatment step, primary clarification, biological treatment and the overall removal in the plant is shown in Table 4.8.

Removal means neither presence in the final effluent nor in the treated sludge. So, the disappearance must be due to other mechanism different to sorption, such as volatilization or degradation.

Volatilization

The amount of compound volatilised during aeration depends on the amount of air getting in contact with wastewater, which is dependant on the type of aeration, and the Henry coefficient in the case of air-water partitioning. Considering the total PPCP amount as the sum of that in solution and that volatilised, the relative fraction volatilised is:

$$\frac{M_G}{M_T} = \frac{S \cdot K_H \cdot Q_{air}}{S + S \cdot K_H \cdot Q_{air}} = \frac{K_H \cdot Q_{air}}{1 + K_H \cdot Q_{air}} \qquad \text{Eq. 4.2}$$

where:

M_G: mass of PPCP volatilised (μg PPCP·$L^{-1}_{wastewater}$),

M_T: total mass of PPCP (μg PPCP·$L^{-1}_{wastewater}$),

S: PPCP concentration in the liquid phase (μg PPCP·$L^{-1}_{wastewater}$),

K_H: Henry coefficient, and

Q_{air}: air getting in contact with wastewater (m^3_{air}·$m^3_{wastewater}$).

In a conventional activated sludge system, the air required ranges from 6 to 10 $m^3 \cdot m^{-3}_{wastewater}$. PPCPs normally have K_H values below 10^{-5}, except the musk fragrances with values in the range of $5 \cdot 10^{-3}$. Even for these substances, the amount volatilised is less than 5%, thus being the removal achieved in the biological reactor mainly due to degradation (Siegrist *et al.*, 2003).

Table 4.3. Input data and PPCPs concentrations in the aqueous and sludge phase by Method I.

Wastewater characterization	**Inlet**	**Pretreat. effluent**	**Primary effluent**	**Final effluent**	**Pretreat. solids**	**Primary sludge**	**Excess sludge**	**Treated sludge**
Q ($m^3 \cdot d^{-1}$)	53,000	**56,000**	**55,000**	53,000	0.5	900	2,300	20
TSS ($kg \cdot m^{-3}$)	0.25	0.25	0.12	0.02	*750*	6	2	450
Mass TSS ($kg \cdot d^{-1}$)	**13,250**	**14,000**	**6,600**	**1,060**	**375**	**5,400**	**4,600**	**9,000**
PPCPs concentrations in the aqueous (S) and sludge (X) phase								
S (HHCB) ($\mu g \cdot L^{-1}$)	2.8 (2.1-3.4)	2.9 (2.3-3.4)	1.6 (1.3-1.8)	0.5 (0.4-0.6)	*2.9 (2.3-3.4)*	*1.6 (1.3-1.8)*	*1.0*	*1.3 (1.2-1.4)*
S (AHTN) ($\mu g \cdot L^{-1}$)	1.2 (0.7-1.7)	1.4 (1.1-1.6)	0.8 (0.6-1.0)	0.2 (0.1-0.2)	*1.4 (1.1-1.6)*	*0.8 (0.6-1.0)*	*0.5*	*0.7 (0.6-0.8)*
S (IBP) ($\mu g \cdot L^{-1}$)	4.2 (2.6-5.7)	4.3 (2.8-5.8)	4.3 (2.8-5.8)	1.3 (0.4-2.1)	*4.3 (2.8-5.8)*	*4.3 (2.8-5.8)*	*0.4 (0.2-0.6)*	*2.4 (1.5-3.2)*
S (E1) ($ng \cdot L^{-1}$)	2.4	2.4	3.4	4.4	*2.4*	*3.4*	*2.5*	*3.0*
S (E2) ($ng \cdot L^{-1}$)	1.6	3.0	2.4	1.0	*3.0*	*2.4*	*1.0*	*2.0*
S (E1+E2) ($ng \cdot L^{-1}$)	**4.0**	**5.4**	**5.8**	**5.4**	**5.4**	**5.8**	**3.5**	**5.0**
X (HHCB) ($\mu g \cdot g^{-1}$)	37	*34*	*31*	*30*	*34*	31	30	*30*
X (AHTN) ($\mu g \cdot g^{-1}$)	19	*12*	*4*	*9*	*12*	4	9	*7*
X (IBP) ($\mu g \cdot g^{-1}$)	0.14	*0.14*	*0.14*	*0.11*	*0.14*	*0.14*	0.11	*0.13*
X (E1) ($ng \cdot g^{-1}$)	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*
X (E2) ($ng \cdot g^{-1}$)	21	*23*	*25*	*35*	*23*	25	35	*30*
X (E1+E2) ($ng \cdot g^{-1}$)	**23**	**25**	**27**	**37**	**25**	**27**	**37**	**32**

Regular type indicates measured data. Italic type indicates estimated data. Boldface type indicates calculated data.
The range of minimum to maximum is indicated in brackets.

Table 4.4. Mass flux of PPCPs through the municipal STP studied obtained by Method I.

Mass flux of musks ($g \cdot d^{-1}$)		**Inlet**	**Pretreat. effluent**	**Primary effluent**	**Final effluent**	**Pret. solids**	**Primary sludge**	**Excess sludge**	**Treated sludge**
HHCB	Dissolved	**148 (111-180)**	**162 (129-190)**	**88 (72-99)**	**27 (21-32)**	**0**	**1.4 (1.2-1.6)**	**2.3**	**0.1**
	Sorbed	**490**	**476**	**205**	**32**	**13**	**167**	**138**	**270**
	Total	**639 (602-671)**	**638 (605-666)**	**293 (276-304)**	**58 (53-64)**	**13**	**169**	**140**	**270**
AHTN	Dissolved	**64 (37-90)**	**78 (62-90)**	**44 (33-55)**	**11 (8-11)**	**0**	**0.7 (0.5-0.9)**	**1.2**	**0**
	Sorbed	**252**	**168**	**26**	**10**	**5**	**22**	**41**	**63**
	Total	**315 (289-342)**	**246 (230-258)**	**73 (59-81)**	**20 (18-20)**	**5**	**22**	**43**	**63**
Mass flux of Ibuprofen ($g \cdot d^{-1}$)									
IBP	Dissolved	**223 (138-302)**	**241 (157-325)**	**237 (154-319)**	**69 (21-111)**	**0**	**3.9 (2.5-5.2)**	**0.9 (0.5-1.4)**	**0.1**
	Sorbed	**1.9**	**2.0**	**0.9**	**0.1**	**0.1**	**0.8**	**0.5**	**1.1**
	Total	**225 (140-304)**	**243 (159-327)**	**237 (155-320)**	**69 (21-111)**	**0.1**	**4.6 (3.3-6.0)**	**1.4 (1.0-1.9)**	**1.3**
Mass flux of E1+E2 ($mg \cdot d^{-1}$)									
E1+E2	Dissolved	**212**	**302**	**319**	**286**	**0**	**5**	**8**	**0**
	Sorbed	**305**	**350**	**178**	**39**	**9**	**146**	**170**	**288**
	Total	**517**	**652**	**497**	**325**	**9**	**151**	**178**	**288**

Boldface type indicates calculated data.
The range of minimum to maximum is indicated in brackets.

Table 4.5. Input data and PPCPs concentrations in the aqueous and sludge phase by Method II.

Wastewater characterization	**Inlet**	**Pretreat. effluent**	**Primary effluent**	**Final effluent**	**Pret. solids**	**Primary sludge**	**Excess sludge**	**Treated sludge**
Q ($m^3 \cdot d^{-1}$)	53,000	**56,000**	**55,000**	53,000	0.5	900	2,300	20
TSS ($kg \cdot m^{-3}$)	0.25	0.25	0.12	0.02	*750*	6	2	450
Mass TSS ($kg \cdot d^{-1}$)	**13,250**	**14,000**	**6,600**	**1,060**	**375**	**5,400**	**4,600**	**9,000**
PPCPs concentrations in the aqueous phase (S)								
S (HHCB) ($\mu g \cdot L^{-1}$)	2.8 (2.1-3.4)	2.9 (2.3-3.4)	1.6 (1.3-1.8)	0.5 (0.4-0.6)	*2.9 (2.3-3.4)*	*1.6 (1.3-1.8)*	*1.0*	***1.3 (1.2-1.4)***
S (AHTN) ($\mu g \cdot L^{-1}$)	1.2 (0.7-1.7)	1.4 (1.1-1.6)	0.8 (0.6-1.0)	0.2 (0.1-0.2)	*1.4 (1.1-1.6)*	*0.8 (0.6-1.0)*	*0.5*	***0.7 (0.6-0.8)***
S (IBP) ($\mu g \cdot L^{-1}$)	4.2 (2.6-5.7)	4.3 (2.8-5.8)	4.3 (2.8-5.8)	1.3 (0.4-2.1)	*4.3 (2.8-5.8)*	*4.3 (2.8-5.8)*	*0.4 (0.2-0.6)*	***2.4 (1.5-3.2)***
S (NPX) ($\mu g \cdot L^{-1}$)	3.2 (1.8-4.6)	3.0 (1.8-4.1)	3.2 (1.6-4.8)	1.4 (0.2-2.6)	*3.0 (1.8-4.1)*	*3.2 (1.6-4.8)*	*1.1 (0.1-2.1)*	***2.2 (0.9-3.5)***
S (SMX) ($\mu g \cdot L^{-1}$)	0.6	0.5	0.6	0.3	*0.5*	*0.6*	*0.3*	***0.5***
S (IPM) ($\mu g \cdot L^{-1}$)	6.6	7.5	7.2	9.3	*7.5*	*7.2*	*8.8*	**8.0**
S (E1) ($ng \cdot L^{-1}$)	2.4	2.4	3.4	4.4	*2.4*	*3.4*	*2.5*	***3.0***
S (E2) ($ng \cdot L^{-1}$)	1.6	3.0	2.4	1.0	*3.0*	*2.4*	*1.0*	***2.0***
S (E1+E2) ($ng \cdot L^{-1}$)	**4.0**	**5.4**	**5.8**	**5.4**	**5.4**	**5.8**	**3.5**	***5.0***
K_d values ($L \cdot kg^{-1}$)*	**HHCB**	**AHTN**	**IBP**	**NPX**	**SMX**	**IPM**	**E1**	**E2**
Primary sludge	4,920	5,300	<20	*<20*	32	<5	*278*	*278*
Biological sludge	1,810	2,400	7.1	*7.1*	32	11	*349*	*349*

Regular type indicates measured data. Italic type indicates estimated data. Boldface type indicates calculated data.
The range of minimum to maximum is indicated in brackets.
*From Ternes *et al.*, 2004.

Table 4.5. Input data and PPCPs concentrations in the aqueous and sludge phase by Method II. *Cont.*

PPCPs concentrations in the sludge (X)	**Inlet**	**Pretreat. effluent**	**Primary effluent**	**Final effluent**	**Pretreat. solids**	**Primary sludge**	**Excess sludge**	**Treated sludge**
X (HHCB) ($\mu g \cdot g^{-1}$)	**13.8 (10.3-16.7)**	**14.3 (11.3-16.7)**	**7.9 (6.4-8.9)**	**0.9 (0.9-1.1)**	**14.3 (11.3-16.7)**	**7.9 (6.4-8.9)**	**1.8**	**4.8 (4.1-5.3)**
X (AHTN) ($\mu g \cdot g^{-1}$)	**6.4 (3.7-9.0)**	**7.4 (5.8-8.5)**	**4.2 (3.2-5.3)**	**0.5 (0.4-0.5)**	**7.4 (5.8-8.5)**	**4.2 (3.2-5.3)**	**1.2**	**2.7 (2.2-3.3)**
X (IBP) ($\mu g \cdot g^{-1}$)	**0.08 (0.05-0.11)**	**0.09 (0.06-0.12)**	**0.09 (0.06-0.12)**	**0.01 (0.00-0.02)**	**0.09 (0.06-0.12)**	**0.09 (0.06-0.12)**	**0.003**	**0.04 (0.03-0.06)**
X (NPX) ($\mu g \cdot g^{-1}$)	**0.06 (0.04-0.09)**	**0.06 (0.04-0.08)**	**0.06 (0.03-0.10)**	**0.01 0.00-0.02)**	**0.06 (0.04-0.08)**	**0.06 (0.03-0.10)**	**0.01 (0.00-0.02)**	**0.04 (0.02-0.06)**
X (SMX) ($\mu g \cdot g^{-1}$)	**0.02**	**0.02**	**0.02**	**0.01**	**0.02**	**0.02**	**0.01**	**0.01**
X (IPM) ($\mu g \cdot g^{-1}$)	**0.03**	**0.04**	**0.04**	**0.10**	**0.04**	**0.04**	**0.10**	**0.07**
X (E1) ($ng \cdot g^{-1}$)	**0.7**	**0.7**	**0.9**	**1.5**	**0.7**	**0.9**	**0.9**	**0.9**
X (E2) ($ng \cdot g^{-1}$)	**0.4**	**0.8**	**0.7**	**0.3**	**0.8**	**0.7**	**0.3**	**0.5**
X (E1+E2) ($ng \cdot g^{-1}$)	**1.1**	**1.5**	**1.6**	**1.9**	**1.5**	**1.6**	**1.2**	**1.4**

Boldface type indicates calculated data.
The range of minimum to maximum is indicated in brackets.

Table 4.6. Mass flux of PPCPs detected through the municipal STP studied obtained by Method II.

Mass flux of musks ($g \cdot d^{-1}$)		Inlet	Pretreat. effluent	Primary effluent	Final effluent	Pretreat. solids	Primary sludge	Excess sludge	Treated sludge
	Dissolved	**148 (111-180)**	**162 (129-190)**	**88 (72-99)**	**27 (21-32)**	**0**	**1.4 (1.2-1.6)**	**2.3**	**0.1**
HHCB	Sorbed	**183 (137-221)**	**200 (158-234)**	**52 (42-59)**	**1**	**5 (4-6)**	**43 (35-48)**	**8**	**43 (37-48)**
	Total	**331 (248-402)**	**363 (287-424)**	**140 (114-158)**	**28 (22-33)**	**5 (4-6)**	**44 (36-50)**	**11**	**43 (37-48)**
	Dissolved	**64 (37-90)**	**78 (62-90)**	**44 (33-55)**	**11 (8-11)**	**0**	**0.7 (0.5-0.9)**	**1.2**	**0**
AHTN	Sorbed	**85 (49-119)**	**104 (81-119)**	**28 (21-35)**	**0.5 (0.4-0.5)**	**2.8 (2.2-3.2)**	**23 (17-29)**	**5.5**	**24 (20-30)**
	Total	**148 (86-209)**	**182 (143-209)**	**72 (54-90)**	**11 (8-11)**	**2.8 (2.2-3.2)**	**23 (18-30)**	**7**	**24 (20-30)**
Mass flux of anti-inflammatories ($g \cdot d^{-1}$)									
	Dissolved	**223 (138-302)**	**241 (157-325)**	**237 (154-319)**	**69 (21-111)**	**0**	**4 (3-5)**	**0.9 (0.5-1.4)**	**0.1**
IBP	Sorbed	**1.1 (0.7-1.5)**	**1.2 (0.8-1.6)**	**0.6 (0.4-0.8)**	**0**	**0**	**0.5 (0.3-0.6)**	**0**	**0.4 (0.3-0.5)**
	Total	**224 (139-304)**	**242 (158-326)**	**237 (154-320)**	**69 (21-111)**	**0**	**4 (3-6)**	**0.9 (0.5-1.4)**	**0.5 (0.3-0.7)**
	Dissolved	**170 (95-244)**	**168 (101-230)**	**176 (88-264)**	**74 (11-138)**	**0**	**3 (1-4)**	**3 (0-5)**	**0.1**
NPX	Sorbed	**0.8 (0.5-1.2)**	**0.8 (0.5-1.1)**	**0.4 (0.2-0.6)**	**0**	**0**	**0.3 (0.2-0.5)**	**0**	**0.3 (0.1-0.5)**
	Total	**170 (96-245)**	**169 (101-231)**	**176 (88-265)**	**74 (11-138)**	**0**	**3 (2-5)**	**3 (0-5)**	**0.4 (0.2-0.6)**
Mass flux of antibiotic ($g \cdot d^{-1}$)									
	Dissolved	**32**	**28**	**33**	**16**	**0**	**0.5**	**0.7**	**0**
SMX	Sorbed	**0.3**	**0.2**	**0.1**	**0**	**0**	**0.1**	**0.1**	**0.1**
	Total	**32**	**28**	**33**	**16**	**0**	**1**	**1**	**0**
Mass flux of X-ray contrast media ($g \cdot d^{-1}$)									
	Dissolved	**350**	**420**	**396**	**493**	**0**	**7**	**20**	**0.3**
IPM	Sorbed	**0.4**	**0.5**	**0.2**	**0.1**	**0**	**0.2**	**0.5**	**0.6**
	Total	**350**	**421**	**396**	**493**	**0**	**7**	**21**	**1**
Mass flux of natural estrogens ($mg \cdot d^{-1}$)									
	Dissolved	**212**	**302**	**319**	**286**	**0**	**5**	**8**	**0**
E1+E2	Sorbed	**15**	**21**	**11**	**2**	**1**	**9**	**6**	**13**
	Total	**227**	**323**	**330**	**288**	**1**	**14**	**14**	**13**

Boldface type indicates calculated data.

The range of minimum to maximum is indicated in brackets.

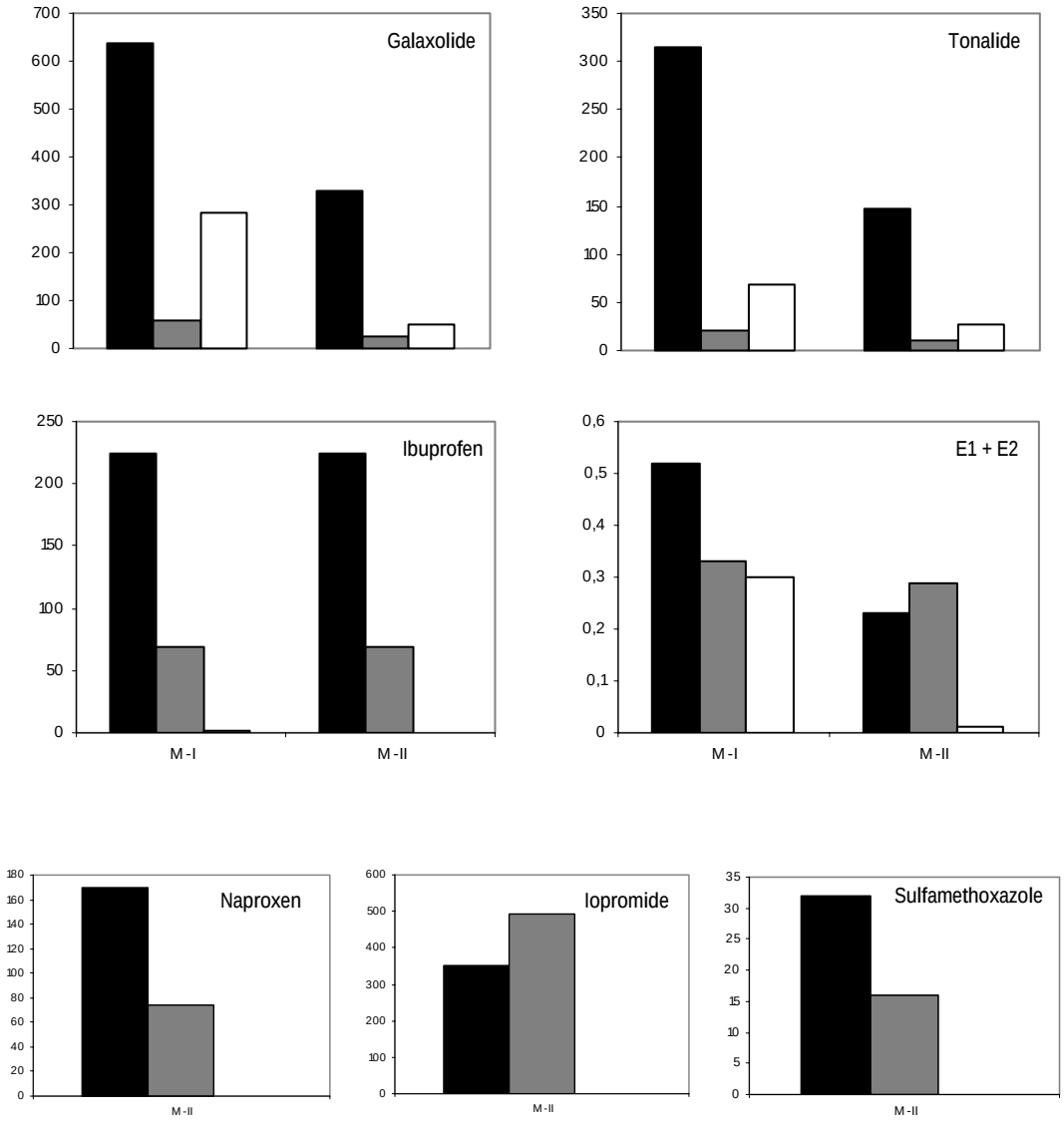

Figure 4.4. Influent (■), effluent (■) and sludge (□) mass flux ($g \cdot d^{-1}$) of PPCPs detected in the STP calculated by Method I and Method II.

Table 4.7. Percentages of PPCP inlet load being degraded and discharged in the final effluent and sludge.

PPCP		Influent ($g \cdot d^{-1}$)	% discharged		% degraded
			Final effluent	*Sludge*	
HHCB	*M-I*	639 (602-671)	9.1 (8.8-9.5)	44.3 (42.2-47.0)	46.6 (44.2-48.3)
	M-II	331 (248-402)	8.2 (8.2-10.9)	14.8 (13.4-16.5)	76.9 (72.3-78.3)
AHTN	*M-I*	315 (289-342)	6.2 (5.8-6.3)	21.6 (19.9-23.5)	72.2 (70.6-74.4)
	M-II	148 (86-210)	7.4 (5.2-9.3)	18.2 (15.7-25.6)	74.0 (64.9-79.2)
IBP	*M-I*	225 (140-304)	31.0 (15.0-36.5)	0.6 (0.4-0.9)	68.7 (62.9-83.9)
	M-II	224 (139-304)	30.8 (15.1-36.5)	0.2	69.0 (63.1-84.5)
NPX	*M-II*	170 (96-245)	43.5 (11.5-56.3)	0.2	56.2 (43.5-88.8)
SMX	*M-II*	32	50	0.3	50
IPM	*M-II*	350	140.9	0.2	-41
E1+E2	*M-I*	0.52	62.9	57.6	-21
	M-II	0.23	126.9	5.7	-33

Table 4.8. Summary of PPCPs removal efficiencies (%) during pre-treatment, primary clarification, biological treatment and overall in the STP (average values).

PPCP		*Pretreatment*	*Primary*	*Biological*	*Overall*
HHCB	*M-I*	6	28	15	47
	M-II	0	55	30	75
AHTN	*M-I*	25	49	3	72
	M-II	0	50	40	74
IBP	*M-I*	0	0	80	70
	M-II	0	0	80	70
NPX	*M-II*	0	0	60	55
SMX	*M-II*	0	0	50	50
IPM	*M-II*	0	0	0	0
E1+E2	*M-I*	-16	-1	-1	-21
	M-II	-36	-9	12	-33

Degradation

Assuming a pseudo-first-order reaction (TSS = constant) for PPCPs elimination, the amount of compound degraded in a specific unit can be estimated from the biodegradation constant rates and the HRT in the unit:

$$\text{Degradation}\,(\%) = 1 - e^{-k \cdot TSS \cdot t} \qquad \text{Eq. 4.3}$$

where:

k: biodegradation constant rate ($L \cdot gTSS^{-1} \cdot h^{-1}$),

TSS: total suspended solids concentrations ($g \cdot L^{-1}$), and

t: HRT in the unit (h).

The biodegradation constant rates can be obtained from the half-life values and considering an average TSS concentration of 2 $g \cdot L^{-1}$. Concerning the HRT, it was considered 1 and 2 h for the primary treatment and 6 and 8 h for the biological treatment. Table 4.9 shows the results obtained.

Table 4.9. Estimated degradation percentage (%) during primary and biological treatment as a function of the HRT in each system and values obtained in this study.

PPCP	k_{biod} (h^{-1})	Primary treatment				Biological treatment			
		HRT		*This study*		*HRT*		*This study*	
		1 h	*2 h*	*M-I*	*M-II*	*6 h*	*8 h*	*M-I*	*M-II*
HHCB	0.071[1]	6.9	13.2	28	55	34.7	43.3	15	30
AHTN	0.023[1]	2.3	4.5	49	50	12.9	16.8	3	40
IBP	0.043[2]	4.2	8.2	0	0	22.7	29.1	80	80
NPX	0.058[2]	5.6	11.0	0		29.4	37.1	60	
SMX	0.014[2]	1.4	2.8	0		8.1	10.6	50	
IPM	0.038[3]	3.7	7.3	0		20.4	26.2	0	
E1	0.462[4]	37.0	60.3	0	0	93.7	97.5	0	12
E2	3.465[4]	96.9	99.9			100.0	100.0		

[1]Artola-Garicano *et al.,* 2003b; [2]Khan and Ongerth, 2004; [3]Kalsch, 1999; [4]Andersen *et al.,* 2003.

Galaxolide and Tonalide

There are significant differences between the results of the mass balances obtained with Method I (measured) and Method II (calculated/estimated). In the latter, the mass fluxes calculated for both musks were approximately half of those obtained with Method I (Figure 4.4). The reason is the difference between the measured and the calculated concentration in the sludge phase, being the corresponding to Method I higher (3-4 times for HHCB and 2-3 times for AHTN) than those of Method II. Taking into account that the measured concentrations are in the same range as those reported in literature for primary, biological and digested sludge, between 2.5 and 81 $\mu g \cdot g^{-1}$ for HHCB (Balk and Ford, 1999; Stevens *et al.,* 2003; Bester, 2004) and from 0.7 to 34 $\mu g \cdot g^{-1}$ for AHTN (Balk and Ford, 1999; Herren and Berset, 2000), the results obtained by Method I seem to be more reliable.

Therefore, as Method I leads to higher amount of musks sorbed to sludge than Method II, the overall removal efficiencies in the STP obtained by Method I should be lower than those calculated by Method II (Table 4.7). However, while it occurred for HHCB, around 50% for Method I and 75% for Method II, similar

elimination was obtained for AHTN by both methods (around 75%). The reason is that the inlet load of AHTN obtained by Method I is 2 times higher than that by Method II (300 vs. 150 $g \cdot d^{-1}$) and the same occurs with the total outlet load (90 vs. 40 $g \cdot d^{-1}$). This fact can be explained analysing the concentrations of AHTN in the sludge. As similar values were obtained in the primary sludge with Method I and II, 4 and 4.2 $\mu g \cdot g^{-1}$, respectively, the differences are in the concentrations at the inlet (19 $\mu g \cdot g^{-1}$ in Method I and 6.4 $\mu g \cdot g^{-1}$ in Method II) and those in the biological sludge (9 $\mu g \cdot g^{-1}$ in Method I and 1.2 $\mu g \cdot g^{-1}$ in Method II). Taking into account that the effect of biological sludge in the treated sludge is approximately 50% (mixing with primary sludge), the differences between Method I and Method II were the same for the inlet and for the outlet, thus leading to similar removal efficiencies.

These substances are mainly present associated to solids, thus being the percentage present in the final effluent (Table 4.7) not affected by the calculation method, being it lower than 10%.

During primary treatment (Table 4.8), the elimination is mainly due to sorption (20% for HHCB and 10% for AHTN), although some degradation and/or volatilization occurred as well (30-55% for HHCB and 50% for AHTN). Similar results were obtained by Simonich *et al.* (2002). Comparing these results with the degradation percentage estimated for primary treatment (Table 4.9), it was observed that the calculated values are significantly lower. This can be due either to an underestimation of the sorbed fraction or to other mechanism being involved in musks elimination.

During biological treatment, higher removal efficiencies were obtained for both compounds with Method II (30% for HHCB and 40% for AHTN) than with Method I (15% for HHCB and 3% for AHTN). Once again, it is due to the fraction sorbed onto sludge, being higher that calculated with Method I. The degradation percentage estimated for biological treatment (Table 4.9) is slightly higher for HHCB (around 40%) and in between for AHTN (around 15%).

Artola-Garicano *et al.* (2003a) reported no substantial biodegradation of musks, since the dissolved concentrations remained almost constant along the STP treatment. They indicated that musks are mostly bound to solids, thus being not directly available for microbial degradation. However, Simonich *et al.* (2002) reported that sorption alone does not account for the removal obtained and that biotransformation or volatilization may be playing a major role in the elimination

of musks in STPs. These different conclusions between studies might be related to differences in the characteristics of the wastewaters (mainly COD) or differences in the operation of the STP (mainly SRT).

Ibuprofen

The measured concentrations of Ibuprofen in the sludge (Table 4.3) are in the lower limit of the range reported in literature for primary, biological and digested sludge, between 0.01 and 4 $\mu g \cdot g^{-1}$ (Khan and Ongerth, 2002).

Conversely to musks, the mass fluxes obtained by both methods for Ibuprofen were almost equal (Figure 4.4). This is due to the hydrophilic nature of this substance, which makes negligible its sorption on solids. Besides, the fraction sorbed to sludge obtained by Method I (measured) is similar to that calculated with Method II.

Consequently, the overall removal efficiency in the STP obtained by both methods was similar (70%), being it clearly due to biological degradation in the activated sludge system (Table 4.8). In total, a 30% of IBP passes through the plant unaltered and less than 0.5% is discharged associated to the sludge (Table 4.7).

Comparing the results with the degradation estimated (Table 4.9), similar values were obtained for primary treatment (no removal, around 5%), but lower removal efficiencies were estimated for biological treatment (around 25%) than those achieved in this study (80%). This fact can be explained by the discrepancy in the reported biodegradation rates, making difficult an accurate prediction (Khan and Ongerth, 2004). These authors reported negligible removal of IBP to sludge and about 50% to biodegradation, slightly lower than the value obtained in this study (70%).

Naproxen

For this compound, only Method II was applied since not measurements in the solid phase were carried out.

Similarly to Ibuprofen, no significant concentrations were estimated for Naproxen associated to solids, between 1 and 100 $ng \cdot g^{-1}$ (Table 4.5). These values are in the range of those reported in literature, from 1 $ng \cdot g^{-1}$ to 1 $\mu g \cdot g^{-1}$ (Khan and Ongerth, 2002).

The overall removal efficiency in the STP was approximately 55%, slightly lower than IBP, being it clearly due to biological degradation in the activated sludge system (Table 4.8). In total, 40% of NPX passes through the plant unaltered and less than 0.2% is discharged associated to the sludge (Table 4.7).

Comparing the results with degradation estimated (Table 4.9), similar values were obtained for primary treatment (no removal, around 10%), but once again lower removal efficiencies were estimated for biological treatment (30-35%) than those achieved in this study (60%). The same explanation as for IBP can be applied.

Khan and Ongerth (2004) reported negligible removal of this compound to sludge and about 55% to biodegradation, exactly the same as in this study.

Sulfamethoxazole

Similarly to Naproxen, only Method II was applied for determining the mass balance of this substance throughout the STP.

The estimated concentrations of Sulfamethoxazole in the sludge ranged between 10 and 20 $ng \cdot g^{-1}$ (Table 4.5); no values of this compound in solid phase have been found in literature.

The overall removal efficiency in the STP was approximately 50% being it clearly due to biological degradation in the activated sludge system (Table 4.8). In total, 50% of SMX passes through the plant unaltered and less than 0.3% is discharged associated to the sludge (Table 4.7).

Comparing the results with the degradation estimated (Table 4.9), similar values were obtained for primary treatment (no removal, around 2%), but once again lower removal efficiencies were estimated for biological treatment (around 10%) than those achieved in this study (50%). The same explanation as for IBP and NPX can be applied.

Khan and Ongerth (2004) reported negligible removal of this compound to sludge and about 22% to biodegradation, quite lower than the value achieved in this study (50%).

Iopromide

Once again, only Method II was applied for determining the mass balance of this substance throughout the STP.

The estimated concentrations of Iopromide in the sludge ranged between 33 and 102 $ng \cdot g^{-1}$ (Table 4.5); no values of this compound in solid phase have been found in literature.

Removal of Iopromide was obtained neither during primary clarification nor in the biological treatment (Table 4.8). This substance passes unaltered through the plant (Figure 4.4), being completely discharged in the final effluent (less than 0.2% is sorbed to sludge). Similar results were obtained in other studies (Putschew *et al.*, 2000; Ternes and Hirsch, 2000).

Comparing the results with the degradation estimated (Table 4.9), similar values were obtained for primary treatment (no reduction, around 5%); however, some degradation should have been achieved in the biological treatment (around 25%) according to the biodegradation rates.

Estrone and 17β-estradiol

E1 was found below the limit of quantification (2 $ng \cdot g^{-1}$); however, E2 was detected up to 35 $ng \cdot g^{-1}$ (Table 4.3). These values are in the same range as those reported in literature, from 2 to 37 $ng \cdot g^{-1}$ for E1 and from 2 to 49 $ng \cdot g^{-1}$ for E2 (Ternes *et al.,* 2002a; Joss *et al.,* 2004). However, the estimated sludge concentrations (Method II) were much lower, between 1 and 2 $ng \cdot g^{-1}$ (Table 4.5). This fact explains the difference between the results obtained by both methods. While in Method I, the total input load is partitioned equally between the final effluent and the sludge, all the estrogens load is discharged in the final effluent according to Method II (Table 4.7).

Similarly to Iopromide, natural estrogens are not removed in the STP (Table 4.7). The outlet mass fluxes are greater than the input loads, this being explained by the cleavage of the glucuronide forms during the STP treatment.

Although a complete removal was estimated from the biodegradation constant rates in both primary and biological treatment (Table 4.9), it is widely reported that natural estrogens are poorly removed in high loaded plants (Ternes, 2001; Kreuzinger *et al.,* 2004; Clara *et al.,* 2005). Their elimination is mostly related to the Sludge Retention Time (SRT) in the aeration tank, being removed when this parameter is higher than 10 d (Kreuzinger *et al.,* 2004; Clara *et al.,* 2005).

4.4. Conclusions

The fate of 8 substances (Galaxolide, Tonalide, Ibuprofen, Naproxen, Sulfamethoxazole, Iopromide, Estrone and 17β-estradiol) has been investigated along the water and sludge train in a municipal sewage treatment plant.

As the removal efficiencies reported (mostly related to disappearance from the liquid phase) differ significantly (Table 1.8), it seems of considerable concern to improve the understanding about the elimination pathways of these substances in STPs. Three mechanisms have been evaluated: volatilisation, sorption and degradation.

Due to the low Henry coefficient of these compounds, volatilisation only accounts up to 5% (for musks) and therefore, only sorption and degradation must be highlighted. In order to differ between both mechanisms, mass balances through the different units of the STP including the sludge phase are needed. In this work, two approaches have been considered for the mass balance calculations: the measured sludge concentrations (Method I) and the calculated sludge concentrations using the solid-water distribution coefficient (Method II).

Although the total mass balance fits quite well for all substances (error up to 15%), the major variability stem from the sludge train. The discontinuous production of dewatered sludge gives the largest contribution to uncertainties for the balance calculation.

An increase in the influent concentrations to primary treatment compared to the influent of the STP was observed for some compounds, which suggests either a contribution of the recycling streams from the sludge treatment processes or the cleavage of glucuronide forms (e.g. estrogens).

While musks are mainly eliminated via sorption (although biodegradation could also play an important role), the removal of anti-inflammatories and Sulfamethoxazole is mainly due to microbial degradation. However, Iopromide and the natural estrogens are not removed in the STP studied.

From the comparison between Method I and Method II, it can be concluded that for sorptive substances, i.e. musks, the determination of the sludge concentration is crucial. The differences between the results obtained by both methods come from the determination of the amount associated to solids. Therefore, an inaccurate estimation of this parameter can lead to

misunderstandings in the establishment of the mechanism responsible for their elimination, sorption or biodegradation.

However, for more hydrophilic compounds, such as Ibuprofen, as they tend to remain in the aqueous phase, the amount sorbed onto sludge is negligible, thus not having a significant effect in the mass balance results. This is the reason for the similar results obtained by Method I and Method II. The removal of these substances is mainly due to microbial degradation.

In the case of Naproxen and Sulfamethoxazole, although no comparison could be made between both methods, considering their hydrophilic nature and according to the results obtained for Ibuprofen, it can be concluded that Method I would lead to similar results as those obtained with the method used (Method II). These compounds are mainly degraded in the plant, being the amount associated to the sludge lower than 1%.

The comparison between the biodegradation percentage obtained in this study and those estimated from biodegradation constant rates differ significantly for some substances. This variable was also found by Khan and Ongerth (2004) as the most critical and uncertain for predictions. They stated that this parameter is particularly sensitive for those compounds with half-life of less than 50 h, which is the case of the substances considered in this study.

To conclude, the mass balance calculations indicate where efforts must be made in order to reduce the amounts of PPCPs discharged into the environment. For sorptive substances, they should be focused in the sludge treatment. On the contrary, for non-sorptive substances, tertiary treatment of the final effluent must be considered. Besides, this work shows that the method used for the mass balance calculations can affect significantly the results. While for hydrophilic substances, Method II seems to be a good option, for sorptive compounds, this method could lead to an underestimation of the fraction sorbed, thus being in this case Method I more suitable.

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Chapter 5

Removal of cosmetic ingredients and pharmaceuticals present in sewage by coagulation-flocculation and flotation processes[1]

Summary

Two physico-chemical processes, coagulation-flocculation and flotation, have been assessed for enhancing the removal of selected Pharmaceutical and Personal Care Products (PPCPs) present in sewage. Seven compounds, representative of three main groups of PPCPs according to their physico-chemical properties, have been selected: lipophilic compounds (the synthetic musks Galaxolide and Tonalide), neutral compounds (the tranquilliser Diazepam and the antiepileptic Carbamazepine) and acidic compounds (the anti-inflammatories Ibuprofen, Naproxen and Diclofenac). During the coagulation-flocculation assays, the main parameters considered were the selection of the additives, their doses and the temperature of operation (12 or 25ºC). Musks, highly lipophilic, and Diclofenac, with significant sorption affinity, were removed around 50-70% at both temperatures independently of the dose and type of coagulant used. However, the rest of the compounds, which are more hydrophilic, were affected to a lesser degree (with maximum reductions below 25%). The exceptions to this behaviour were Carbamazepine and Ibuprofen, which were not removed under any condition tested. During the flotation assays, the parameters studied were the initial content of fat in wastewaters and the temperature. Again, musks were removed to a greater degree (35-60%), followed by Diazepam (40-50%) and Diclofenac (20-45%) and, to a lesser extent, Carbamazepine (20-35%), Ibuprofen (10-25%) and Naproxen (10-30%). The best results were always obtained at 25ºC, although in some cases the operation at 12ºC gave similar values. The removal of musks and neutral compounds was higher in wastewaters with a high fat content (around 150 $mg \cdot L^{-1}$).

[1] **Carballa, M., Omil, F. and Lema, J.M.** (2005). Removal of cosmetic ingredients and pharmaceuticals in sewage primary treatment. *Water Research,* 39 (19): 4790-4796.

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5.1. Introduction

Four main mechanisms (sorption, biodegradation, volatilization and photooxidation) can be considered when studying PPCPs removal in STPs, although in some cases it is difficult to differentiate the effect of each one. The efficiency of these removal mechanisms greatly depends on the physico-chemical properties and the chemical structure of the selected compound.

- *Volatilisation:* The values of Henry's law constant (see Table 1.6) for every PPCP considered, except the polycyclic musk fragrances, are uniformly less than 10^{-5}, implying that partitioning of the compounds to the atmosphere by volatilisation is negligible (Struijs *et al.,* 1991). Only for musks, this path can be relevant, as it is confirmed by the presence of these substances in the atmosphere. Kallenborn *et al.* (1999) detected 0.14 $ng \cdot m^{-3}$ of Galaxolide and 0.052 $ng \cdot m^{-3}$ of Tonalide in Norwegian air samples. Peck and Hornbuckle (2004) reported concentrations of HHCB and AHTN in the atmospheric air of Lake Michigan area ranging between 1.1-4.6 $ng \cdot m^{-3}$ and 0.5-2.9 $ng \cdot m^{-3}$, respectively. They pointed out volatilisation as one of the major loss mechanisms of musks in lakes, estimating a loss of 290 $kg \cdot y^{-1}$ of both polycyclic fragrances from Lake Michigan.

- *Photooxidation:* Some PPCPs have been found to be photodegraded under UV light, such as Diclofenac (Buser *et al.,* 1998) or musks fragrances (Buerge *et al.,* 2003; Sanchez-Prado *et al.,* 2004). However, the contribution of this process to the total removal of PPCPs in STPs appears to be very low, although it could be predominant in lakes, in the case that sorption and chemical degradation be neglected.

- *Biodegradation:* It is one of the most important mechanisms of PPCPs removal in STPs. However, the complete mineralization of PPCPs has not been reported yet. Often the substances are transformed yielding sometimes more refractory metabolites. For some substances considered in this work, studies have been carried out in literature to study their biodegradation, such as Carbamazepine (Stamatelatou *et al.,* 2003), Ibuprofen (Zwiener *et al.,* 2002), Iopromide (Kalsch, 1999) and estrogens (Ternes *et al.,* 1999). There are several factors influencing the biodegradation of PPCPs, such as microbial activity, temperature or the Sludge Retention Time (SRT).

- *Sorption:* One important factor that determines the fate of organic contaminants in wastewater treatment systems is their distribution between the

dissolved, colloidal and particulate phases. The efficiency of pollutants removal from water is influenced by their ability to interact with solid particles, both natural (clay, sediments, microorganisms) or added to the medium (active carbon, coagulants), because this facilitates their removal by physico-chemical (settling, flotation) or biological processes (biodegradation). However, hydrophilic compounds with low sorption coefficients tend to remain in the liquid phase, which favours their mobility through the STP and the receiving environment (Ohlenbusch *et al.*, 2000).

5.1.1. Sorption onto solids

The sorption of PPCPs onto solids depends basically on their physico-chemical properties, such as lipophilicity or acidity. Drug molecules often have many functional groups (e.g., carboxylic acids, aldehydes and amines) which makes their binding capacities to solids dependent on pH or the presence of other constituents (e.g., complexation) in the solid matrix.

The sorbed amount of contaminants also depends on particle size and its roughness (specific surface). Smaller material (i.e. colloids) will possess a greater sorption affinity for PPCPs compared to sediments or suspended particles (Holthaus *et al.,* 2002; Bowman *et al.,* 2002).

A high content of organic matter in the solids enhances sorption of contaminants, since higher octanol-water partitioning coefficients lead to strong interactions with natural organics. The implication of this is that trace pollutants in water and wastewater treatment systems are likely to be found associated with colloids because in natural systems most colloids have an organic coating. Colloid-micropollutants partitioning has been well-documented between commercially available and naturally derived natural organic matter (NOM) and non-ionic contaminants such as polyaromatic hydrocarbons (PAHs) (Chefetz *et al.,* 2000; Peuravuori, 2001; Gustafsson *et al.,* 2001). More recently, colloid sorption of estrogenic substances has been investigated. For example, Bowman *et al.* (2002) determined that the distribution coefficients between colloids present in estuary systems and E2 were two orders of magnitude higher than those corresponding to sediments, and Yamamoto *et al.* (2003) concluded that NOM surrogates have significant sorption capacity for a variety of estrogenic compounds. However, differences in structure (Drewes *et al.,* 2002), elemental composition (Fujita *et al.,* 1996) and fluorophoric properties (Baker, 2001)

between NOM and wastewater organic materials have been reported, suggesting that solids present in wastewaters may have properties that either facilitate or impede the sorption of organic contaminants.

These sorptive interactions are important when a treatment strategy is designed. If a high partitioning of contaminant onto organics is expected, it might affect the treatment applied and the final use of sludge. Other point which could be considered is the sorption to colloids, since colloidal material is often difficult to remove from final effluents without advanced treatment processes, being finally discharged in the receiving waters. Finally, temporal and spatial variations in the colloidal-aqueous phase distribution can be expected during biological wastewater treatment as well as the influence of microbial degradation.

5.1.2. Sorption coefficients

In order to determine the sorbed amount of a substance to solids, several coefficients can be used with some limitations.

The octanol-water partition coefficient (K_{ow}), defined as the ratio between the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature, is often used to characterise the affinity of a given substance to organic matter. The greater this coefficient is, the higher is the affinity. This coefficient has been used to determine the effectiveness of sorption (Rogers, 1996) in the following way:

- if log K_{ow} is less than 2.5, the compound has a low sorption potential (i.e. it will not adsorb onto soil particles and will not be very lipophilic),
- if log K_{ow} is between 2.5 and 4, the compound has a medium sorption potential, and
- if log K_{ow} is greater than 4, the compound has a high sorption potential and it is very lipophilic.

The organic carbon partition coefficient (K_{oc}), defined as the ratio between the concentration of a chemical sorbed to organic carbon and in water at equilibrium and at a specified temperature, could be more adequate to characterise the partition of a given substance between the liquid and the solid phase. For example, Holbrook *et al.* (2004) stated that the affinity for E2 and EE2 by the colloidal organic carbon is greater than what would be expected from simple partitioning and the coefficients (K_{oc}) are greater than their respective K_{ow}

coefficients. These results are in agreement with Yamamoto *et al.* (2003), who observed no relationship between K_{oc} and K_{ow} coefficients for a selected set of compounds and adsorbents. Besides, Lai *et al.* (2000) reported sorption of some estrogens to sediments with zero organic carbon content. These facts limit the application possibilities of K_{ow} and K_{oc} as a sorption characteristic and requires testing each adsorbent separately.

The solid-water distribution coefficient (K_d), defined as the ratio between the concentrations of a given compound in the solid and in the aqueous phase at equilibrium conditions (Eq. 5.1), appears to be the most suitable parameter to explain the sorption processes of PPCPs (Schwarzenbach *et al.*, 2003; Ternes *et al.,* 2004):

$$K_d = \frac{X}{S} \qquad \text{Eq. 5.1}$$

where:

K_d: solid-water distribution coefficient ($L \cdot kg^{-1}$),

X: concentration in the solid phase ($\mu g\ PPCP \cdot kg\ solid^{-1}$), and

S: concentration in the aqueous phase ($\mu g\ PPCP \cdot L^{-1}$).

This coefficient takes into account the two main sorption mechanisms (Ternes *et al.,* 2004):

- *Absorption*: It is the incorporation of a substance in one state into another of a different state. In this case, it refers to the hydrophobic interactions of the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms and the lipid fractions of the sludge. It is related to the substance lipophilicity, characterised by the K_{ow} value.
- *Adsorption*: It is the physical adherence or binding of ions and molecules onto the surface of another molecule. In this case, it refers to the electrostatic interactions of positively charged groups of chemicals with the negatively charged surfaces of the microorganisms (Siegrist *et al.,* 2003; Golet *et al.,* 2003). It is related to the tend of a substance to be ionised or dissociated in aqueous phase, characterised by the dissociation constant (pK_a), which is a numeric representation of the relative proton transfer for a substance, i.e. its likelihood of donating a proton.

Considering that the organic matter fraction (volatile suspended solids, VSS) of the sludge is relevant (Schwarzenbach *et al.,* 2003), when comparing several types of sludges it is better to normalize the distribution coefficient (K_d) to the organic content (K_{do}). This normalization to the organic matter of the sludge takes into account the higher sorption potential of sludge with higher organic matter content.

Calculations with the distribution coefficients are usually suitable since the retention times are higher than the time necessary to reach the sorption equilibrium. Therefore, from equation 5.1, the distribution of a single compound between the solid and the liquid phase can be determined, as expressed by equations 5.2 and 5.3, respectively.

$$\%\ \text{solid phase} = \frac{K_d \cdot SS}{1 + K_d \cdot SS} \times 100 \qquad \text{Eq. 5.2}$$

$$\%\ \text{liquid phase} = \frac{1}{1 + K_d \cdot SS} \times 100 \qquad \text{Eq. 5.3}$$

where:

K_d: solid-water distribution coefficient ($L \cdot kg^{-1}$), and

SS: concentration of suspended solids ($kg \cdot L^{-1}$).

According to their physico-chemical properties, PPCPs can be divided into three main groups: lipophilic (with high K_{ow} values), neutral (non-ionic) and acidic (hydrophilic and ionic) compounds. Substances representative of each group have been considered in this work: two fragrances (Galaxolide and Tonalide), one tranquilliser (Diazepam), one antiepileptic (Carbamazepine) and three anti-inflammatories (Ibuprofen, Naproxen and Diclofenac). The K_d values for the substances considered in this work are indicated in Table 1.6. It can be observed that the polycyclic musks, Galaxolide and Tonalide, have the highest values of log K_d (around 3.5 $L \cdot kg^{-1}$), mainly as a result of their lipophilicity (high log K_{ow} values, around 5.3-5.9). So, the sorption of these substances will be mostly due to *absorption*. In contrast, Diclofenac, despite its low K_{ow}, also shows a quite high sorption capacity (log K_d of 1.2- 2.7 $L \cdot kg^{-1}$), which points out that the mechanism which controls the sorption in this case is different. The pK_a value of this compound (4.0 - 4.2) indicates that in aqueous phase it will be partly ionized, being the sorption governed by electrostatic interactions, i.e. *adsorption*.

But in all cases, the sorption capacity is not only dependant on the physico-chemical properties of the substance, but also on environmental conditions and solids concentration and composition.

5.1.3. Physico-chemical processes

The treatment processes applied to liquid streams have been traditionally classified in pretreatment, primary, secondary and tertiary treatment, depending on the solids size. Apart from these conventional processes, there are other treatment operations which use chemical additives to enhance the removal of contaminants, such as coagulation-flocculation and flotation.

The natural partitioning PPCPs-solids can be influenced by the presence of other substances in the medium or modified by the addition of some chemicals (coagulants, flocculants, tensoactives, etc.). In this way, the influence of physico-chemical processes on PPCPs removal during sewage primary treatment is described in this chapter.

Coagulation-flocculation

Coagulation-flocculation processes enhance the removal of suspended solids and colloids, since the high stability of these systems makes difficult their separation by spontaneous settling (Metcalf and Eddy, 1991; Degremont 1991).

The addition of metal salts and polyelectrolites (organic flocculants of cationic or anionic type) causes the agglomeration of these particles, allowing in this way their elimination by decantation or filtration (Li and Gregory, 1991). Two main effects are achieved with the addition of metal salts (mainly iron and aluminium). Firstly, they may effectively destabilize colloids, and the further addition of flocculants enhances the efficiency of large aggregates formation, easily separated by settling. Secondly, the addition of trivalent cations may also enhance the possibility of negative charged molecules removal by electrostatic interactions.

Lipophilic trace pollutants in water and wastewater treatment systems are likely to be found associated with colloids because in natural systems most colloids have an organic coating (Stumm and Morgan, 1996). In addition, positive charged molecules can be associated to these colloids by means of low strength Van der Waals bonds. Therefore, it could be expected that the addition of

coagulants would enhance the removal of those PPCPs, either lipophilic or easily ionised in aqueous phase.

Flotation processes

Flotation techniques, in which finely suspended particles are separated by adhering rising bubbles to the surface, have proved efficient, practical and reliable separation methods for the removal of fats, as well as other contaminants, such as oils, biomolecules and suspended solids from water (Zouboulis and Avranas, 2000).

The flotation process relies on the surface chemistry of the material to be separated. The naturally hydrophobic materials are ideal candidates. This process involves a number of physical phenomena simultaneously occurring with several variables influencing the process. It has been theoretically predicted that the collection efficiency of emulsions will be increased by increasing the droplet size and decreasing the bubble size (Medrzycka, 1993).

Dissolved Air Flotation (DAF) technology has been increasingly applied for fat and particle removal in water and wastewater treatment. The other flotation techniques, such as dispersed (diffusers) and electrolytic flotation, are not so commonly used because of their lower removal efficiencies. In DAF systems, tiny air bubbles attach to the fat globules or to the particles carrying them to the surface from where they are collected and finally discharged into a sludge channel. There are three basic flow sheets for DAF processes: i) total pressurization of influent wastewater, ii) partial pressurization of influent wastewater, and c) recycle pressurization, where a stream consisting of 20-50% of clarified effluent flow is being recycled, pressurized an mixed with the raw influent. The latter mode is the preferred process in most Sewage Treatment Plants (STPs). Fat droplets with size higher than 40 μm can be effectively removed by applying DAF units. In order to overcome the smaller droplet sizes and the hydrophilic nature of most wastewater contaminants, coagulants and flocculants are used as additives (Edzwald *et al.,* 1992; Valade *et al.,* 1996).

Together with solids and fat separation, other pollutants like lipophilic PPCPs can be removed from the wastewaters based on their solubilisation in the lipid fractions or sorption onto small aggregates, which can be efficiently removed by dissolved air flotation. For instance, Paxeus (2004) associated the removal of

Carbamazepine (about 50%) in a STP with the presence of an unusual high content of silicone oil in the wastewaters.

Literature information about the removal of PPCPs (not only the compounds studied in this chapter, but also concerning the other substances considered in this study) by physico-chemical processes is scarce. When some data is available, it is related to either a post-treatment (Romero *et al.,* 2003) or to drinking water treatment, and they are normally combined with other technologies, such as activated carbon or filtration (Ternes *et al.,* 2002; Boyd *et al.,* 2003; Stackelberg *et al.,* 2004). Results can not be compared since the type and content of solids and organic matter in the raw waters of drinking water facilities differs considerably from municipal wastewaters. Anyway, some of this literature is following summarized.

Romero *et al.* (2003) studied different technologies (infiltration-percolation, ring filtration, sand filtration and coagulation-flocculation plus sand filtration) after a classical activated sludge treatment. They observed that the coagulation-flocculation process (PAX-18, 40 $mg \cdot L^{-1}$) is less effective for the removal of musks (1% for Galaxolide and 32% for Tonalide) in comparison with sand or ring filtration and infiltration-percolation, up to 28, 53 and 95%, respectively. They attributed the greatest efficiency of infiltration-percolation system to the highest wastewater retention time in the biofilter, thus improving the adsorption mechanisms.

Boyd *et al.* (2003) studied the fate of some pharmaceuticals during drinking water facilities with different treatment technologies in Lousiana and Ontario, and they reported that conventional drinking water processes (coagulation-flocculation (aluminium and Percol LT22 as coagulants)/sedimentation step with PAC addition) do not remove Naproxen. Adams *et al.* (2002) reported no significant removal of selected antibiotics with aluminium or ferric salt coagulation. Similarly, Ternes *et al.* (2002) reported no significant elimination of selected pharmaceuticals, Carbamazepine (13%) and Diclofenac (4%), using ferric chloride coagulation in lab-scale experiments (≈20 $mg \cdot L^{-1}$) and investigations in waterworks (6-13 mg $Fe^{3+} \cdot L^{-1}$).

Stackelberg *et al.* (2004) reported little or no removal of HHCB, AHTN, CBZ, IBP, ROX and SMX during conventional drinking water treatment, which includes coagulation-flocculation/sedimentation with PAC addition and filtration. He stated that sorption efficiencies depend on competition with other organic

compounds; therefore, the adsorption capacity for PPCPs in a facility that processes raw water that contains substantial amounts of many naturally occurring and anthropogenic organic compounds is expected to be smaller than that in laboratory and pilot-scale experiments in which fresh activated carbon and deionized water were used.

Schäfer and Waite (2002) reported minimal removal of E1 during laboratory experiments of coagulation with ferric chloride (5-50 $mg \cdot L^{-1}$). This was expected as coagulation tends to favour the removal of large and hydrophobic compounds. They also reported low adsorption to the iron hydroxide precipitates. However, they stated that the interaction and removal may change if natural organics are present, suggesting that the experiments must be done with raw waters. Snyder (2002) investigated the influence of several drinking water technologies (PAC, O_3, H_2O_2/O_3, softening and coagulation with aluminium) on the estrogens removal. They reported elimination of E2 around 43% during coagulation process. However, no reduction was observed for EE2. Kobuke *et al.* (2002) observed that estrogenic activity was somewhat eliminated by the coagulation-flocculation process (around 50%), although not complete. This can be due to the fact that most of the components responsible for the occurrence of estrogenic activity are low molecular organic compounds, which are not removed by this process.

5.1.4. Objective

The aim of this work is to improve the removal efficiencies of three groups of PPCPs (musks, neutral and acidic pharmaceuticals), with different sorption properties, during sewage primary treatment by coagulation-flocculation and flotation processes. This objective is based on the hypothesis that the distribution of PPCPs between the solids and the aqueous phase can be modified by the addition of some chemicals (coagulants, flocculants, tensoactives, etc.). The influence of the main operational parameters, such as the type and dose of coagulant, the fat content of the wastewaters and the temperature has been studied.

5.2. Materials and Methods

5.2.1. Wastewaters

The wastewaters used were collected in the municipal STP considered in this work (Galicia, NW of Spain). The inlet to the primary clarifier was used for the coagulation-flocculation experiments, whereas the inlet to the fat separator was used for the flotation assays. The main characteristics of these wastewaters are: 500-900 $mg \cdot L^{-1}$ (TS), 200-500 $mg \cdot L^{-1}$ (VS), 100-400 $mg \cdot L^{-1}$ (TSS), 100-300 $mg \cdot L^{-1}$ (VSS), 200-800 $mg \cdot L^{-1}$ (COD_t), 100-500 $mg \cdot L^{-1}$ (COD_s) and 60-70 $mg \cdot L^{-1}$ (fat).

5.2.2. Coagulation-flocculation assays

Coagulation-flocculation assays were carried out in a Jar-Test device (Figure 5.1), in vessels of 1 liter of liquid volume (800 mL of sample). The influence of three additives was studied: ferric chloride ($FeCl_3$, 50 $g \cdot L^{-1}$), aluminium sulphate ($Al_2(SO_4)_3$, 50 $g \cdot L^{-1}$) and aluminium polychloride (PAX, 17.5% w/w). The assays were conducted at two temperatures, 12 and 25ºC, simulating winter and summer conditions, respectively. The test included an initial 3 min period of rapid stirring (150 rpm), after the addition of the coagulant and lime for neutralization, followed by 5 min of slow mixing (50 rpm) for emulsion breaking and floc formation, and finally 1 h period without mixing for floc separation. The influence of the type and dose of coagulant and the temperature was studied.

Figure 5.1. Jar-Test device.

Since the objective of the work was to enhance PPCPs removal during sewage primary treatment, all the experiments were carried out at the neutral pH necessary for the further biological process. Besides, a pH adjustment would

mean higher costs of treatment. Moreover, the parameters selected to characterise the performance of the assays were the solids and COD concentrations in the supernatant, being their removal efficiencies high enough to expect an improvement with a pH adjustment. Finally, pH could only influence the removal of anti-inflammatories (with functional groups susceptible to be protonated or deprotonated), since musks, Carbamazepine and Diazepam are non-ionic compounds (neutral pH).

5.2.3. Flotation assays

Flotation assays were carried out in a unit (Figure 5.2) consisting of a pressurized vessel of 2 L (where air is dissolved into water) and a flotation cell of 1 L (800 mL of sample) (Metcalf&Eddy, 1991). The pressurized cell has two inlets (for air and water), and one outlet for the pressurized liquid. Besides, a manometer was set up in the air line to check the pressure. The dissolved air was then introduced into the flotation cell where the fine air bubbles produced by depressurization helped the flotation of flocs. The influence of the fat content in the wastewaters and the temperature was studied. The assays were carried out in duplicate.

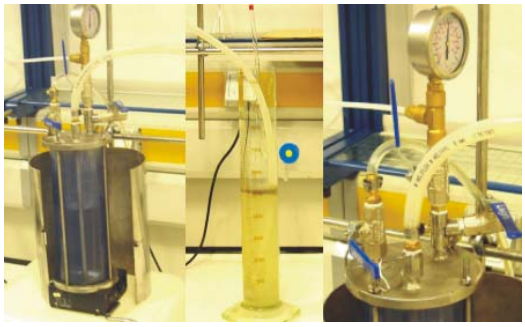

Figure 5.2. Flotation unit.

Two types of wastewaters with different concentrations of fat were used: a low fat (LF) and high fat (HF) water, with approximately 60 and 150 mg fat·L^{-1}, respectively. While the LF wastewaters were directly taken from the STP, the HF wastewaters were synthetically prepared by adding fat (as liquid butter) to the LF wastewaters spiked with PPCPs (section 5.2.4). This fact allowed evaluating exclusively the influence of the wastewater fat content, since its main characteristics (type and solids content) did not change.

The amount of fat was slowly added under stirring to 5 L of wastewater previously spiked with PPCPs, keeping the solution stirred during 2-3 hours until fat homogenisation was observed. After this time, the determination of fat content was carried out. If it was the selected (around 150 $mg \cdot L^{-1}$), the solution was ready for the flotation experiment; if not, the same procedure was repeated again.

5.2.4. PPCPs

The PPCPs considered in this work were Galaxolide, Tonalide, Carbamazepine, Diazepam, Ibuprofen, Naproxen and Diclofenac. For each experiment, two solutions of PPCPs, one containing musks plus the neutral pharmaceuticals, and the other one with the acidic substances were spiked to 10 L of urban wastewater in order to achieve a final concentration of 10-15 $\mu g \cdot L^{-1}$. Once prepared, the solution was kept during the night at 4ºC in order to get a uniform concentration in the liquid phase (equilibrium between the soluble and the sorbed amount). After that, the resulting PPCPs concentrations were measured (Table 5.1), ranging from 1.5 to 18 $\mu g \cdot L^{-1}$. These values include both the background content (already present in sewage) and the spike.

Table 5.1. Measured concentrations (in $\mu g \cdot L^{-1}$) of PPCPs in the spiked samples of urban wastewater used in coagulation-flocculation and flotation assays.

PPCP	Coagulation-Flocculation	Flotation	
		LF	*HF*
Galaxolide	2 - 4	3 - 4	1 - 2
Tonalide	1 - 3	2 - 3	1 - 2
Diazepam	10 - 13	10 - 16	7 - 12
Carbamazepine	10 - 12	11 - 13	8 -11
Ibuprofen	13 - 15	10 - 13	12 - 13
Naproxen	16 - 18	9 - 13	10 - 18
Diclofenac	14 - 18	10 - 18	12 - 23

For some substances (e.g. musks), negative differences from the theoretical concentrations were observed, which can be explained by the sorption onto the vessel walls, solids and fat. In contrast, for anti-inflammatories, the concentrations measured were higher than the theoretical values, being this fact due to the background content of the raw wastewaters used.

5.2.5. Analytical methods

TS, VS, TSS, VSS, COD and fat were analysed according to Standard Methods (APHA-AWWA-WPCF, 1999) as described in Chapter 2. pH was determined using a selective electrode and temperature with a digital thermometer.

The soluble content of the PPCPs studied was determined according to section 2.2.1 of Chapter 2. The values given for the different experiments correspond to the average value of two aliquots of each single sample.

5.2.6. Calculations

The removal efficiencies of coagulation-flocculation and flotation assays were calculated considering the initial concentration of PPCPs measured in the waters (Table 5.1) as reference and not the theoretical amount spiked because the negative differences with the theoretical amount can not be directly correlated with solids or fat removal. Therefore, the results obtained represent the minimum value since some of the initial losses are expected to be due to solids and fat sorption.

In the case of HF wastewaters, the concentration of the substance measured before the fat addition was considered, since in this case the loss can be only attributed to absorption on fat.

Summarising, the calculation of the removal efficiency in the coagulation-flocculation, LF and HF flotation experiments is indicated in equations 5.4, 5.5 and 5.6, respectively.

$$\frac{S_{ci} - S_{cf}}{S_{ci}} \times 100 \qquad \text{Eq. 5.4}$$

$$\frac{S_{LFi} - S_{LFf}}{S_{LFi}} \times 100 \qquad \text{Eq. 5.5}$$

$$\frac{S_{LFi} - S_{HFf}}{S_{LFi}} \times 100 \qquad \text{Eq. 5.6}$$

where:

S_{ci}: initial concentration in coagulation-flocculation assay ($\mu g \cdot L^{-1}$),

S_{cf}: final concentration in coagulation-flocculation assay ($\mu g \cdot L^{-1}$),

S_{LFi}: initial concentration in LF flotation assay ($\mu g \cdot L^{-1}$),

S_{LFf}: final concentration in LF flotation assay ($\mu g \cdot L^{-1}$), and

S_{HFf}: final concentration in HF flotation assay ($\mu g \cdot L^{-1}$).

5.3. Results and Discussion

5.3.1. Coagulation-flocculation assays

The coagulation-flocculation experiments were performed in three steps: i) adjustment of additive dose range (without spike of PPCPs); ii) influence of coagulant dose and temperature; and iii) influence of type of coagulant.

Selection of coagulant dose range

Preliminary assays with $FeCl_3$, $Al_2(SO_4)_3$ and PAX were performed at 12 and 25ºC without PPCPs addition in order to adjust the dose range for each coagulant. Several concentrations of $FeCl_3$ (100-500 $mg \cdot L^{-1}$), $Al_2(SO_4)_3$ (100-500 $mg \cdot L^{-1}$) and PAX (250-1250 $mg \cdot L^{-1}$) were tested and the parameters monitored were the SS and COD concentrations in the supernatant. Figure 5.3 shows the results obtained at 25ºC for each coagulant. Similar results were achieved at 12ºC (data not shown).

Although the differences are not significant in the range considered, it can be observed that the lower solids and COD content in the final supernatant was obtained in the dose range of 200-300 $mg \cdot L^{-1}$ for $FeCl_3$, 250-350 $mg \cdot L^{-1}$ for $Al_2(SO_4)_3$ and 700-950 $mg \cdot L^{-1}$ for PAX.

Influence of coagulant dose and temperature

Once adjusted the dose range for each coagulant, the next step was to study the influence of coagulant dose (on selected ranges) and temperature on PPCPs removal. Similar experiments as those carried out in the previous section were performed with $FeCl_3$ (200-300 $mg \cdot L^{-1}$), $Al_2(SO_4)_3$ (250-350 $mg \cdot L^{-1}$) and PAX (700-950 $mg \cdot L^{-1}$) at 12ºC and 25ºC.

In addition, a blank assay (an experiment without additive) was carried out in the Jar-test set-up to study the removal of these compounds merely associated with the sedimentation of solids in the beakers.

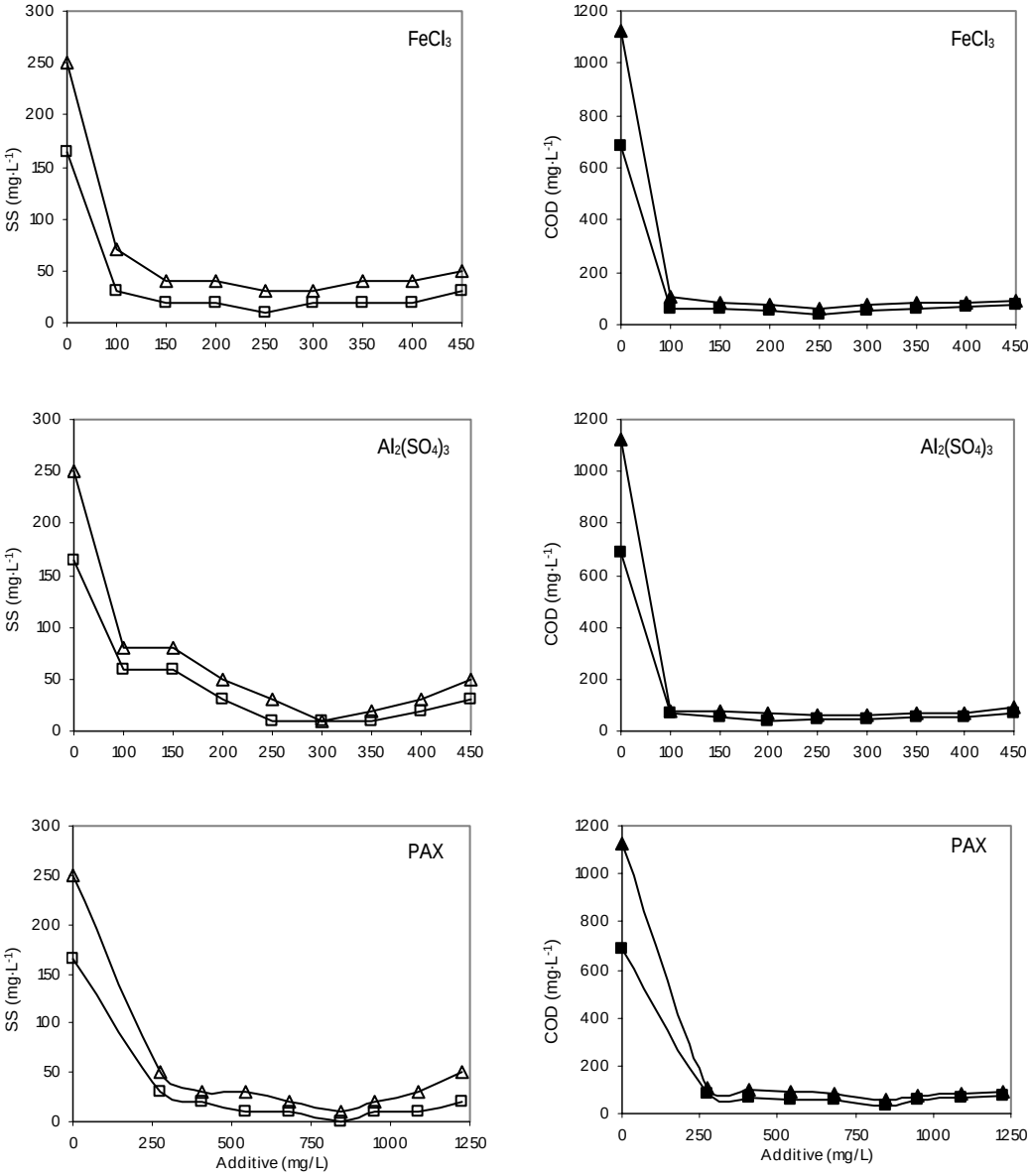

Figure 5.3. SS and COD concentrations ($mg \cdot L^{-1}$) in the supernatant after coagulation-flocculation assays at 25ºC versus coagulant dose applied. Symbols: TSS (Δ); VSS (□); COD_t (▲) and COD_s (■).

The results obtained for musks with ferric chloride in terms of average values of four doses of coagulant and the standard deviation are summarized in Figure 5.4.

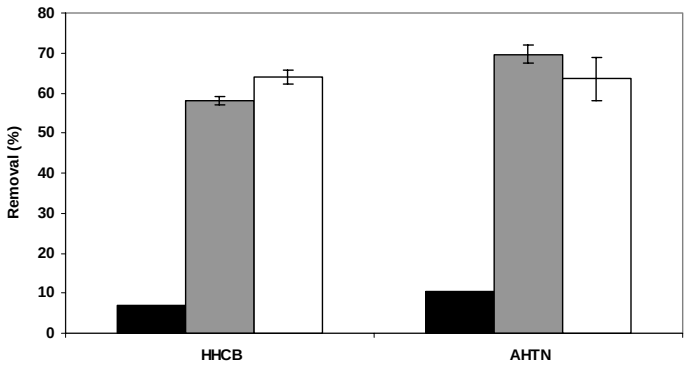

Figure 5.4. Influence of $FeCl_3$ dose and temperature on musks removal (%) after coagulation-flocculation assays. Symbols: No additive (■); $FeCl_3$ at 12ºC (■) and $FeCl_3$ at 25ºC (□).

It can be concluded that there is no significant influence (less than 5%) either of the coagulant dose or of the temperature on musks elimination during coagulation-flocculation assay with ferric chloride. Similar results were obtained for the other compounds and additives considered (data not shown).

Influence of type of coagulant

Given that the previous results were independent of the temperature and coagulant dose in the selected range (Figure 5.4), the same type of assays were repeated but only at 25ºC with the following coagulant concentrations: 250 mg $FeCl_3 \cdot L^{-1}$, 300 mg $Al_2(SO_4)_3 \cdot L^{-1}$ and 850 mg $PAX \cdot L^{-1}$. The concentrations of PPCPs in the final supernatant and the removal efficiencies obtained in the experiment are indicated in Table 5.2 and Figure 5.5, respectively.

Except for Carbamazepine (CBZ) and Ibuprofen (IBP), which were not affected by the addition of any coagulant (Table 5.2), Figure 5.5 shows that the use of additives increased the removal efficiencies of the rest of PPCPs tested compared to the blank.

Table 5.2. Concentrations ($\mu g \cdot L^{-1}$) of PPCPs in the supernatant obtained after coagulation-flocculation assays at 25ºC.

PPCP	*No additive*	*$FeCl_3$*	*$Al_2(SO_4)_3$*	*PAX*
HHCB	3.4	0.9	1.1	0.8
AHTN	2.7	0.7	0.7	0.4
DZP	15.1	7.4	8.1	9.6
CBZ	12.3	10.5	10.7	10.4
IBP	15.0	11.7	12.5	13.2
NPX	18.5	13.2	15.8	16.0
DCF	13.9	5.5	6.0	9.5

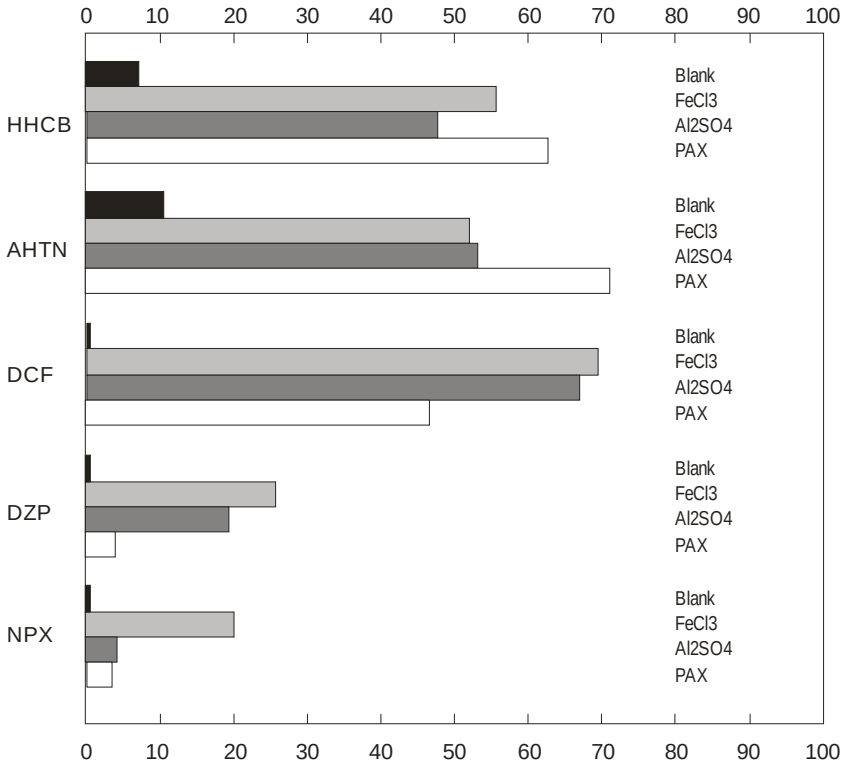

Figure 5.5. Removal efficiencies (%) of PPCPs considered after coagulation-flocculation assays at 25ºC.

In the case of musks, while ferric chloride and aluminium sulphate lead to a similar elimination of both substances (around 50%), the use of aluminium

polychloride improved the removal efficiencies up to 63% for Galaxolide (HHCB) and to 71% for Tonalide (AHTN). Conversely, the elimination of Diclofenac (DCF) was higher with ferric chloride and aluminium sulphate (around 70%), although PAX also gave a significant reduction (around 50%). The concentrations of Diazepam (DZP) and Naproxen (NPX) were reduced by 20-25%. While for Diazepam there were no significant differences between ferric chloride and aluminium sulphate, Naproxen was only removed with ferric chloride. In both cases, PAX was the less effective additive (below 5%).

Discussion

The different behaviour obtained in coagulation-flocculation assays for each compound can be explained by the different physico-chemical properties of the PPCPs considered. The mechanism which controls the PPCPs removal during coagulation-flocculation processes is the sorption onto solids. Therefore, the different eliminations obtained are related to the different affinities of the compounds considered to solids. The maximum removal efficiency expected for each substance could be estimated from its concentration in the solid (Eq. 5.2) and liquid phase (Eq. 5.3) and the solids removal efficiency (Table 5.3).

Table 5.3. Solid-liquid distribution of PPCPs based on the use of K_d values (5-10% accuracy).

PPCP	K_d ($L \cdot kg^{-1}$)	No additive		$FeCl_3$		$Al_2(SO_4)_3$		PAX	
		% liquid	*% solid*	*% liquid*	*% solid*	*% liquid*	*% solid*	*% liquid*	*% solid*
HHCB	4900	41	60	27	73	25	75	15	85
AHTN	5250	39	61	26	74	24	76	14	86
CBZ	1	100	0	100	0	100	0	100	0
DZP	44	99	1	98	2	97	3	95	5
IBP	7	100	0	100	0	100	0	99	1
NPX	7	100	0	100	0	100	0	99	1
DCF	500	87	13	78	22	77	23	63	37

Based on this approach, the good removal of musks is concordant with their high ability to attach to solid particles (log K_d values between 3.3-3.7), mainly due to their lipophilic nature, which enhances their removal by absorption. Therefore, their elimination is somehow related to the solids reduction during the experiment (around 80%). The maximum removal estimated range from 60-85%, very close to those obtained in the experiments (50-70%). These results are quite

higher than those reported by Romero *et al.* (2003) and Stackelberg *et al.* (2004) for coagulation-flocculation processes during drinking water treatment.

Diclofenac was also very well removed (log K_d ranged from 1.2-2.7), but in this case, the mechanism responsible of the elimination is different from musks. The acidic nature of this compound ($pK_a \sim 4$) provokes that in aqueous phase it remains partially ionized, which enhances its removal by means of specific electrostatic interactions, i.e. adsorption. The maximum removal efficiency expected for Diclofenac (Table 5.3) ranged from 10-35%, quite lower than those obtained in the experiments (50-70%). This fact would indicate that the coagulant enhances the binding of Diclofenac to the suspended solids throughout the trivalent cations, thus allowing a further removal from the water phase. This result differs from those reported by Ternes *et al.* (2002), who obtained no significant elimination of Diclofenac (4%) during lab and full-scale experiments with drinking water using ferric chloride.

Diazepam and Naproxen removal was also improved by the action of coagulants (20-25%), although in a lower extent than Diclofenac, which can be explained by their lower K_d values. However, the mechanisms involved in the elimination of these substances may be different. While Diazepam is a non-ionised molecule at ambient pH, thus being sorbed by means of non-ionic interactions (absorption), Naproxen contains functional groups which can be protonated and deprotonated, being then its sorption due to electrostatic interactions (adsorption). Boyd *et al.* (2003) reported no removal of Naproxen during conventional drinking water processes (coagulation-flocculation with aluminium and Percol LT22 as coagulants followed by a sedimentation step with PAC addition).

Finally, Carbamazepine and Ibuprofen were not separated at any conditions tested, which is in accordance with their very low K_d values. Similar results were obtained by Ternes *et al.* (2002) for Carbamazepine (removal of 13% with ferric chloride). The slightly different behaviour between Ibuprofen and Naproxen may be due to their slight different acidity.

The effect of pH on PPCPs removal has not been considered in this work for the reasons stated previously (Section 5.2.2.). Another point would be not having performed the neutralisation with lime during the assays. The pH value after coagulant addition ranged from 5.2-6.1 for $FeCl_3$ (250 $mg \cdot L^{-1}$), 5.4-6.2 for $Al_2(SO_4)_3$ (300 $mg \cdot L^{-1}$) and 4.6-5.8 for PAX (850 $mg \cdot L^{-1}$). Except for the lowest

value obtained with the addition of PAX, the others were one or two units below the neutral range, so no significant differences may be expected if the neutralisation had not been performed.

5.3.2. Flotation assays

The flotation experiment was performed in two steps: i) Determination of air-solids ratio (without spike of PPCPs); and ii) influence of fat content in wastewaters and temperature.

Determination of air-solids ratio

Preliminary assays were carried out to determine the pressurized liquid flow necessary to produce a proper fat separation in the flotation cell. This value was adjusted to 200 ml operating inside the pressurized cell at 6.4 atm. These conditions imply the following average air-solids ratios (A/S): 0.07 (12ºC) and 0.01 (25ºC). These values are in the range of those reported in literature (Metcalf and Eddy, 1991; Bueno *et al.*, 1997) for wastewater treatment (0.01-0.1).

Influence of fat content in wastewaters and temperature

Two types of wastewaters with different concentrations of fat were used: a low fat (LF) and high fat (HF) wastewater, with approximately 60 and 150 mg fat·L^{-1}, respectively. The temperatures were the same as in coagulation-flocculation assays, 12 and 25ºC. The assays were performed in duplicate.

Comparing the initial concentrations of PPCPs in LF and HF wastewaters (Table 5.1), it can be observed that the presence of high fat content in the wastewater caused a significant reduction in the initial soluble concentration of some substances, since both the raw wastewater and the spike of PPCPs were the same. This reduction was especially significant for musks (around 60%), but also for Carbamazepine and Diazepam (20-30%). Only the anti-inflammatories were not affected.

The concentrations of the substances in the final effluent of the two experiments are indicated in Table 5.4.

Table 5.4. Concentrations of PPCPs ($\mu g \cdot L^{-1}$) in the final effluent of flotation assays.

PPCP	Experiment 1				Experiment 2			
	12ºC		25ºC		12ºC		25ºC	
	LF	*HF*	*LF*	*HF*	*LF*	*HF*	*LF*	*HF*
HHCB	1.7	2.0	2.2	2.3	2.5	0.9	1.8	0.8
AHTN	1.6	1.5	1.6	1.9	1.9	0.7	1.3	0.6
CBZ	9.8	8.9	10.2	8.5	8.9	6.6	8.9	6.9
DZP	8.6	7.7	8.7	8.9	6.9	4.8	5.9	4.5
IBP	7.8	10.0	7.3	8.0	11.5	10.3	10.0	10.0
NPX	10.1	13.0	9.2	10.7	8.6	7.2	6.5	5.7
DCF	13.8	16.5	12.4	13.5	-	6.7	5.3	3.6

Figure 5.6 shows the average removal efficiencies of both experiments for the different PPCPs considered when LF wastewaters were used.

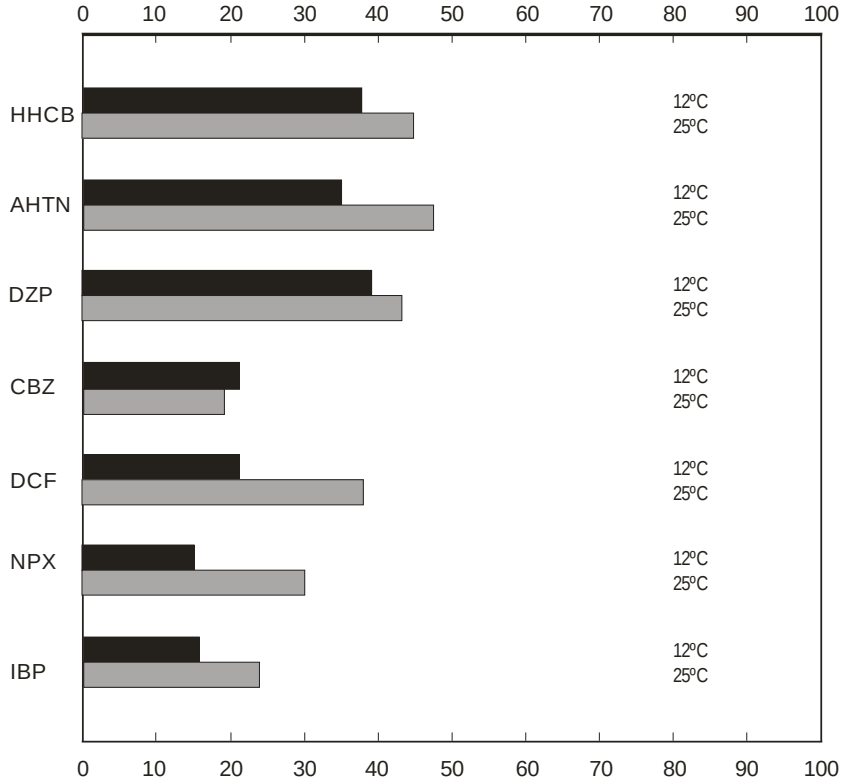

Figure 5.6. Removal efficiencies (%) from the aqueous phase obtained in the flotation assays carried out with LF wastewaters (60 $mg \cdot L^{-1}$).

It can be observed that both musks were substantially reduced at both temperatures (35-45%), with the highest removal efficiencies being obtained at 25ºC. The elimination of Diazepam was similar to that obtained for musks (around 40%), although no significant difference was observed between both temperatures. However, according to its lower lipophilicity (log K_{ow} around 2.4), Carbamazepine was removed to a lesser extent (around 20%) independently of the temperature. The anti-inflammatories were also affected by flotation, the highest removals being those obtained for Diclofenac (20-40%). For these three compounds, temperature influenced removal significantly and, as for musks, the highest values were obtained at 25ºC.

Figure 5.7 shows the average removal efficiencies of both experiments for the different PPCPs studied when HF wastewaters were used.

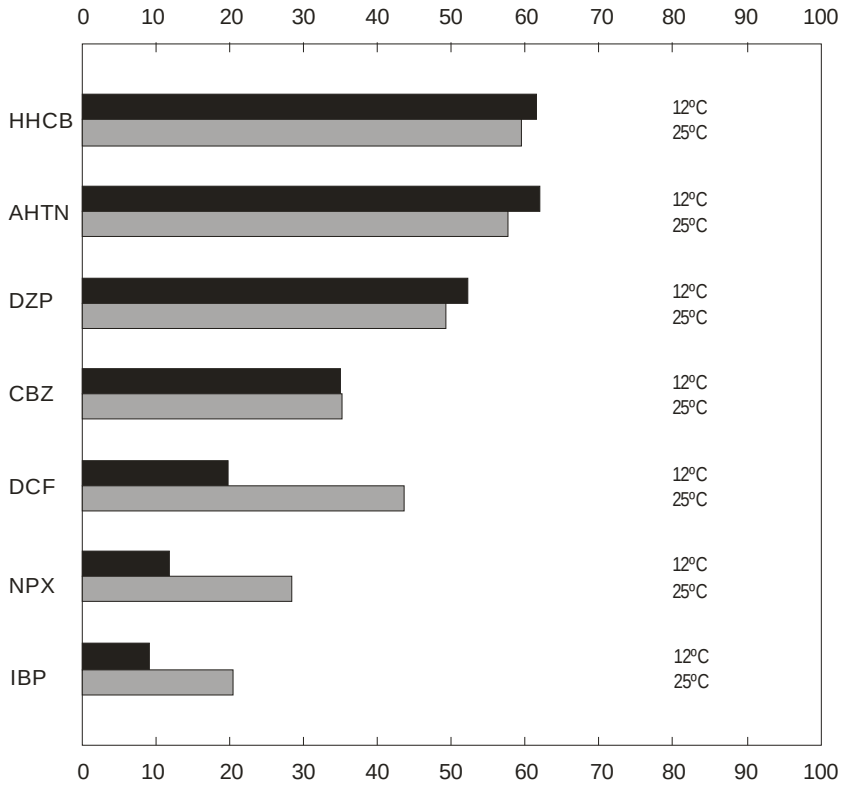

Figure 5.7. Removal efficiencies (%) from the aqueous phase obtained in the flotation assays carried out with HF wastewaters (150 mg·L^{-1}).

It can be observed that the elimination of musks is higher (around 60%) under these conditions and that temperature did not significantly influence removal. This behaviour was also observed for Carbamazepine and Diazepam, with removals increasing to 35 and 50%, respectively. As for musks, these rates were uninfluenced by temperature. Since the soluble content of anti-inflammatories was independent on the fat content in the wastewaters, their removal patterns were similar to those observed in the assays with LF wastewaters: 20-45% for Diclofenac, 10-30% for Naproxen and 10-20% for Ibuprofen. Temperature clearly influences the elimination of these compounds, being the best results obtained once again at 25ºC.

Discussion

The different behaviour obtained in flotation assays for each compound can be again explained considering their physico-chemical properties. As in coagulation-flocculation, both sorption mechanisms (absorption and adsorption) are the responsible of PPCPs removal, but in this case absorption must be highlighted.

As the PPCPs elimination during the flotation process is related to the solids and fat reduction, Figure 5.8 shows the average solids and fat removal during the flotation assays.

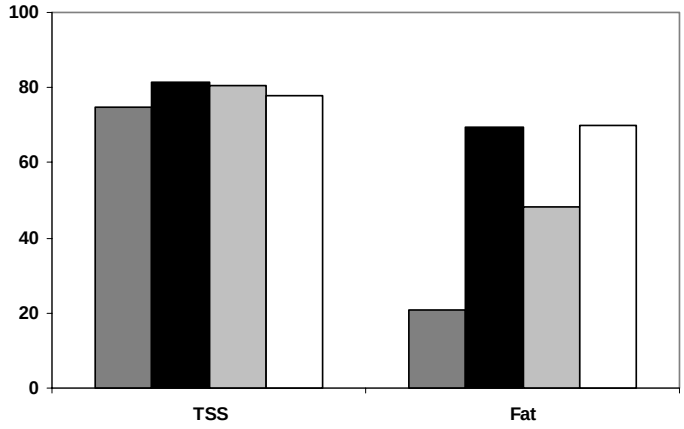

Figure 5.8. Removal efficiencies (%) of solids and fat during flotation assays with LF (60 mg·L^{-1}) and HF (150 mg·L^{-1}) wastewaters. Symbols: LF at 12ºC (■); HF at 12ºC (■); LF at 25ºC (■) and HF at 25ºC (□).

Due to their lipophilic character (log K_{ow} of 5.7-5.9), musks were very well removed with both LF (35-45%) and HF (60%) wastewaters. The mechanism involved in their elimination is the absorption rather than adsorption, thus being their removal enhanced when HF wastewaters were used (Figure 5.7). This fact is also confirmed with the greater fat reduction achieved (Figure 5.8) for HF wastewaters (around 70%) compared to LF wastewaters (20-50%). Their high lipophilicity also explains the decrease in the soluble content of both musks when the concentration of fat in the wastewaters was high (Table 5.1).

An increase in musks elimination with temperature (from 12 to 25ºC) was observed in the assay with LF wastewaters (Figure 5.6). It was probably due to the higher fat reduction achieved at 25ºC in comparison with 12ºC (Figure 5.8). This fact is also confirmed by the no effect observed with HF wastewaters, since the fat removal remained in the same level at both temperatures (around 70%).

The limited removal of Carbamazepine (20-35%) and Diazepam (40-50%) is explained by their lower lipophilicity compared to musks, with log K_{ow} of 2.5 and 2.8, respectively. These values also explain the little reduction (20-30%) observed in their initial concentrations when the concentration of fat in the wastewaters was high. For these substances, no influence of temperature was observed regardless of the initial concentration of fat.

The anti-inflammatories are widely reported as polar and highly hydrophilic compounds (Stumpf *et al.,* 1999; Zwiener *et al.,* 2002; Strenn *et al.,* 2004). As already stated, the sorption mechanism implied in the removal of these substances is by means of electrostatic interactions (adsorption). Therefore, their reduction is related to the solids elimination during the assay since fat globules are not charged. It can be observed in Figure 5.8 that the solids removal during the flotation experiments remained almost constant (around 80%), independently of fat content and temperature. This fact explains the same removal observed for these compounds, ranging from 10 to 30% for Ibuprofen and Naproxen, and slightly higher from Diclofenac (20-45%), regardless of fat concentration (Figure 5.6 and 5.7). More precisely, the lowest removal efficiencies were achieved with LF waters at 12ºC, which also corresponds with the smallest solids reduction. What it is more difficult to justify is the clear influence of temperature observed in this case, being the better results obtained at 25ºC independently of the fat content. The same explanation as for musks can not be applied, since it would justify the results obtained with LF waters but not when HF waters were used.

Besides, it was previously stated that the elimination of anti-inflammatories is more correlated with solids than with fat removal. Although it can not be confirmed because it was not measured, this difference could be due to a variation in the pH value, since it would change the ratio between the protonated and deprotonated forms of these substances, thus modifying their adsorption.

Paxeus (2004) reported removal of about 50% for Carbamazepine in one of the STPs surveyed in his study and he pointed out the presence of unusual high content of silicone oil as the main reason. So, he tried to model the removal of this compound by sorption to the sludge lipid fraction. With the data of pK_a and log K_{ow} from literature, and assuming that the sludge lipid fraction was n-octanol and no other interactions but just partition was taking place, he reported reductions of Carbamazepine of 7, 24 and 30% when the content of n-octanol is 20% (30% of VSS), 65% (all VSS are n-octanol) and 100% (when all sludge is n-octanol), respectively. However, for the anti-inflammatories, no significant elimination (1-4%) was achieved. These results confirmed the hypothesis that the mechanisms responsible for Carbamazepine and the anti-inflammatories elimination are the absorption and adsorption, respectively.

In the current study, the lipid fraction of the solids present in the wastewaters used was not determined. Anyway, the effect expected from this factor would have been the same for both assays with LF and HF wastewaters because the type and solids content was equal (the same raw wastewater was used). Only between Experiment 1 and 2 some influence could have occurred because they were carried out in different time periods and thus a variation in solids composition could have been expected. However, taking into account the standard deviations of the results obtained in Experiment 1 and 2 (less than 20%), it could be concluded that there were no significant differences between the type and solids content of both experiments.

5.4. Conclusions

Two physico-chemical treatment technologies, coagulation-flocculation and flotation, were applied for removing selected PPCPs (Galaxolide, Tonalide, Diazepam, Carbamazepine, Ibuprofen, Naproxen and Diclofenac) commonly present in sewage. The mechanism responsible for the elimination of these substances during these processes is sorption, being thus the different removal efficiencies obtained strongly correlated with the specific affinity of each

compound to solids, which is characterised by the solid-water distribution coefficient (K_d). Two different sorption mechanisms can be distinguished, depending on the type of interaction: non-ionic (absorption) or ionic/electrostatic (adsorption). Therefore, the different behaviour observed is attributed to the different chemical structures and properties of each single substance.

During coagulation-flocculation assays, influence neither of temperature nor of coagulant dose was observed for any compound tested, being the most suitable additive dependant of each substance.

In the flotation assays, the effect of temperature was negligible, except for the anti-inflammatories. The influence of the fat content in wastewaters depends on each single compound.

Musks, which have the greatest log K_d values (3.3-3.7 $L \cdot kg^{-1}$), were the substances best removed by both processes, with efficiencies up to 70% in the coagulation-flocculation assays, being PAX the most suitable additive, and up to 60% in the flotation experiments, with higher reduction when HF wastewaters were used. Their lipophilic nature points out absorption as the main mechanism involved in their elimination.

Diclofenac, which has the second greatest log K_d value (1.2-2.7 $L \cdot kg^{-1}$), was also well removed by both processes, with efficiencies up to 70% in the coagulation-flocculation assays, being $FeCl_3$ the most suitable additive, and up to 45% in the flotation experiments, regardless of the fat content in the wastewaters. Since this compound contains functional groups susceptible to be ionised, but also a quite high log K_{ow} value (4.6), its elimination must be due to both absorption and adsorption.

Diazepam, which has the next greatest log K_d value (1.3-1.6 $L \cdot kg^{-1}$), was better removed during flotation (up to 50%) than in coagulation-flocculation (up to 25% with $FeCl_3$) assays. The reason could be that the mechanism which governs its elimination is absorption since, similarly to musks, it is a neutral molecule.

Despite having similar K_d values (0.9-1.2 $L \cdot kg^{-1}$), Ibuprofen and Naproxen showed a different behaviour during coagulation-flocculation assays. While Ibuprofen was not removed at any conditions tested, Naproxen did so, although in a low extent (up to 20% with $FeCl_3$). However, both were eliminated during flotation experiment with removal efficiencies ranging from 10 to 30%,

independently of the fat content in wastewaters. Similarly to Diclofenac, these substances contain functional groups in their molecules, thus being their removal mainly due to adsorption.

Carbamazepine was the PPCP considered with the lowest log K_d value (0.1 $L \cdot kg^{-1}$). This resulted in no elimination during coagulation-flocculation assays at any conditions tested and a moderate removal during flotation experiments between 20 and 35%, being the greatest value obtained when HF wastewaters were used. Considering its non-ionised molecule, its reduction must be caused by absorption.

Summarizing the results obtained, it can be concluded that coagulation-flocculation process can be successfully applied for the removal of Galaxolide, Tonalide and Diclofenac, and in less extent, Naproxen and Diazepam. Although PAX gives the best results for musks, considering that the concentration of PAX required is quite high and that a single additive should be selected for all substances, the option of ferric chloride appears to be the most suitable.

Flotation assays can be successfully applied for the elimination of all substances considered. Taking into account that they were carried out without the addition of extra chemicals, which could have increased the removal efficiencies obtained, it seems that from an environmental and also an economic point of view (with less sludge to treat) the application of this process could be an interesting tool to achieve an important reduction of PPCPs in sewage.

This work shows that the behaviour of PPCPs when applying coagulation-flocculation and flotation processes to sewage can be different from that obtained in drinking water treatment. Moreover, it shows that a physico-chemical treatment including coagulation-flocculation and flotation units can achieve a high degree of removal of the considered PPCPs, with lipophilic, neutral and acidic characteristics. Taking into account that some PPCPs, as well as other micropollutants present in sewage, appear to be not readily biodegradable (Clara *et al.*, 2004; POSEIDON final report, 2005), enhancing their removal in the sewage primary treatment could be an interesting strategy for minimizing costs in the biological and tertiary treatment of STPs. Besides, these processes may be also useful for specific wastewaters, characterised by low volumes and higher concentrations of PPCPs (e.g. hospital wastewaters), as a pre-treatment step prior to the discharge in the municipal sewer.

5.5. References

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Chapter 6

Fate of PPCPs during anaerobic digestion of sewage sludge[1]

Summary

The behaviour of 13 substances belonging to different therapeutical classes has been studied during anaerobic digestion of sewage sludge: two musks (Galaxolide and Tonalide), one tranquilliser (Diazepam), one antiepileptic (Carbamazepine), three anti-inflammatories (Ibuprofen, Naproxen and Diclofenac), two antibiotics (Sulfamethoxazole and Roxithromycin), one X-ray contrast medium (Iopromide) and three estrogens (17β-estradiol, Estrone and 17α-ethinylestradiol). Two parallel processes have been carried out, one in mesophilic range (37ºC) and the other in thermophilic range (55ºC). The influence of temperature and Sludge Retention Time (SRT) has been analysed. Among the substances considered, the higher removal efficiencies were achieved for the antibiotics, natural estrogens, musks and Naproxen. For the other compounds, the values ranged between 20% and 60%, except for Carbamazepine, which showed no or very low (<20%) elimination. The removal of Diazepam, Diclofenac and 17α-ethinylestradiol occurred after sludge adaptation. In general, no influence of SRT and temperature on PPCPs removal was observed.

[1] **Carballa, M., Omil, F., Ternes, T.A. and Lema, J.M.** (2006). Fate of PPCPs during anaerobic digestion of sewage sludge. *Water Research,* (submitted).

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6.1. Introduction

The management of wastewater sludge from Sewage Treatment Plants (STPs) represents one of the major challenges in wastewater treatment today. The cost of the sludge treatment amounts to more than the cost of the liquid in many cases (Odegaard, 2004).

Sludge stabilization is obtained with treatments reducing their organic content (aerobic or anaerobic digestion) or blocking their fermentation ability (lime addition). Among them, the Anaerobic Digestion (AD) process has been proven to be the most efficient technology to stabilize sewage slugde (Ray *et al.,* 1990), especially for STPs with more than 20,000-30,000 p.e. The interest in this process has been focussed on an increase in the process efficiency and a reduction in the investment and operation costs.

Paulsrud and Nedland (1997) proposed a strategy for land application of sewage sludge in Norway based on the production of high quality biosolids with a low level of toxic elements and the effective control of odour nuisance and health risks. These authors cited several years of experience with six different processes for stabilisation and disinfection of sewage sludge with documented satisfactory performance, proper design and operation. Anaerobic digestion was incorporated in two of these six processes.

Advantages of anaerobic digestion include: solids reduction of up to 60%, production of renewable energy (methane) from sewage sludge and improvement of the dewatering and handling properties of digested sludge (Monteiro, 1997). Operating problems (poor process stability) caused by the slow growth of methanogenic bacteria and loss of efficiency due to foaming have been cited as ones of the most common problems in anaerobic digestion (Pitt and Jenkins, 1990; Pagilla *et al.,* 1996).

6.1.1. Mesophilic versus Thermophilic conditions

The mesophilic AD is one of the most widely adopted processes for treatment of primary and secondary sludge generated from STPs. However, because of the increased demands on sewage sludge treatment (hygienization, dewatering, storage and sludge reduction), the mesophilic process is being supplemented or complemented with a thermophilic treatment.

The mesophilic digestion usually requires over a 20-d retention time and it is not very efficient in the reduction of volatile solids and the deactivation of

pathogenic organisms, thus producing Class B sludge, which cannot be reused without site and application restrictions. To overcome these limitations, interest in thermophilic digestion has increased (Fang and Chung, 1999; Zabranská *et al.,* 2000).

The thermophilic AD brings an acceleration of the biochemical reactions, a greater degree of hygienization and a higher efficiency in the degradation of organic matter in comparison with the mesophilic process. Many mesophilic bacteria have their thermophilic homologues, but they may not be always present in the mesophilic sludge (Uemura and Harada, 1993). Such bacteria need a sufficient adaptation period after the temperature change to transform enzymes, proteins, nucleic acids, lipids and other cell components to thermophilic states. The growth rates of thermophilic bacteria are 2-3 times higher than those of mesophilic bacteria (Van Lier, 1995) and a gradual increase of methanogenic activity corresponds with increasing temperature from the mesophilic to the thermophilic range (Chen, 1983). However, the biomass yield of thermophilic bacteria is substantially lower, which may be attributed to the higher maintenance energy demands (Zinder, 1986).

The better performance of thermophilic digestion in the reduction of volatile solids and deactivation of pathogenic organisms leads to Class A sludge, which can be used without any restrictions and represents the highest quality product in terms of pathogen content and vector attraction. In contrast, the effluent quality is poor with high concentrations of Volatile Fatty Acids (VFA) that cause offensive odours (Fisher and Greene, 1945) and the process requires additional energy to heat the digester (Kim *et al.,* 2002). Furthermore, the thermophilic digestion is a little more sensitive to operational conditions, such as temperature, the organic loading rate and the characteristics of the influent sludge.

Concerning the ability to dewater the residual sludge, there is no a common trend in literature; while some authors state that the thermophilic process cause an improvement of the dewatering properties of digested sludge (Garber, 1982), others indicate the opposite (Kim *et al.,* 2002).

Although each process has its unique advantages, depending on the digestion environment, microorganisms and process configuration, all these features of the thermophilic process are of great technological importance, because they enable to operate the digestion with a higher loading rate or use a smaller volume of digester. Therefore, the change of temperature from mesophilic to thermophilic

conditions leads to a better utilization of the existing facilities and consequently avoids the overloading of the digesters. The higher degradation efficiency is connected with higher biogas production and a lower content of volatile solids in the stabilized sludge.

Tables 6.1 and 6.2 show a summary of operational conditions and yields, respectively, obtained in literature for mesophilic and thermophilic digesters.

6.1.2. Primary versus Biological sludge

Most STPs employing anaerobic digestion use common tanks for the digestion of mixtures of primary and biological sludge. The Volatile Solids (VS) reduction rate is slowed by even small additions of biological solids, particularly Waste Activated Sludge (WAS). The WAS is a dilute suspension of microbial cells and cell debris. Because the potential substrates are "membrane enclosed" within viable cells, WAS becomes more difficult to degrade compared with Primary Sludge (PS). Two serious problems are commonly encountered in the application of mesophilic AD to WAS: low VS reduction and foaming. However, thermophilic AD has been found to enhance hydrolysis of the complex biological materials of WAS (Garber*,* 1977) and to reduce foaming (Rimkus *et al.,* 1982).

6.1.3. Micropollutants

As it has been pointed out in Chapter 5, some micropollutants sorb onto the sludge solids during wastewater treatment. Depending on the efficiency of each sludge treatment technology on their removal, these compounds will be recycled with the supernatant or disposed with the sludge. Sludge solids could be applied to agricultural soil in order to improve its structure and fertility. The contaminants contained in the sludge can remain in the soil from months to years because of their sorption onto the organic, mineral and amorphous phases of the soil and their slow rates of biodegradation (Wilson *et al.,* 1997). There is evidence that certain sewage sludge-derived compounds in soil have the potential to be taken up by plants and animals and accumulate in the terrestrial food chain (Wild *et al.,* 1994), as well as to leach into the groundwater (Kreuzinger *et al.,* 2004a; Oppel *et al.,* 2004). Moreover, some of these substances have the potential to cause adverse effects on plants, soil microbes and invertebrates above certain concentrations (Jensen *et al.,* 2001).

Table 6.1. Operational conditions of mesophilic and thermophilic digesters treating sewage sludge (in $g \cdot L^{-1}$, except VFA in $mg \cdot L^{-1}$).

	TS	VS	TSS	VSS	COD_t	VFA	pH	TA	Reference
Mesophilic	37	19	4	2	23	160	7.2	3.5	Cecchi *et al.,* 1992[a]
	18	12	-	-	14	260 - 480	7.4	1.4 - 3.2	Tapana *et al.,* 2000
	20 - 50	-	-	-	-	90 - 95	7.1	3.7	Pagilla *et al.,* 1997[b]
	38	17	-	-	-	206	7.3 - 7.6	12.7	De la Rubia *et al.,* 2002[b]
	20 - 29	11 - 16	19 - 27	10 - 15	17 - 24	-	7.4	-	Zabranská *et al.,* 2000
	-	-	-	-	-	200 - 250	-	-	Han *et al.,* 1997
	-	-	-	-	-	-	-	-	Oles *et al.,* 1997
	19 - 84	12 - 57	-	-	20 - 85	456	7.4 - 8.1	-	Fujishima *et al.,* 2000
	15 - 38	-	-	-	-	-	6.8 - 7.3	-	Killilea *et al.,* 2000
	-	-	30 - 32	16 - 19	21 - 40	<50	7.1	4.0 - 4.3	Govin *et al.,* 1991
	-	14 - 19	-	-	-	482 - 676	7.6 - 7.8	5.9 - 7.0	Song *et al.*, 2004[b]
	12 - 16	8 - 11	10 - 14	7 - 10	-	192 - 227	7.0 - 7.2	2.1 - 2.5	Watanabe *et al.,* 1997
Thermophilic	65	37	-	-	-	959	7.4	4.5	Cecchi *et al.,* 1992[a]
	27 - 28	14 - 16	25 - 26	14 - 15	22 - 25	-	7.6 - 7.7	-	Zabranská *et al.,* 2000
	-	14 - 17	-	-	-	1,285 - 1,889	8.0 - 8.2	6.3 - 7.4	Song *et al.*, 2004[b]
	16 - 23	10 - 17	12 - 19	8 - 14	-	1,721 - 3,593	7.0 - 7.3	1.9 - 2.4	Watanabe *et al.,* 1997

[a]Expressed in $g \cdot kg^{-1}$; [b]VFA expressed as acetic acid.

Table 6.2. Yields of mesophilic and thermophilic digesters treating sewage sludge.

	Reduction (%)		SGP		%CH_4	Reference
	VS	*COD_t*	*Biogas*	*Methane*		
Mesophilic	27	-	0.30	-	62	Cecchi *et al.,* 1992[a]
	44 - 56	36	0.81 - 0.91	0.49 - 0.55	61	Tapana *et al.,* 2000[b]
	54 - 62	-	0.74 - 0.93	-	-	Pagilla *et al.,* 1997[b]
	50 - 54	-	-	0.40 - 0.70	-	De la Rubia *et al.,* 2002[b]
	53 - 54	-	-	-	66	Zabranská *et al.,* 2000
	32 - 47	-	-	0.50	65 - 72	Han *et al.,* 1997[b]
	48	-	0.39	-	-	Oles *et al.,* 1997[a]
	51 - 57	-	-	0.28 - 0.33	-	Fujishima *et al.,* 2000[a]
	50	-	-	-	-	Killilea *et al.,* 2000 (4 STPs)
	41	-	0.96	0.60	63	Malina, 1961[b]
	45	-	1.14	0.71	62	Toya, 1984[b]
	42	45	-	0.60 - 0.80		Govin *et al.,* 1991[b]
	35 - 52	-	-	0.41 - 0.50	62 - 67	Song *et al.*, 2004[b]
	-	-	0.54	-	66 - 67	Dohanyos *et al.,* 2004[a]
	50 - 63	-	0.50 - 0.58	0.30 - 0.35	61 - 63	Watanabe *et al.,* 1997
Thermophilic	-	-	0.19	-	65	Cecchi *et al.,* 1992[a]
	54 - 56	-	-	-	66	Zabranská *et al.,* 2000
	44	-	0.81	0.52	63	Malina, 1961[b]
	50	-	1.05	0.65	62	Toya, 1984[b]
	41 - 52	-	-	0.35 - 0.48	62 - 65	Song *et al.*, 2004[b]
	-	-	0.71	-	66 - 67	Dohanyos *et al.,* 2004[a]
	22 - 53	-	0.13 - 0.53	0.07 - 0.33	53 - 62	Watanabe *et al.,* 1997

SGP: Specific Gas Production ($m^3 \cdot kg\ VS^{-1}$); [a]Expressed per kg VS fed; [b]Expressed per kg VS destroyed.

Although some inorganic compounds, such as heavy metals, are analyzed on a routine basis, the characterization and long-term observation of organic contaminants in sludge has received little attention so far, probably due to the absence of limit values. It is well known that sewage sludge contains many xenobiotic (anthropogenic) organic chemicals which might have a negative impact on soil organisms and fertility (Klöpffer, 1996; Lega *et al.,* 1997; Halling-Sorensen *et al.,* 1998). Among these, non polar and highly lipophilic compounds like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dioxins (PCDD) and dibenzofurans (PCDF) are important contaminants as they persist for a long time in the different environmental compartments. The European Union (EU) has produced a Working Document on Sludge (EU 2000; EU 2004), in which limit concentration values in the sludge to be used on land for certain classes of compounds are proposed. These are the so-called sum of halogenated organic compounds (AOX), linear alkylbenzene sulfonates (LAS), di(2-ethylhexyl)phthalate (DEPH), nonylphenol and nonylphenol ethoxylates with 1 or 2 ethoxy groups (NPE), polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and –furans (PCDD/Fs).

Quite recently, other groups of compounds exhibiting similar chemical and physical properties like those mentioned above have been described to occur as ubiquitous contaminants. Among them, some PPCPs, such as musks and hormones, are included. The problem is that not only those substances with high partitioning to sludge are recycled with it, but also do the compounds which tend to remain in the water phase, since the water content in the sludge to be treated is very high (>90%).

A recent review published by the UK Environment Agency noted that no quantitative data were found on concentrations of pharmaceuticals in sewage sludge although this is a potential route for lipophlic substances to the terrestrial environment (Ayscough *et al.,* 2000). Surprisingly, little attention has been directed to the total estrogenic load present in the solid phases discharged from biological treatment facilities. The vast majority of studies have focused on monitoring estrogenic compounds concentrations contained in the liquid phase of sewage and effluents (Kolpin *et al.,* 2002; Komori *et al.,* 2004; Cargouet *et al*., 2004). However, based on their sorption coefficients, a relatively high percentage of these compounds are expected to partition onto solids before appreciable

degradation occurs (Layton *et al.,* 2000; Johnson and Sumpter, 2001). Similarly, there have been no reports on the estrogenic activity of biosolids following aerobic and/or anaerobic digestion of sewage sludge. Principal reasons for the lack of such data are likely the inherent difficulties associated with the analysis of sludge samples.

Effects of some PPCPs, e.g. endocrine disrupters and antibiotics, in the aquatic environment are well-documented (Petersen *et al.,* 1997; Jobling *et al.,* 2002), but not much is known about the behaviour of these compounds in soils. As said, intake by plants, leaching into the groundwater and negative impact on the terrestrial organisms are not excluded.

Another interesting question is whether the presence of these micropollutants in sewage sludge has an adverse effect on the anaerobic digestion process used for its stabilization. Anaerobic digestion is vulnerable to various organic compounds in wastewater, such as surfactants (Gavala and Ahring, 2002) and chlorinated phenols (Kim *et al.,* 1997). Methanogens are generally considered to be the most sensitive microorganism group participating in the anaerobic process and their activity is usually the rate-limiting step of the process (Speece, 1983). Thus, it is possible that PPCPs may affect methanogens physiology and growth and eventually lead to a less efficient process. The PPCPs impact on anaerobic digestion has not been studied sufficiently. Hilpert *et al.* (1981) studied the sensitivity of methanogens to 28 antibiotics (by the agar diffusion test), stating that methanogens are sensitive to some of them.

6.1.4. Objective

The objective of this chapter is to study the fate of selected PPCPs during conventional anaerobic digestion of sewage sludge in order to know not only the final amounts being discharged with the treated sludge, but also their influence, if any, on the anaerobic digestion process. Besides, the performances of the mesophilic and thermophilic digestions were examined to clarify their unique characteristics related to sewage sludge stabilization. Two parameters have been analysed: the temperature and the SRT.

6.2. Materials and Methods

6.2.1. Sewage sludge

Raw sewage sludge used in this work was collected from an urban STP located in Santiago de Compostela (NW of Spain). A mixture (70:30, v/v) of primary and secondary sludge collected from the thickener and the flotator, respectively, was used as feeding of the anaerobic digestion pilot plant. The main characteristics of this feeding are indicated in Table 6.3.

Table 6.3. Main characteristics of the raw sludge ($g \cdot L^{-1}$).

	TS	VS	TSS	VSS	COD_t	COD_s
Prim. sludge	50 - 145	25 - 85	50 - 125	25 - 70	45 - 120	1 - 8
Biol. sludge	15 - 40	10 - 35	10 - 35	10 - 30	10 - 50	1 - 7
Mixture	35 - 110	25 - 65	30 - 95	20 - 60	30 - 110	1 - 8

6.2.2. PPCPs

The fate and behaviour of the 13 substances considered in this work have been studied during anaerobic treatment of sewage sludge. Several spiking solutions containing the different substances (I-Musks, Carbamazepine and Diazepam; II-Anti-inflammatories; III-Antibiotics; IV-Iopromide and V-Estrogens) were added to the sludge mixture before feeding the anaerobic digesters in order to ensure their presence in the raw sludge. The spiked concentrations of PPCPs ranged between 4 and 400 $\mu g \cdot L^{-1}$ (Table 6.4) and they were selected according to the levels reported in literature in that moment (Table 1.9).

Table 6.4. Spiked concentrations ($\mu g \cdot L^{-1}$) of PPCPs in sewage sludge.

HHCB	AHTN	CBZ, DZP	IBP, NPX, DCF	IPM, SMX, ROX	E1, EE2	E2
400	200	20	10	40	4	8

In order to obtain a homogenous spike in the entire volume of sludge, the spiking procedure consisted of the following steps: i) 2-hour sedimentation once prepared the mixture of primary and biological sludge; ii) collection of the liquid supernatant; iii) addition of the spikes to the supernatant under continuous stirring; and, iv) return of this liquid to the sludge mixture tank with vigorous shaking of the whole tank.

6.2.3. Anaerobic digestion pilot plant

Two lab-scale (10 L) continuously stirred (Heidolph, RZR2041) anaerobic digesters have been installed and started up in February 2002 (Figure 6.1). One of them is operated in the mesophilic range (37ºC) and the other in the thermophilic one (55ºC). The temperature is maintained by hot water circulation through the external jacket of the digesters. The feeding, common for both reactors, is stored at 4ºC in a fridge, from where it is pumped to each digester using peristaltic pumps (Masterflex, 77200-52). In order to maintain the high Sludge Retention Time (SRT) required for sludge digestion (10-30 d), both pumps are programmed to feed the digesters three times per day. Simultaneously, the digested sludge is pumped out and collected in tanks. Four parameters are controlled online: Temperature (Desin Instruments, SR-RZH), pH (Desin Instruments, EPH-M12-FLAT), stirring speed and biogas production (Veiga *et al.,* 1990).

Figure 6.1. Anaerobic digestion pilot plant.

The anaerobic digestion pilot plant was started-up in February 2002. A 20% of methanogenic sludge coming from an anaerobic UASB reactor operated under mesophilic conditions with sacharose was used as inoculum. The initial amount of total COD in each reactor was around 100 g, prepared as a mixture (1:1) of primary and secondary sludge, and the final volume was completed to 10 L.

During a first period (22 days), the digesters were run under batch conditions until the elimination of this initial load was accomplished. Apart from the on-line daily measurements, the pilot plant operation was monitored in terms of solids, total COD, alkalinity (partial and total), VFA and biogas composition twice or three times per week. The next step of the start-up strategy was to apply a continuous feeding at a very low Organic Load Rate (OLR) during 1 month approximately (SRT of 50 days). More parameters were included in the monitoring of the operation, such as the soluble COD and the carbon and nitrogen content. Finally, a gradual increase of the inlet OLR was applied in order to attain the operational conditions previously established, a SRT of 30 and 20 days for the mesophilic and thermophilic digester, respectively.

The whole start-up period lasted around three months. Afterwards, the digesters started to be fed with sludge previously spiked with PPCPs and three stages of operation were applied to each digester by selecting the SRT (Table 6.5).

Table 6.5. Operational stages of the mesophilic and thermophilic digester.

	SRT (d)*	Duration (months)
Mesophilic digester	30	5
	20	4
	10	2
Thermophilic digester	20	6
	10	3
	6	2

*SRT=HRT (no purge has been made).

In each operational stage, once the steady-state was achieved (after a period corresponding to 1-2 SRT), 2-4 samples of digested sludge were taken for PPCPs analysis. All the samples were taken as 5-day composite samples preserved by refrigeration (4ºC) and with the addition of hydrochloric acid to pH<2 to stop biological activity.

6.2.4. Analytical methods

TS, VS, TSS, VSS, COD, TOC, TC, TN, TKN, VFA and alkalinity were analyzed according to Standard Methods as described in Chapter 2. The biogas production and composition was monitored as described in Chapter 2.

The soluble content of PPCPs in the digested sludge has been measured for all the compounds considered according to the methodology described in section 2.2.1 of Chapter 2. However, the concentrations in the sludge have been only determined for some of them: musks, anti-inflammatories and estrogens. In addition, some extra measurements of antibiotics, Carbamazepine and Iopromide have been performed in few sludge samples.

6.2.5. Calculations

In order to develop the PPCPs mass balances during the anaerobic digestion of the sludge, it is important to know the different factors which must be considered in the calculations. To facilitate the balance understanding, Figure 6.2 shows the flow scheme followed by the sludge through the pilot plant.

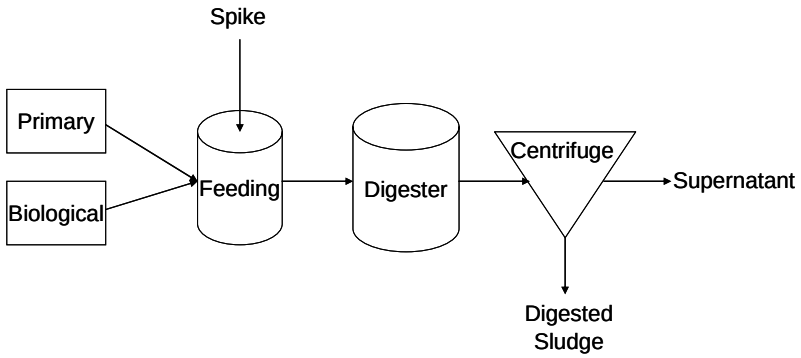


Figure 6.2. Flow-scheme of the sludge through the anaerobic digestion pilot plant.

Background concentration

Most of the PPCPs studied in this work (HHCB, AHTN, CBZ, IBP, NPX, IPM, SMX, E1, E2 y EE2) have been detected in the STP considered, and therefore they were already present in the primary and biological sludge used in the preparation of the anaerobic digesters feeding. Both types of sludge have high water content (90-97%), thus being the total PPCP concentration in the sludge the sum of the liquid and the solid contributions (Equation 6.1):

$$C_i = X_i \cdot TSS_i + S_i \qquad \text{Eq. 6.1}$$

where:

i: P (primary) or B (biological sludge),

C_i: total concentration in each type of sludge ($\mu g \cdot L^{-1}$),

X_i: PPCP concentration in the sludge (μg PPCP$\cdot kg$ TSS^{-1}),

TSS_i: total suspended solids content in each type of sludge ($kg \cdot L^{-1}$), and

S_i: PPCP concentration in the aqueous phase (μg PPCP$\cdot L^{-1}$).

The concentrations in the aqueous phase have been determined for all substances detected in the STP (Chapter 3). However, only few of them (HHCB, AHTN, IBP, E1, E2 and EE2) have been once determined in the sludge. In order to use the same approach for all compounds, it was decided to calculate the concentration in the sludge from that in the aqueous phase, using the solid-water distribution coefficient (K_d), as defined in equation 6.2.

$$X_i = K_{d,i} \cdot S_i \qquad \text{Eq. 6.2}$$

where:

i: P (primary) or B (biological sludge),

X_i: PPCP concentration in the sludge (μg PPCP$\cdot kg$ TSS^{-1}),

$K_{d,i}$: solid-water distribution coefficient ($L \cdot kg$ TSS^{-1}), and

S_i: PPCP concentration in the water (μg PPCP$\cdot L^{-1}$).

The concentrations in the effluent of the primary clarifier and the aeration tank were considered as S_P (soluble content in the primary sludge) and S_B (soluble content in the biological sludge), respectively. The average value of the four sampling campaigns described in Chapter 3 has been used.

The K_d values used in the calculations depend of each single compound:

- CBZ and IPM: the values reported by Ternes *et al.* (2004) were used. For primary sludge, the maximum value indicated, 20 $L \cdot kg^{-1}$ for CBZ and 5 $L \cdot kg^{-1}$ for IPM, was considered.
- SMX: the values reported by Theiss (2004) in biological sludge were used. Due to the lack of information, the same value was also used for primary sludge.

- HHCB, AHTN, IBP, E1, E2 and EE2: for these substances, the concentrations in primary and biological sludge have been measured during one sampling campaign (April 2002). Therefore, it was possible to calculate their K_d values in primary and biological sludge, being them used in the calculation of their background content. The K_d values obtained for musks are higher than those reported by Ternes *et al.* (2004), but similar to those indicated by Simonich *et al.,* (2002). The reason why the K_d values obtained in real STP are higher than those obtained in batch experiments could be related to the "free" sites of the sludge available to sorption. The sludge used in the batch experiments comes from real STPs, thus having already some PPCP sorbed. This means that there are fewer sites available for "new" sorption.

The total background concentration (C_{raw}) is the sum of the primary (C_P) and the biological (C_B) sludge contributions as indicated in the following expression:

$$C_{raw} = \frac{C_P \cdot V_P + C_B \cdot V_B}{V_T} = \frac{V_P \cdot S_P \cdot \left(K_{d,P} \cdot TSS_P + 1\right) + V_B \cdot S_B \cdot \left(K_{d,B} \cdot TSS_B + 1\right)}{V_P + V_B} \qquad \text{Eq. 6.3}$$

where:

C_{raw}: total background concentration ($\mu g \cdot L^{-1}$),

C_P: total concentration in primary sludge ($\mu g \cdot L^{-1}$),

V_P: volume of primary sludge (L),

C_B: total concentration in biological sludge ($\mu g \cdot L^{-1}$),

V_B: volume of biological sludge (L), and

V_T: total volume of raw sludge (L).

Inlet concentration

The total inlet concentration (C_{in}) of each PPCP is the sum of the background (C_{raw}) and the spike (C_{spike}).

Outlet concentration

Since the digesters are completely stirred, their outlet comprises the digested sludge and the aqueous phase. This effluent was centrifuged in order to simulate a high efficiency solids separation step and two streams were obtained: the liquid (supernatant), which is usually recycled to the primary treatment (water line) of

the STP, and the digested sludge, which is finally disposed. Both must be considered in the calculation of the total outlet concentration (C_{out}) of each PPCP as indicated in equation 6.4:

$$C_{out} = X_{out} \cdot SS_{out} + S_{out} \qquad \text{Eq. 6.4}$$

where:

C_{out}: total outlet concentration ($\mu g \cdot L^{-1}$),

X_{out}: PPCP concentration in the digested sludge ($\mu g \cdot kg\ TSS_{out}^{-1}$),

TSS_{out}: total suspended solids concentration in the digested sludge ($kg \cdot L^{-1}$), and,

S_{out}: PPCP concentration in the supernatant ($\mu g \cdot L^{-1}$).

The PPCPs concentrations in the supernatant (S_{out}) have been measured in all samples. However, only musks, anti-inflammatories (except Naproxen) and estrogens have been determined in most sludge samples. For the other PPCPs, except Diazepam, sludge measurements have been exceptionally performed in the last stage of operation of each digester (Table 6.3). Therefore, in those samples for which there are no sludge measurements, the sludge concentration (X_{out}) must be calculated from the K_d value and the concentration in the supernatant (Equation 6.2).

The K_d values used in these calculations were those obtained for digested sludge (Carballa *et al.*, 2006), except for Diazepam, because this compound has not been determined in the sludge samples (only in aqueous phase). Therefore, for this substance, the average between the values reported for primary and biological sludge was used (Ternes *et al.,* 2004).

Removal efficiency and accuracy analysis

Removal efficiencies for all PPCPs were calculated taking into account the total concentration at the inlet (C_{in}) and that at the outlet (C_{out}), as indicated in equation 6.5.

$$\text{Removal}\,(\%) = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \qquad \text{Eq. 6.5}$$

where:

C_{in}: total inlet concentration ($\mu g \cdot L^{-1}$), and

C_{out}: total outlet concentration ($\mu g \cdot L^{-1}$).

A statistical selection of the results obtained was performed according to the following criteria (Annex II):

- Consistency in the measurements of liquid and sludge phase.
- Consistency in the K_d values calculated.
- Liquid and sludge data confirmation: the approach followed consisted of calculating the outlet sludge concentration (X_{out}) as the difference between the inlet (C_{in}) and the outlet liquid concentration (S_{out}), which means that no degradation takes place. The same procedure was applied to calculate S_{out} from C_{in} and X_{out}. Finally, the K_d values were determined for each method and compared. In case of obtaining similar values, it would mean that degradation did not occur, and viceversa.

6.3. Results and Discussion

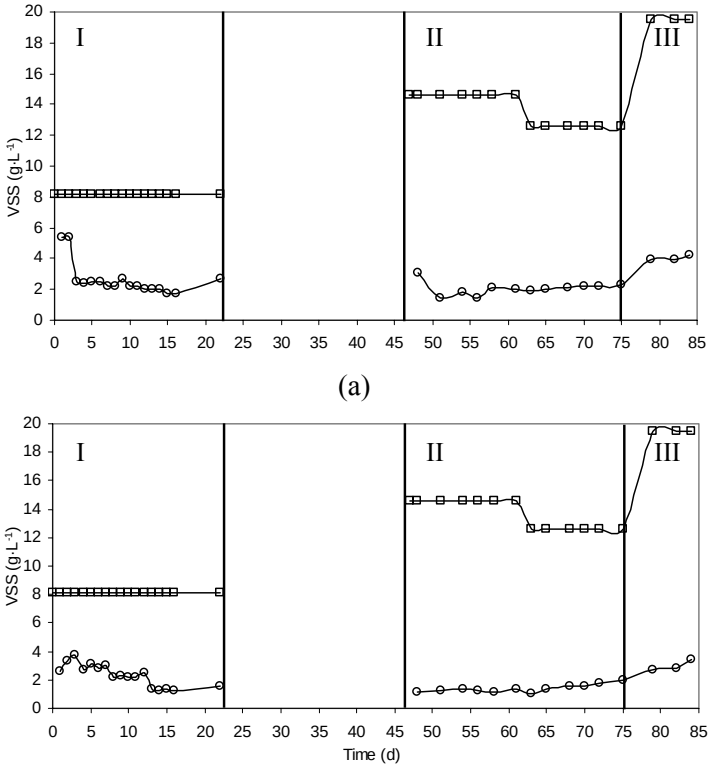
6.3.1. Start-up of the anaerobic digestion pilot plant

During the start-up of the anaerobic digestion pilot plant, apart from the on-line measurements previously mentioned, the pilot plant operation was also monitored in terms of solids, total COD, alkalinity (partial and total), VFA and biogas composition. Figures 6.3 and 6.4 show the VSS and total COD profile, respectively, in the feeding and digested sludge during the start-up period, and Figure 6.5 shows the conditions in the digesters in terms of pH, total alkalinity and VFA/TA ratio.

At the end of the batch operation (days 0-22), higher elimination of solids was achieved in the thermophilic reactor (around 80%) compared to the mesophilic one (around 65%). However, the removal of COD_t was similar in both digesters (80-85%). The pH remained in the neutral range (6.0-7.0) in both digesters (Figure 6.5); however, a small accumulation of VFA (data not shown) occurred during the first 10 days of operation of both reactors, up to 1.0 and 1.5 $g \cdot L^{-1}$ in thermophilic and mesophilic unit, respectively. This fact led to a temporary increase of the VFA/TA ratio (0.6-0.8), which decreased at the end of this period to 0.3-0.4. However, due to the low TA concentrations observed in both reactors (around 1 $g \cdot L^{-1}$), it was decided to increase this value up to 3 $g \cdot L^{-1}$ by adding sodium bicarbonate.

Once started the continuous feeding of sludge (day 47), more operational parameters were monitored, such as the soluble COD and the carbon and nitrogen content. During this stage, the elimination of solids (Figure 6.3) and COD_t (Figure 6.4) was similar in both digesters, around 85% and 90%, respectively. However, the COD_s removal (data not shown) was higher under mesophilic conditions (65% vs. 50%).

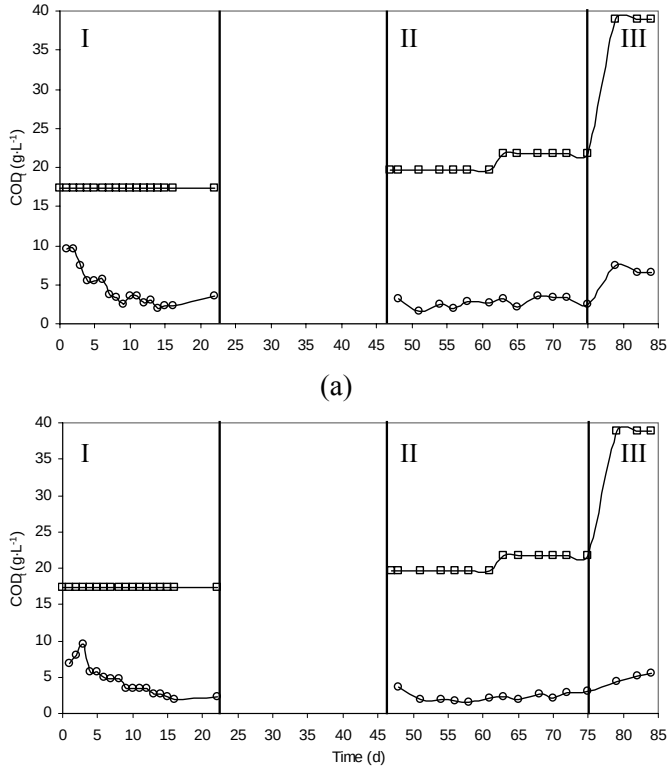
Finally, it was observed that during the last days of the start-up period (75-86), in which the OLR was gradually increased to get the first operational conditions established, the operation of both reactors remained stable.



(a)

(b)

Figure 6.3. VSS concentrations in the feeding (□) and digested sludge (o) of mesophilic (a) and thermophilic (b) digester during the start-up period. I-Bath operation; II-SRT 50 d; III-SRT 30 d.



(a)

(b)

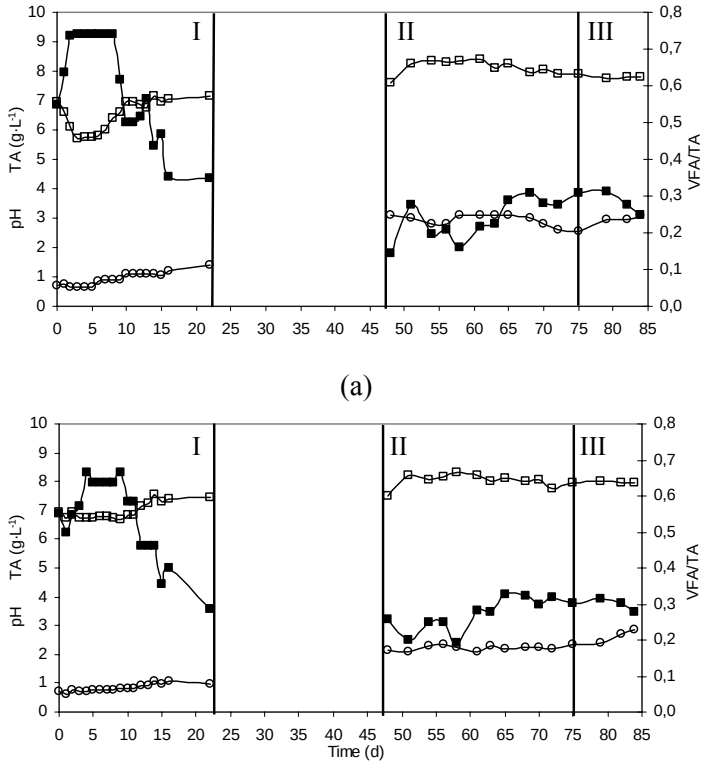
Figure 6.4. COD_t concentrations in the feeding (□) and digested sludge (o) of mesophilic (a) and thermophilic (b) digester during the start-up period. I-Bath operation; II-SRT 50 d; III-SRT 30 d.

6.3.2. Operation of the anaerobic digestion pilot plant

Once the stability of the plant was achieved, three stages of operation with different SRT were performed in each digester (Table 6.5).

Feeding characteristics

The sludge used as feeding of the anaerobic digesters was collected in a real STP, thus being its properties (solids, organic matter, etc.) dependent on the operational conditions of the STP as well as on the characteristics of the wastewaters treated. The solids and COD_t concentration profiles in primary and biological sludge during the experimental period (from February 2002 to August 2004) are shown in Figures 6.6 and 6.7, respectively.



(a)

(b)

Figure 6.5. pH (□), TA (o) and VFA/TA ratio (■) in the mesophilic (a) and thermophilic (b) digester during the start-up period. I-Bath operation; II-SRT 50 d; III-SRT 30 d.

It can be observed that TSS concentrations (Figure 6.6) ranged from 60 to 80 $g \cdot L^{-1}$ in the primary sludge except between days 400 and 500, which were higher (around 120 $g \cdot L^{-1}$). Something similar occurred in the biological sludge with average values of 20 $g \cdot L^{-1}$, except in winter 2002 and summer 2003 in which the levels were higher (35 $g \cdot L^{-1}$). The COD_t concentrations (Figure 6.7) ranged between 50-80 $g \cdot L^{-1}$ in primary sludge and 20-40 $g \cdot L^{-1}$ in biological sludge. Once again, periods with higher levels were observed.

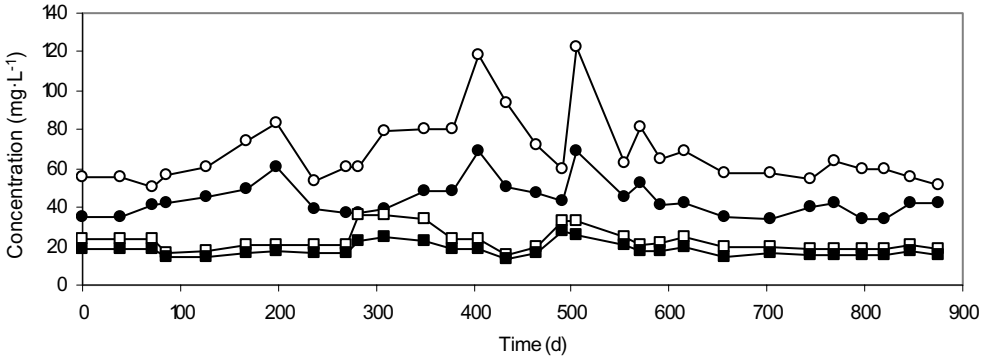


Figure 6.6. TSS and VSS concentrations in primary and biological sludge during the experimental period. (o) $TSS_{primary}$; (●) $VSS_{primary}$; (□) $TSS_{biological}$, (■) $VSS_{biological}$.

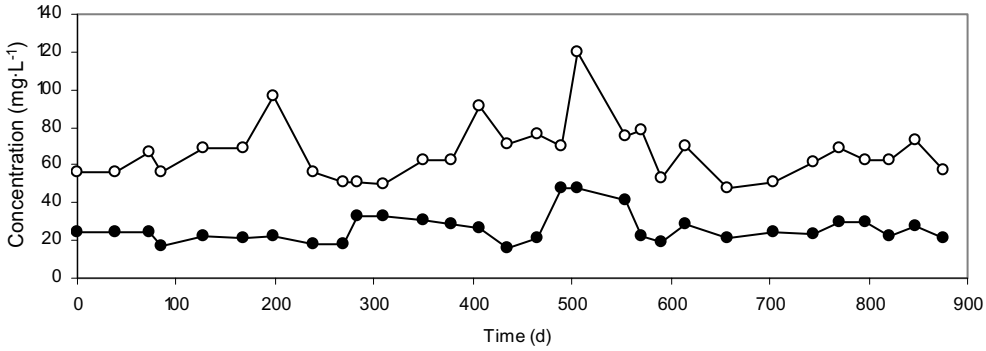


Figure 6.7. COD_t content of primary (o) and biological (●) sludge during the experimental period.

Digesters performance

During the experimental period, three stages of operation with increasing OLR were performed in the mesophilic and thermophilic digester. Figures 6.8a and 6.8b show the OLR profiles at the inlet, outlet and in the biogas of the mesophilic and thermophilic process, respectively.

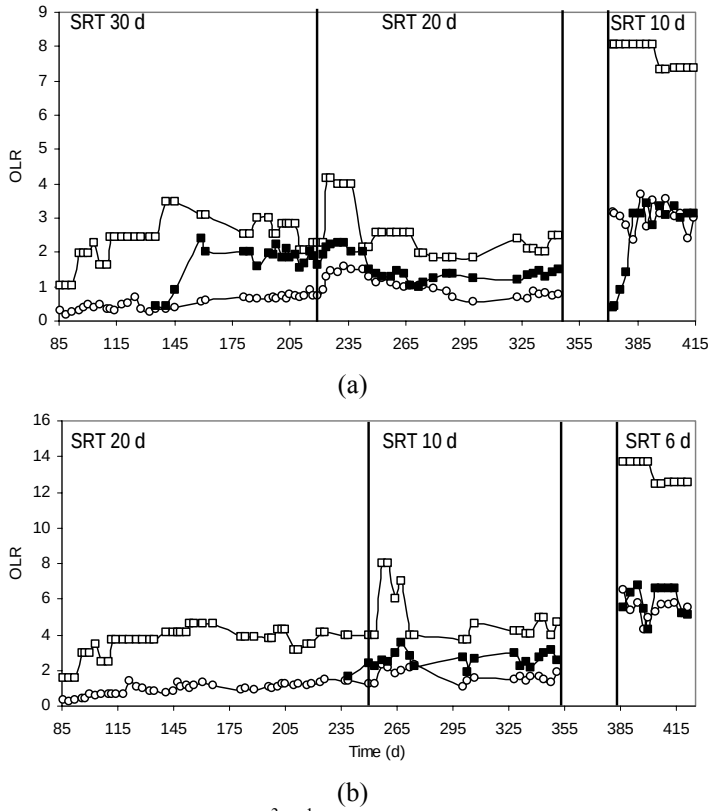


Figure 6.8. OLR (kg $COD_t \cdot m^{-3} \cdot d^{-1}$) at the inlet (□), outlet (o) and in the biogas (■) of mesophilic (a) and thermophilic (b) digester during the experimental period.

The OLR_{in} was similar during the two first stages of operation of the mesophilic (2-3 $kg \cdot m^{-3} \cdot d^{-1}$) and thermophilic (3-4 $kg \cdot m^{-3} \cdot d^{-1}$) digester. Although the OLR_{in} was different in both reactors, the values in the effluent and biogas were similar, around 1 and 2 $kg \cdot m^{-3} \cdot d^{-1}$, respectively. However, the values of OLR_{in} in the last stage were much higher, around 7-8 $kg \cdot m^{-3} \cdot d^{-1}$ for mesophilic range and 13-14 $kg \cdot m^{-3} \cdot d^{-1}$ for the thermophilic one. Accordingly, the values in the effluent and biogas were higher in the thermophilic reactor (4-6 $kg \cdot m^{-3} \cdot d^{-1}$) than in the mesophilic one (2.5-3.5 $kg \cdot m^{-3} \cdot d^{-1}$).

A summary of the operation of each digester is shown in Table 6.6.

Table 6.6a. Effluent quality and performance of the mesophilic digestion process.

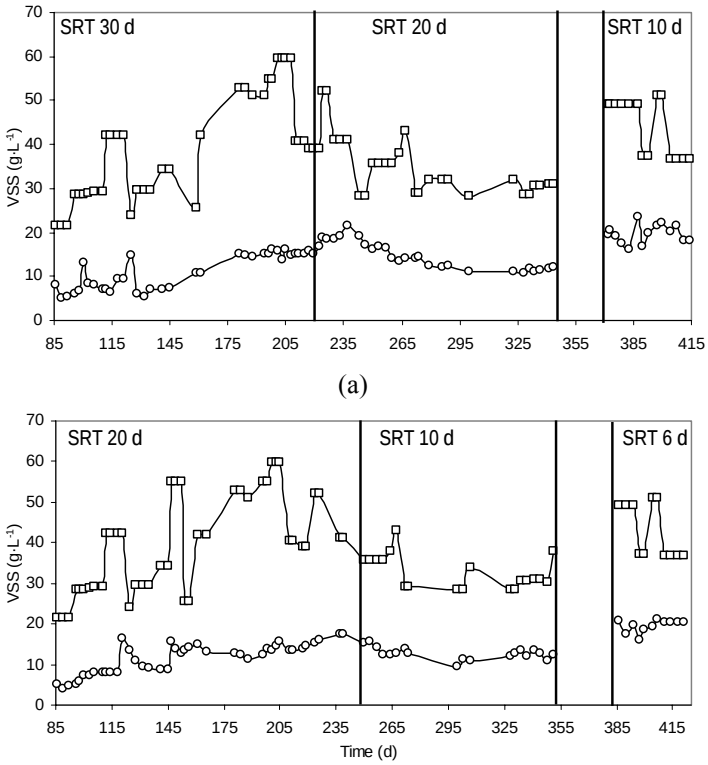
SRT (d)	**30**	**20**	**10**
OLR_{in} (kg $COD_t \cdot m^{-3} \cdot d$)	2.6 ± 0.4	2.1 ± 0.2	5.9 ± 1.4
OLR_{in} (kg $VS \cdot m^{-3} \cdot d$)	1.8 ± 0.3	1.7 ± 0.2	4.1 ± 1.3
TS ($g \cdot L^{-1}$)	28.1 ± 2.4	37.3 ± 4.3	40.2 ± 3.3
VS ($g \cdot L^{-1}$)	15.6 ± 1.4	12.6 ± 1.2	20.4 ± 2.1
TSS ($g \cdot L^{-1}$)	26.9 ± 1.3	33.4 ± 3.2	36.5 ± 3.8
VSS ($g \cdot L^{-1}$)	15.3 ± 0.7	12.3 ± 1.2	19.9 ± 2.4
COD_t ($g \cdot L^{-1}$)	21.6 ± 2.5	16.4 ± 3.0	30.5 ± 4.6
COD_s ($g \cdot L^{-1}$)	1.9 ± 0.4	1.8 ± 0.3	3.2 ± 0.8
$N\text{-}NH_4^+$ ($g \cdot L^{-1}$)	1.1 ± 0.2	0.7 ± 0.1	1.4 ± 0.2
pH	8.5 ± 0.2	7.8 ± 0.3	8.0 ± 0.3
TA ($g \cdot L^{-1}$)	7.2 ± 1.1	4.1 ± 0.6	6.2 ± 0.4
VFA/TA	0.23 ± 0.05	0.33 ± 0.10	0.30 ± 0.04
VFA (mg acetic$\cdot L^{-1}$)	44 ± 91	55 ± 81	182 ± 274
Acetic	32 ± 64	55 ± 81	133 ± 182
Propionic	12 ± 42	0 ± 1	40 ± 78
Daily production ($L \cdot d^{-1}$)	12.1 ± 1.6	10.2 ± 1.9	19.4 ± 3.6
GPR ($m^3 \cdot m^{-3} \cdot d^{-1}$)	1.21 ± 0.16	1.02 ± 0.19	1.94 ± 0.36
$\%CH_4$	63.2 ± 2.2	59.2 ± 3.7	61.5 ± 2.4
$\%CO_2$	34.1 ± 1.8	33.7 ± 2.1	31.6 ± 2.3
SGP (m^3 $CH_4 \cdot kg$ VS_{rem}^{-1})	0.67 ± 0.19	0.54 ± 0.16	0.58 ± 0.27

Table 6.6b. Effluent quality and performance of the thermophilic digestion process.

SRT (d)	**20**	**10**	**6**
OLR_{in} (kg $COD_t \cdot m^{-3} \cdot d$)	3.8 ± 0.4	4.3 ± 0.5	12.8 ± 0.5
OLR_{in} (kg $VS \cdot m^{-3} \cdot d$)	2.6 ± 0.4	3.4 ± 0.4	9.2 ± 0.4
TS ($g \cdot L^{-1}$)	36.3 ± 3.9	41.6 ± 6.1	45.5 ± 4.8
VS ($g \cdot L^{-1}$)	16.4 ± 2.2	13.2 ± 1.7	21.2 ± 2.4
TSS ($g \cdot L^{-1}$)	31.2 ± 1.9	41.5 ± 4.8	39.7 ± 2.8
VSS ($g \cdot L^{-1}$)	14.3 ± 1.6	12.0 ± 1.2	19.5 ± 1.6
COD_t ($g \cdot L^{-1}$)	23.6 ± 3.1	15.0 ± 2.1	31.3 ± 3.0
COD_s ($g \cdot L^{-1}$)	6.4 ± 1.5	3.0 ± 0.3	8.5 ± 1.0
$N\text{-}NH_4^+$ ($g \cdot L^{-1}$)	1.3 ± 0.2	0.6 ± 0.1	1.5 ± 0.1
pH	8.6 ± 0.2	7.7 ± 0.1	8.3 ± 0.2
TA ($g \cdot L^{-1}$)	7.6 ± 1.1	4.3 ± 1.0	6.2 ± 0.3
VFA/TA	0.35 ± 0.07	0.48 ± 0.11	0.39 ± 0.08
VFA (mg acetic$\cdot L^{-1}$)	873 ± 610	439 ± 324	1,065 ± 559
Acetic	329 ± 310	143 ± 193	201 ± 144
Propionic	517 ± 362	262 ± 237	711 ± 390
Daily production ($L \cdot d^{-1}$)	14.9 ± 2.1	20.2 ± 2.8	37.3 ± 5.9
GPR ($m^3 \cdot m^{-3} \cdot d^{-1}$)	1.49 ± 0.21	2.02 ± 0.28	3.73 ± 0.59
$\%CH_4$	62.4 ± 2.7	58.3 ± 3.0	66.6 ± 3.1
$\%CO_2$	33.8 ± 2.4	34.1 ± 3.7	31.3 ± 3.4
SGP (m^3 $CH_4 \cdot kg$ VS_{rem}^{-1})	0.80 ± 0.36	0.65 ± 0.24	0.54 ± 0.09

Solids reduction

Figure 6.9 shows the VSS concentrations in the feeding and digested sludge of mesophilic (a) and thermophilic (b) reactor, respectively.



(a)

(b)

Figure 6.9. VSS concentrations in the feeding (□) and digested sludge (o) of mesophilic (a) and thermophilic (b) digester.

The feeding showed the normal fluctuations related with the STP operation (20-60 $g \cdot L^{-1}$); whereas the VSS concentration in the effluent remains constant in both digesters, around 10 $g \cdot L^{-1}$ in the two first stages and up to 20 $g \cdot L^{-1}$ in the last one. These values are in the range of those reported (Table 6.1) for digested sludge (Govin *et al.,* 1991; Zabranská *et al.,* 2000).

The VSS removal ranged from 50% at the 10-d SRT to 68% at the 30-d SRT in the mesophilic range, whereas in the termophilic digester, it varied from 53% at the 6-d SRT to 72% at the 20-d SRT. These values are slightly higher than those

reported in literature (Table 6.2) for mesophilic (27-62%) and thermophilic (44-56%) digesters.

For the same VSS removal, the SRT required in the mesophilic digester is higher than that in the thermophilic one. This fact indicates that the capacity of the system is increased by using higher temperatures, which is mainly due to the higher reaction rate achieved at those temperatures.

The levels of VSS were constantly maintained throughout the operation at 15 $g \cdot L^{-1}$ for the mesophilic and 10 $g \cdot L^{-1}$ for the thermophilic process, despite the wide variation in the influent characteristics of the feed sludge, as shown in Figure 6.9. This implies that anaerobic digestion processes have large potentials for the stable reduction of VSS, which is considerably dependent on the feed sludge characteristics but it is not influenced by the temperature conditions. However, the higher VSS removal obtained in thermophilic range is due to the greater specific hydrolysis rate of this process in comparison with the mesophilic one (Maibaum and Kuehn, 1999; Song *et al.,* 2004). This difference becomes significant in relation to the decrease in the retention time.

COD reduction

Figure 6.10 shows the total COD concentrations in the feeding and digested sludge of mesophilic (a) and thermophilic (b) reactor, respectively. Once again, the feeding showed the normal fluctuations related with the STP operation (40-100 $g \cdot L^{-1}$); whereas the COD_t concentration in the effluent remained constant in both digesters, around 20 $g \cdot L^{-1}$ in the first stages and up to 30 $g \cdot L^{-1}$ in the last one. These values are in the range of those reported in literature (Table 6.1) for digested sludge (Govin *et al.,* 1991; Tapana and Pagilla, 2000; Zabranská *et al.,* 2000).

The COD_t removal ranged from 51% at the 10-d SRT to 73% at the 30-d SRT in the mesophilic range, whereas in the termophilic digester, it varied from 56% at the 6-d SRT to 69% at the 20-d SRT. Similarly to VSS reduction, for the same COD_t removal, the SRT required in the mesophilic digester is higher than that in the thermophilic one.

COD_s values in the effluent of the thermophilic process were a little more dependent on the change in the feed characteristics and they were higher than those of the mesophilic process (Figure 6.11). The average values of COD_s during the two first stages of operation were 2 and 6 $g \cdot L^{-1}$, for the mesophilic and

thermophilic digester, respectively, which are in the same range as those reported in literature (Zabranská *et al.,* 2000; Song *et al.,* 2004). However, an increase of these values was observed in the last stage of both digesters, up to 4 $g \cdot L^{-1}$ in mesophilic range and 10 $g \cdot L^{-1}$ in the thermophilic one.

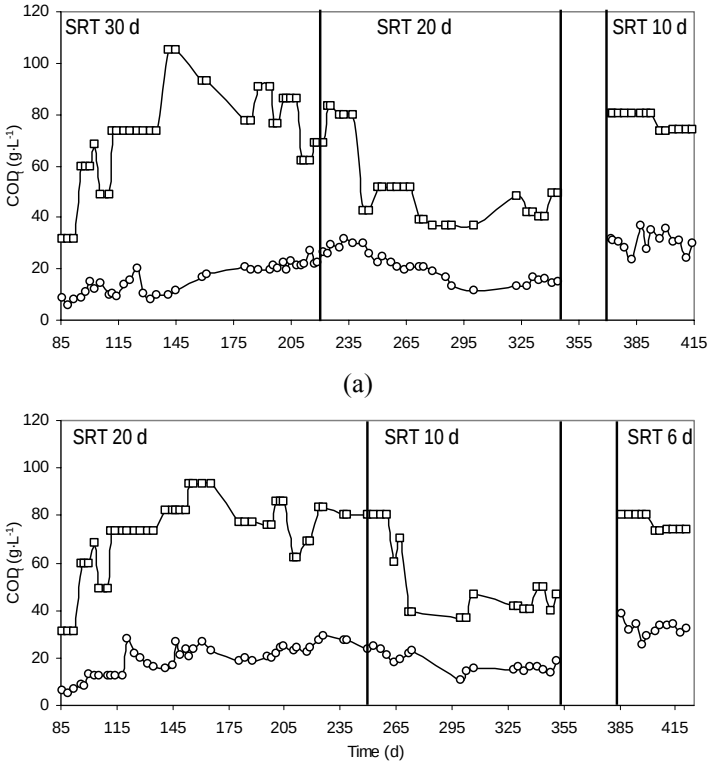


Figure 6.10. COD_t concentrations in the feeding (□) and digested sludge (o) of mesophilic (a) and thermophilic (b) digester.

VFA, alkalinity and pH

The pH value remained basically constant during the experimental period in both digesters (Figure 6.12). The pH of the thermophilic process (Figure 6.12b) was slightly higher (around 8.5) than that of the mesophilic one (Figure 6.12a), which was 8.0 approximately.

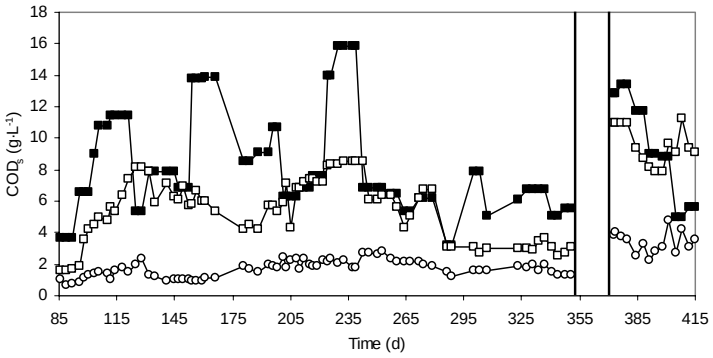


Figure 6.11. COD_s concentrations in the feeding (■) and digested sludge of mesophilic (o) and thermophilic (□) digester.

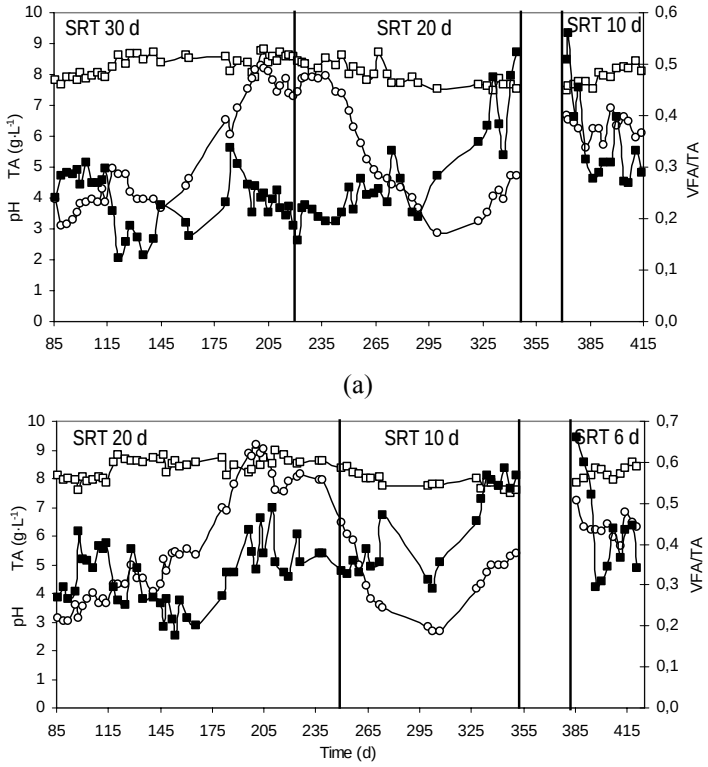


Figure 6.12. pH (□), TA (o) and VFA/TA (■) ratio in the mesophilic (a) and thermophilic (b) digester.

During the experimental period, the alkalinity level in the thermophilic digester (Figure 6.12b) was slightly higher than that in the mesophilic one (Figure 6.12a). The average values of TA in each stage of operation varied from 4.1-7.2 and 4.3-7.6 $g \cdot L^{-1}$ for the mesophilic and thermophilic process, respectively. The higher values obtained in the thermophilic digester explain the also higher pH values for this process.

The increased alkalinity, and thus pH, in the thermophilic digester is in agreement with previous studies (Gallert and Winter, 1997; Yu *et al.,* 2002; Song *et al.,* 2004).

It is well known that the alkalinity in anaerobic digestion can be generated from the degradation of nitrogenous organic compounds, sulphate reduction, release of ortho-phosphate and increase of VFAs (van Haandel, 1994; Munch and Greenfield, 1998). In this study, only the ammonia nitrogen and VFA concentrations were measured and their concentrations in the digesters during the experimental period are shown in Figure 6.13 and 6.14, respectively.

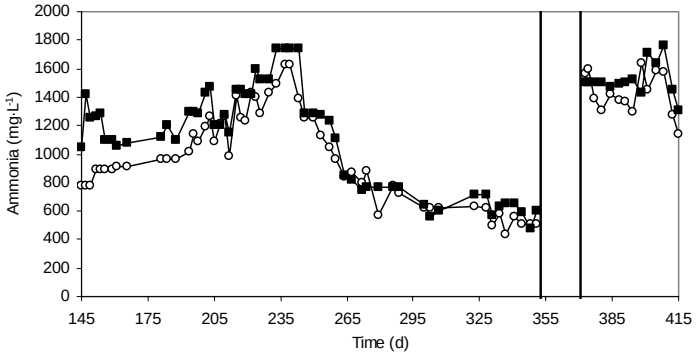


Figure 6.13. Ammonia nitrogen concentrations in the mesophilic (o) and thermophilic (■) digester.

The ammonia nitrogen concentrations were higher in the thermophilic digestion process (400-1,800 $mg \cdot L^{-1}$) than those of the mesophilic one (400-1,600 $mg \cdot L^{-1}$). This indicates that the activity for the degradation of nitrogenous organic compounds under thermophilic conditions was higher than that under the mesophilic ones (Sánchez *et al.,* 2000). The maximum values of ammonia nitrogen concentration in both mesophilic (1,600 $mg \cdot L^{-1}$) and thermophilic (1,800 $mg \cdot L^{-1}$) reactors exceed the threshold level (1,000 $mg \cdot L^{-1}$) considered as

inhibitory for methane production (Hashimoto, 1986; Koster and Lettinga, 1988). However, it is also reported that methanogenic bacteria can acclimate to ammonia nitrogen concentrations up to 3,100 $mg \cdot L^{-1}$, thus minimizing potential toxic effects on the methane production (Koster and Lettinga, 1988; Fujishima *et al.,* 2000).

The increase in ammonia nitrogen content observed between day 200 and 250 (Figure 6.13) explains the also higher TA values obtained (Figure 6.12) in these days. Taking into account that each alkalinity-equivalent corresponds to 50 g of calcium carbonate (commonly used to express the alkalinity), and that ammonia nitrogen has one equivalent per mol, each gram of ammonia nitrogen would lead to an increase of alkalinity of 2.8 g, approximately. This fits quite well with the behaviour observed in this study, since the increase in ammonia nitrogen content was about 800-1,000 $mg \cdot L^{-1}$, which would correspond with 2.2-2.8 g $CaCO_3 \cdot L^{-1}$. The increase observed in the TA was around 3-4 g $CaCO_3 \cdot L^{-1}$.

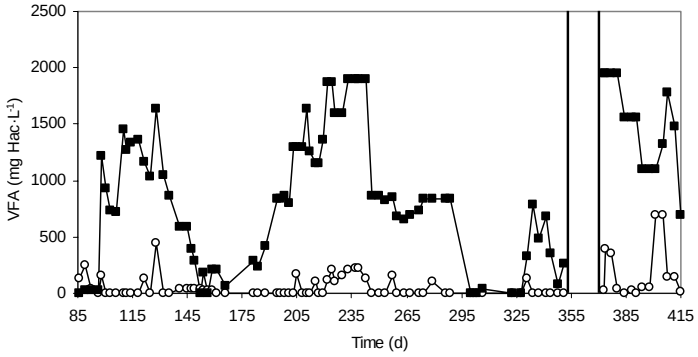


Figure 6.14. VFA concentrations in the mesophilic (o) and thermophilic (■) digester.

The VFA level in the thermophilic process was generally higher than that in the mesophilic one (Figure 6.14), which is consistent with the COD_s data. This clearly shows that the mesophilic digestion was superior to the thermophilic one in terms of effluent quality, which can be explained by the low substrate affinity of some thermophilic organisms (Fang and Chung, 1999; Kim *et al.,* 2002). The main component of the VFA in the mesophilic process was acetic, but in the thermophilic process it was propionic (Table 6.6). From literature (Fang and Chung, 1999; Kim *et al.,* 2002), the higher level of propionate in the thermophilic digester occurred under higher hydrogen partial pressures and the acetate from

higher organic loading rate conditions. This indicates that acetogens and hydrogenotrophs under thermophilic conditions are more sensitive to changes in their environments. The VFA content of the COD_s weas around 3.5% (10.3% maximum) and 13.6% (maximum 23.8%) for the mesophilic and thermophilic digestion process, respectively. These values are lower than those reported by Song *et al.* (2004), 22.7% for the mesophilic reactor and 30.3% for the thermophilic one.

The VFA-to-alkalinity ratio indicates the buffering capacity of the system for a rapid change of pH (Figure 6.12). It has been reported that the buffering capacity was sufficient when the VFA-to-alkalinity ratio was maintained below 0.4 (Zhao and Kugel, 1996). In this study, the VFA/TA ratio of the mesophilic process was almost constant, around 0.15-0.35. However, in the thermophilic digester, the VFA/TA values were slightly higher (0.20-0.50) as well as more unstable than in the mesophilic one. This is mainly due to the higher VFA concentrations observed in the thermophilic reactor (Figure 6.14), which indicate that the single-stage mesophilic anaerobic digestion could have better buffering capabilities than the thermophilic digestion. An increase of VFA/TA values was observed in both digesters at the end of stage II and beginning of stage III, up to 0.55 in the mesophilic process and 0.65 in the thermophilic one. This increase was probably a result of the decrease in the TA (Figure 6.12) since no accumulation of VFA was observed (Figure 6.14). Finally, the values stabilised at 0.30-0.40 during the last days of the experiment.

Gas production and methane content

Daily biogas production varied from 10.2 $L \cdot d^{-1}$ at 20-d SRT to 19.4 $L \cdot d^{-1}$ at 10-d SRT in the mesophilic process, and from 14.9 $L \cdot d^{-1}$ at 20-d SRT to 37.3 $L \cdot d^{-1}$ at 6-d SRT in the thermophilic one. Therefore, the Gas Production Rate (GPR) of the thermophilic process (1.5-3.7 $m^3 \cdot m^{-3} \cdot d^{-1}$) is higher than that of the mesophilic one (1.0-1.9 $m^3 \cdot m^{-3} \cdot d^{-1}$). These values are in the range of those reported in literature (Table 6.2).

The average methane content of the biogas from the mesophilic process was slightly higher, around 65%, than that of the thermophilic one (60%). Although these results are contradictory with the pH and alkalinity values, they are explained by the higher influence of carbon dioxide solubility, which is lower under thermophilic conditions (Gallert and Winter, 1997). Other studies state that

the methane content of the biogas was mainly affected by the type of substrate, rather than the temperature conditions (Zabranská *et al.*, 2000; Ahn and Forster, 2000). Figure 6.15 shows the Specific Gas Production (SGP), based on the VS removed, in both digesters during the experimental period. The average SGP of the thermophilic process (632 L CH_4·kg VS_{rem}^{-1}) was higher than that of the mesophilic one (591 L CH_4·kg VS_{rem}^{-1}) (Table 6.4). Malina (1961) and Toya (1984) also found similar results. However, Song *et al.* (2004) reported the opposite attributing the lower SGP of thermophilic process to the higher maintenance energy of the anaerobic thermophilic microorganisms (Borja *et al.,* 1995: Kim *et al.,* 2002) as well as the higher hydrogen content of the biogas (Gallert and Winter, 1997). These authors also stated that the specific methane yield of the mesophilic process is a little more sensitive to the influent characteristics of the feed sludge, thus indicating the higher capacity of mesophilic methanogens for coping with the variation of the influent characteristics compared to the thermophilic ones. But this behaviour was not observed in this study.

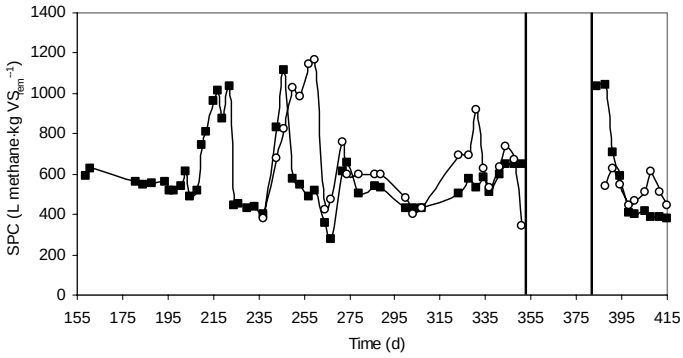


Figure 6.15. Specific methane production in the mesophilic (■) and thermophilic (o) digester.

Discussion

The sludge feeding composition was not constant and dependant on the operation of the sludge train in the sewage treatment plant. Average solids concentration in the feed was kept around 50 g·L^{-1}. This value appears to be the optimum (Killilea *et al.,* 2000), as lower concentrations result in less efficient solids removal in the digesters and higher concentrations tend to cause mechanical problems with pumps, heat exchangers and mixing units.

Despite the variation of the feeding characteristics, the operation of both reactors was stable. Even if the operative conditions applied in the thermophilic digester were more drastic respect to the mesophilic ones, the process was stable in terms of the organic loading rate (OLR), as can be seen when considering the values of the stability parameters reported (Figure 6.12).

The higher VFA concentrations observed in the thermophilic range seem to make no influence on the process stability: the pH and TA values were in the normal ranges for these conditions during the whole experimental period (Figure 6.12b). IWPC (1979) stated that if digestion is proceeding satisfactorily, pH control is unnecessary since the natural buffering capacity of the digested sludge (based on bicarbonate and ammonia ions) usually maintains the pH close to the optimum of 7. pH correction is usually not necessary except at the start-up or after a shock feed to the digester.

The difference between the thermophilic and mesophilic output sludge volatile solids was not significant and did not reach the expected value corresponding to the increment of specific methane production in the thermophilic digester. Zabranská *et al.* (2000) studied the activity of anaerobic biomass in thermophilic and mesophilic conditions at different loading rates and they found that the activity of thermophilic sludge was always higher than that of mesophilic sludge. Besides, the maximum degradation capacity of the thermophilic sludge is reached at a much higher loading (3 g COD·g VSS^{-1}) in comparison with 1 g COD·g VSS^{-1} for the mesophilic sludge. Therefore, the thermophilic sludge was more stable in the same situation and could degrade the substrate without a lag phase and with a higher methane content (52%). The kinetic test of the production and subsequent utilization of volatile fatty acids also proved the higher specific degradation capacity, the concentration of VFA under thermophilic conditions being always lower and utilized in a shorter time due to accelerated conversion ability.

As a summary, Figure 6.16 shows the removal efficiencies of volatile solids and COD in the mesophilic and thermophilic digester during the experimental period.

It can be concluded that the higher organic load rate (OLR) applied, the lower removal efficiencies obtained in terms of solids and COD under both mesophilic and thermophilic conditions. The solids removal efficiency decreases from 70% to 50%, approximately, when lower SRT is used, independently of temperature.

However, when both digesters were operated at the same SRT, the influence of temperature on solids removal was clear, being the better efficiencies achieved when the temperature was higher, i.e. thermophilic range.

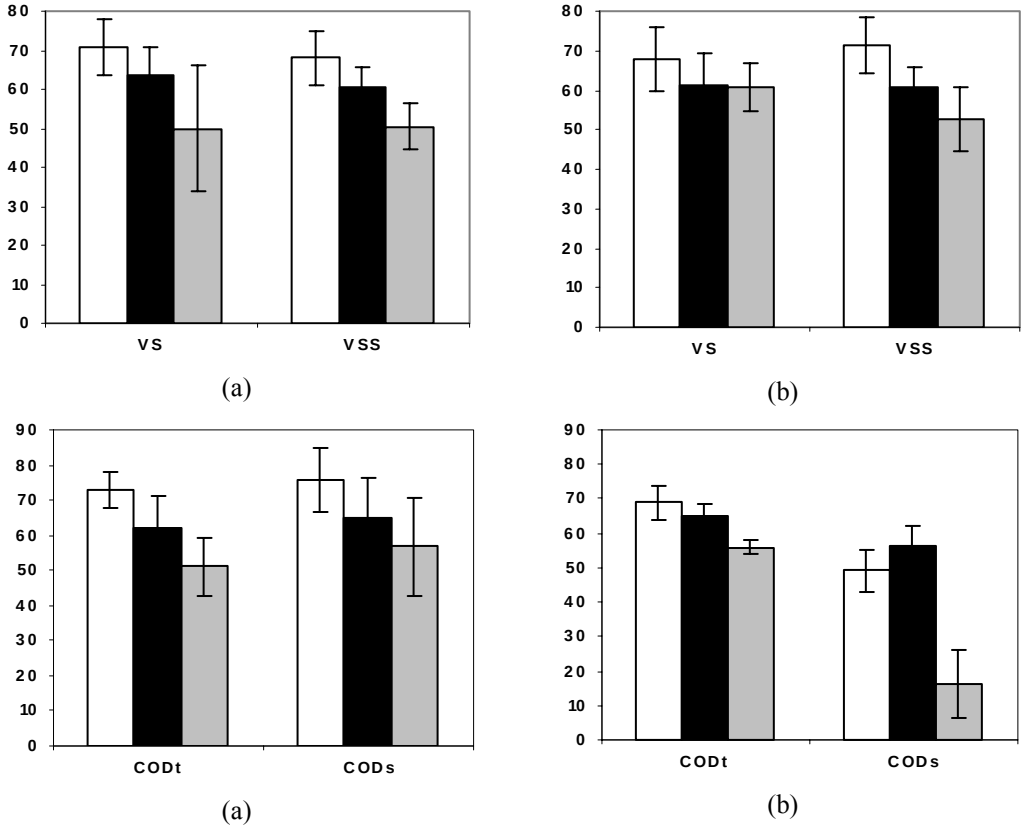


Figure 6.16. Volatile solids and COD removal (%) in the mesophilic (a) and thermophilic (b) digester. Mesophilic: □ SRT 30 d; ■ SRT 20 d; ■ SRT 10 d. Thermophilic: □ SRT 20 d; ■ SRT 10 d; ■ SRT 6 d.

While COD_t removal decreases from 70% to 50-55% when increasing OLR, independently of temperature, the reduction in COD_s removal efficiencies is more important in thermophilic than in mesophilic conditions, from 50% to 20% and from 75% to 55%, respectively. Similarly to solids, when both reactors were operated at the same SRT, the COD_t removal was slightly higher in the thermophilic process; however, the elimination of COD_s was similar in both reactors or slightly higher in the mesophilic range.

Therefore, it seems possible to conclude that the thermophilic anaerobic digestion process can be advantageously applied to sewage sludge. At least, the data reported here demonstrate the stability of the process at low SRT and the higher degree of stabilization of the digested sludge.

Operational problems

The main difficulty was related to the sludge pumping. The high concentrations of solids present in the feeding obstructed the tubes and caused mechanical problems in the pumps. The solution was to feed the digesters manually three times per day.

Another problem was the availability of primary sludge, causing that sometimes the feed consisted only of secondary sludge, which is characterized by a lower biogas potential. Wise (1983), referring to the study of Hang *et al.* (1978), reports that at the same value of OLR and at 35ºC the specific methane production (m^3 of methane per kg of VS fed to the digester) was 0.44 for primary sludge and 0.19 for secondary sludge. Moreover, in terms of VS removal, the yields reported were 66.3% for primary sludge in front of 26.2% for activated sludge.

The digesters were mechanically stirred. However, if the stirring is not very efficient, the sludge retention time can be higher than the hydraulic one (Cecchi *et al.,* 1992), thus affecting the operation.

Finally, indicate that bulking/foaming, a problem which often appears during anaerobic digestion of sludge, was rarely observed in the thermophilic reactor. In contrast, it appeared in the mesophilic one. Pagilla *et al.* (1997) studied the causes and effects of foaming in anaerobic digesters and they found that it can be attributed to the presence of filamentous bacteria in the activated sludge. Chemical treatment of the activated sludge eliminated the bulking problem and additional chemical treatment was required to eliminate the foaming problem in the digester.

6.3.3. Fate of PPCPs during anaerobic treatment of sludge

For each stage of digesters operation, the mass balance of PPCPs was performed following the method described in section 6.2.5. The results obtained are shown in the following sections.

Background concentration

For those compounds detected in the STP considered, the background concentration in the raw sludge was determined. Table 6.7 shows the values of the different parameters used in the calculations as well as the total concentration in the raw sludge (C_{raw}).

Table 6.7. Background content of PPCPs in the raw sludge. TSS ($g \cdot L^{-1}$); log K_d ($L \cdot kg^{l}$); S ($\mu g \cdot L^{-1}$); X ($\mu g \cdot g^{-1}$); C ($\mu g \cdot L^{-1}$).

	Primary sludge			***Biological sludge***			
TSS	54 - 83			17 - 36			C_{raw}
	$log\ K_{d,P}$	S_P	X_P	$log\ K_{d,B}$	S_B	X_B	
HHCB	4.2	1.6	25.4	4.1	1.0	12.6	1,286 ± 37
AHTN	4.0	0.8	8.0	4.3	0.5	10.0	449 ± 14
CBZ	1.3	0.3	0.01	0.1	0.3	0.0	0.50 ± 0.01
IBP	1.6	4.3	0.2	2.4	0.4	0.1	12
NPX	1.6	3.2	0.1	2.4	1.1	0.3	11
IPM	0.7	7.2	0.04	1.0	8.8	0.09	10
SMX	2.4	0.6	0.16	2.4	0.3	0.06	9
E1	2.8	0.003	0.002	2.9	0.003	0.002	0.12
E2	4.0	0.002	0.024	4.0	0.001	0.010	1.20 ± 0.04
EE2	3.6	0.001	0.004	3.8	0.001	0.006	0.23 ± 0.01

The musks were the substances with the highest concentrations in the raw sludge, around 1,300 $\mu g \cdot L^{-1}$ for Galaxolide and 450 $\mu g \cdot L^{-1}$ for Tonalide. The concentrations of Ibuprofen, Naproxen, Iopromide and Sulfamethoxazole were similar (around 10 $\mu g \cdot L^{-1}$); while the levels of the other compounds detected (Carbamazepine and estrogens) were lower (< 1 $\mu g \cdot L^{-1}$).

Inlet concentration

The total inlet concentration (Table 6.8) is the sum of the background (Table 6.7) and the spike (Table 6.4). With the exception of fragrances, the C_{in} for the other compounds ranged between 4 and 50 $\mu g \cdot L^{-1}$. The contribution of the background concentration was lower than 25% except for Ibuprofen and Naproxen, which was 50%, and for Galaxolide and Tonalide whose concentrations in the raw sludge were four and two times higher, respectively.

Table 6.8. Spiked, background and total concentrations (in $\mu g \cdot L^{-1}$) of PPCPs in the feeding of the anaerobic digesters.

	C_{spike}	C_{raw}	C_{in}
HHCB	400	1,286 ± 37	1,686 ± 37
AHTN	200	449 ± 14	649 ± 14
CBZ	20	0.50 ± 0.01	≈ 20
DZP	20	n.d.	20
IBP	10	12 ± 0	≈ 22
NPX	10	11 ± 0	≈ 21
DCF	10	n.d.	10
IPM	40	10 ± 0	≈ 50
SMX	40	9 ± 0	≈ 49
ROX	40	n.d.	40
E1	4	0.12 ± 0.00	≈ 4
E2	8	1.20 ± 0.04	≈ 9
EE2	4	0.23 ± 0.01	≈ 4

n.d.: not detected.

Outlet concentration

The PPCPs concentrations measured in the liquid (supernatant) and solid phase (digested sludge) are shown in Annex II.

A statistical selection of data has been carried out following the criteria explained in section 6.2.5. The values dismissed have been highlighted.

Mass balance results

Table 6.9 shows the results of PPCPs mass balance in the mesophilic and thermophilic process for each experimental period. The error was calculated as the standard deviation when the number of data was higher than two or as the average error when only two values were available.

Since E2 can be naturally oxidized to E1 (the reverse reaction could theoretically occur under anaerobic conditions), the combined concentrations of E1 and E2 were considered in the mass balance calculations.

PPCPs removal

Figures 6.17 and 6.18 show the average removal efficiencies of PPCPs in each experimental period of the mesophilic and thermophilic digester, respectively.

Table 6.9. Mass flux of PPCPs (μg·d^{-1}) and removal efficiency (%) in the mesophilic and thermophilic digester.

Mass flux of PPCPs (μg·d^{-1})			Mesophilic digester			Thermophilic digester		
			SRT 30 d	*SRT 20 d*	*SRT 10 d*	*SRT 20 d*	*SRT 10 d*	*SRT 6 d*
HHCB	*Inlet*		564	819	1,718	821	1,707	2,921
	Outlet	*Dissolved*	0.7 ± 0.4	0.6 ± 0.0	2.0 ± 0.2	1.0 ± 0.3	2.7 ± 1.1	4.2 ± 0.2
		Sorbed	217 ± 24	288 ± 41	526	257 ± 75	413 ± 148	587
	Removal (%)		59.8 ± 4.1	64.8 ± 5.0	69.3	68.5 ± 9.2	75.7 ± 8.7	79.8
AHTN	*Inlet*		214	321	656	313	668	1,115
	Outlet	*Dissolved*	0.4 ± 0.2	0.2 ± 0.0	1.0 ± 0.1	0.3	1.7 ± 1.3	4.0 ± 0.6
		Sorbed	102 ± 9	125 ± 15	248	85.4	120 ± 14	243
	Removal (%)		52.0 ± 4.3	61.0 ± 4.8	62.0	72.7	81.8 ± 1.9	77.9
CBZ	*Inlet*		7	10	21	10	21	35
	Outlet	*Dissolved*	5.1 ± 0.2	6.5 ± 1.0	7.9	6.5 ± 0.7	14.0 ± 1.0	15.1
		Sorbed	6.1 ± 1.2	10.7 ± 2.3	10.2	6.1 ± 1.1	14.2 ± 4.1	12.1
	Removal (%)		0	0	11.6	0	0	21.7
DZP	*Inlet*		7	10	20	10	20	34
	Outlet	*Dissolved*	3.3 ± 0.6	3.9 ± 0.4	4.5 ± 0.5	3.8 ± 0.0	7.2	6.1
		Sorbed	2.4 ± 0.1	4.1 ± 0.2	5.1 ± 0.7	3.2 ± 0.2	9.4	7.7
	Removal (%)		15.5 ± 4.8	19.8 ± 5.6	52.1 ± 5.9	30.4 ± 2.3	16.8	59.4
IBP	*Inlet*		7	11	22	11	22	37
	Outlet	*Dissolved*	2.6 ± 0.9	3.0 ± 0.4	4.8 ± 0.2	3.2 ± 0.4	7.7 ± 0.9	12.0 ± 1.0
		Sorbed	2.6 ± 0.2	3.7 ± 0.8	3.7	3.2 ± 1.1	3.5 ± 0.7	5.4
	Removal (%)		28.8 ± 8.8	38.5 ± 11.2	61.6 ± 0.7	40.9 ± 13.8	49.1 ± 6.5	53.6 ± 2.5
NPX	*Inlet*		7	10	21	10	21	35
	Outlet	*Dissolved*	0.8 ± 0.2	0.8 ± 0.3	1.9 ± 0.0	0.4 ± 0.1	0.9 ± 0.3	3.8 ± 0.4
		Sorbed	0.4 ± 0.0	0.5 ± 0.2	0.7 ± 0.0	0.3 ± 0.1	0.6 ± 0.1	2.0
	Removal (%)		82.4 ± 3.9	87.5 ± 5.4	87.4	93.6 ± 2.4	93.0 ± 1.5	83.5 ± 1.0

Table 6.9. Mass flux of PPCPs (μg·d^{-1}) and removal efficiency (%) in the mesophilic and thermophilic digester. *Cont.*

Mass flux of PPCPs (μg·d^{-1})			Mesophilic digester			Thermophilic digester		
			SRT 30 d	*SRT 20 d*	*SRT 10 d*	*SRT 20 d*	*SRT 10 d*	*SRT 6 d*
DCF	*Inlet*		3	5	10	5	10	17
	Outlet	*Dissolved*	1.3 ± 0.4	1.8 ± 0.1	1.2 ± 0.1	0.8 ± 0.7	4.2 ± 0.1	1.8 ± 0.4
		Sorbed	2.0 ± 0.1	4.4 ± 0.2	1.1 ± 0.0	2.6 ± 0.9	4.2 ± 1.3	2.0
	Removal (%)		0	0	77.6 ± 0.1	31.2 ± 6.4	16.9 ± 12.5	77.4 ± 2.7
IPM	*Inlet*		17	25	50	25	50	85
	Outlet	*Dissolved*	8.5 ± 0.7	16.8 ± 0.3	33.1	-	27.7 ± 4.2	55.6
		Sorbed	2.3 ± 0.5	6.3 ± 0.1	8.4	-	14.3 ± 1.7	3.4
	Removal (%)		35.0 ± 7.0	7.8 ± 1.4	17.0	-	16.4 ± 5.6	30.6
SMX	*Inlet*		16	24	49	24	49	83
	Outlet	*Dissolved*	0.1 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	0.6	0.1 ± 0.0	0.6 ± 0.3
		Sorbed	0.0 ± 0.0	0.0 ± 0.0	0.3	0.3	0.0 ± 0.0	0.5 ± 0.0
	Removal (%)		99.5 ± 0.6	99.8 ± 0.1	98.8 ± 0.2	96.3	99.7 ± 0.0	98.6 ± 0.3
ROX	*Inlet*		13	20	40	20	40	68
	Outlet	*Dissolved*	0.0 ± 0.0	0.4 ± 0.1	-	0.0 ± 0.0	0.6 ± 0.1	-
		Sorbed	0.0 ± 0.0	1.2 ± 0.4	-	0.0 ± 0.0	0.6 ± 0.0	-
	Removal (%)		99.6 ± 0.1	91.8 ± 2.9	-	99.8 ± 0.0	97.9 ± 1.5	-
E1+E2	*Inlet*		4	7	13	7	13	23
	Outlet	*Dissolved*	0.2 ± 0.0	-	0.1	0.3 ± 0.0	-	0.6
		Sorbed	1.3 ± 0.4	-	0.6	0.9 ± 0.0	-	2.0
	Removal (%)		66.3 ± 8.5	-	94.6	81.0 ± 0.4	-	88.6
EE2	*Inlet*		1	2	4	2	4	7
	Outlet	*Dissolved*	0.1	-	0.1	0.4 ± 0.1	-	0.1
		Sorbed	0.7	-	0.6	1.0 ± 0.1	-	0.5
	Removal (%)		40.5	-	94.6	38.1 ± 6.4	-	91.3

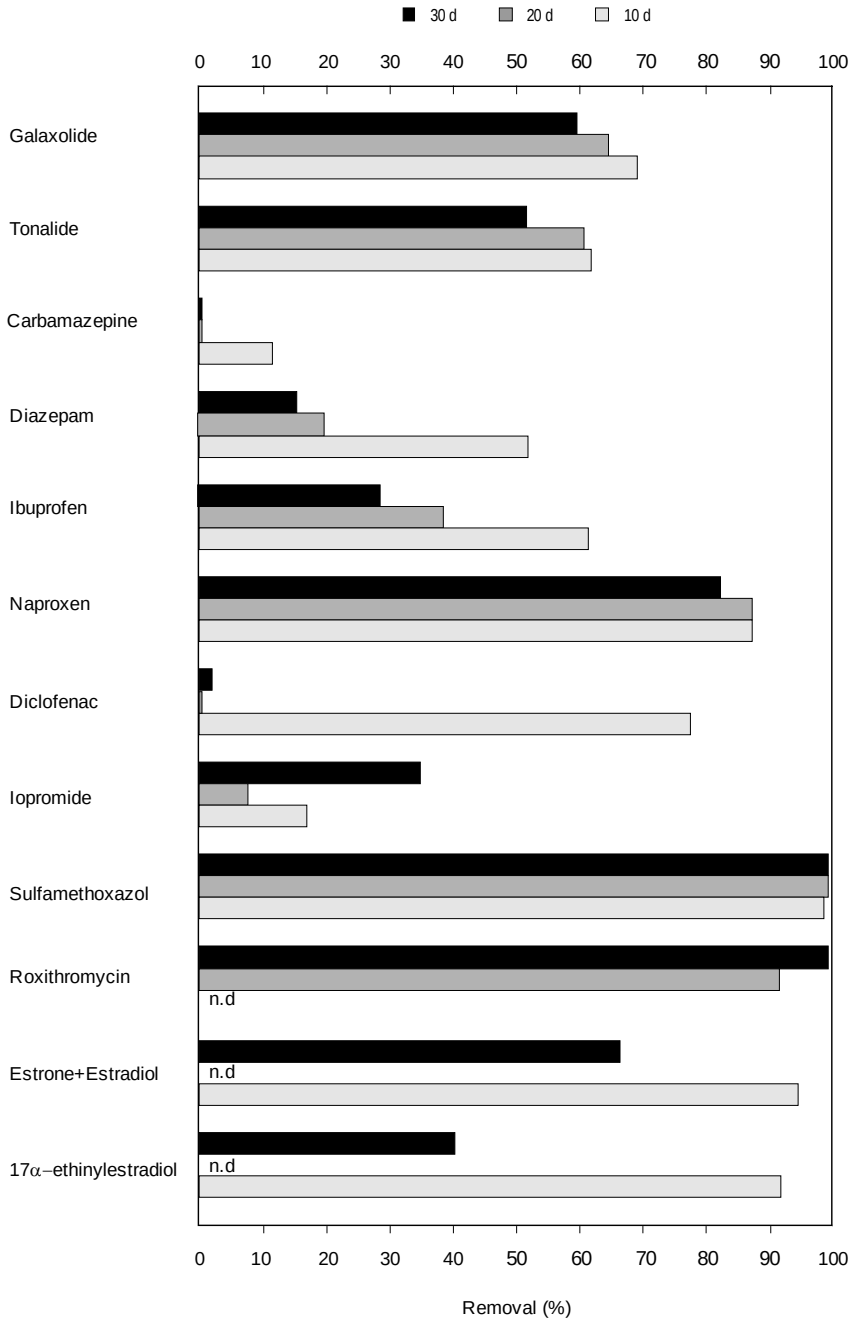


Figure 6.17. Removal of PPCPs (%) during mesophilic anaerobic digestion of sludge. n.d.: no data.

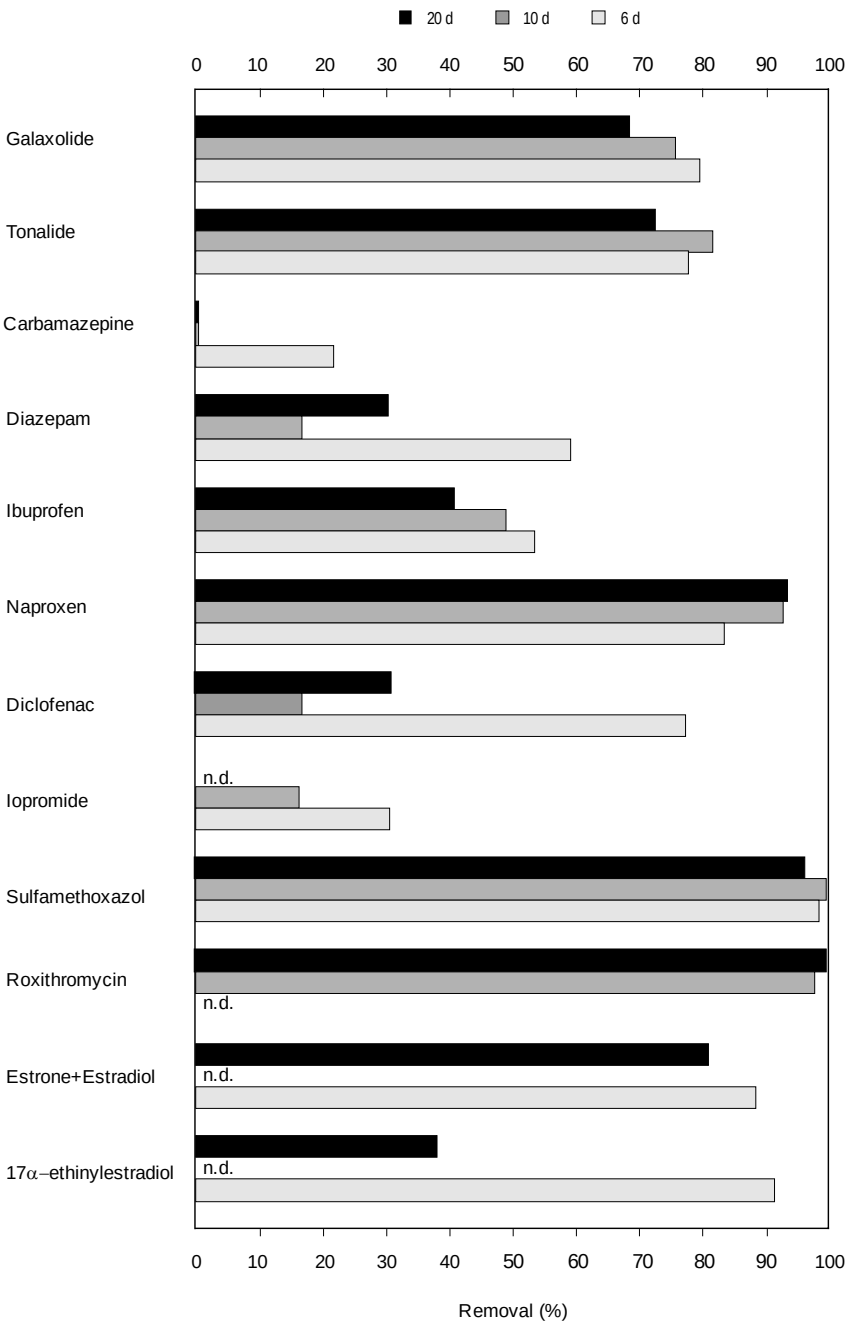


Figure 6.18. Removal of PPCPs (%) during thermophilic anaerobic digestion of sludge. n.d.: no data.

The main mechanisms involved in PPCPs removal during anaerobic digestion are sorption and biodegradation, since volatilization and photodegradation are negligible.

Table 6.10 shows the percentage (related to the feeding load) of PPCPs leaving the anaerobic digesters dissolved (in the supernatant) and sorbed (in the digested sludge). In addition, the average removal efficiencies obtained in the mesophilic and thermophilic process are indicated.

It can be observed that those compounds with high sorption affinity, such as musks, Diclofenac and estrogens, are present mainly associated to solids. For the other substances, a similar distribution between the liquid and solid phase was obtained, except IPM which is present in the liquid phase.

Next, the results obtained for each single substance will be discussed considering two factors: SRT and temperature.

Galaxolide

Galaxolide was significantly removed in both digesters (Table 6.11), with removal efficiencies ranging from 60 to 70% in the mesophilic process and from 70 to 80% in the thermophilic one.

Table 6.11. Summary of Galaxolide removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	60	65	69	n.d.
Thermophilic	n.d.	69	76	80

Considering the standard deviations of the results, no significant influence of temperature and SRT was observed.

This elimination was checked following the data confirmation process, because the K_d values obtained from the liquid concentrations (around 15 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 30,000 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

Table 6.10. Distribution of PPCPs in the effluent of the mesophilic and thermophilic digester and removal efficiencies.

	Mesophilic digester			Thermophilic digester		
	Dissolved (%)	*Sorbed (%)*	*Removed (%)*	*Dissolved (%)*	*Sorbed (%)*	*Removed (%)*
HHCB	0.1 ± 0.0	34.8 ± 4.0	64.6 ± 4.8	0.1 ± 0.0	25.2 ± 5.7	74.7 ± 5.7
AHTN	0.1 ± 0.1	41.5 ± 5.4	58.3 ± 5.5	0.2 ± 0.1	22.4 ± 4.7	77.5 ± 4.6
CBZ	58.5 ± 18.5	80.9 ± 29.7	0 - 12	58.3 ± 13.1	54.4 ± 17.5	0 - 21
DZP	36.2 ± 12.6	33.6 ± 7.8	20 – 50*	30.6 ± 11.1	33.9 ± 12.3	20 – 60*
IBP	28.4 ± 7.8	29.2 ± 10.9	43.0 ± 16.9	32.2 ± 3.0	19.9 ± 8.0	47.9 ± 6.4
NPX	9.5 ± 1.8	4.7 ± 1.2	85.8 ± 2.9	6.4 ± 3.9	3.9 ± 1.6	90.0 ± 5.7
DCF	30.4 ± 16.4	55.2 ± 39.8	0 – 75*	22.9 ± 16.8	35.3 ± 21.0	20 – 75*
IPM	61.1 ± 9.7	18.5 ± 6.0	19.9 ± 13.8	60.4 ± 7.1	16.3 ± 17.4	23.5 ± 10.0
SMX	0.4 ± 0.4	0.2 ± 0.4	99.4 ± 0.5	1.1 ± 1.2	0.6 ± 0.6	98.2 ± 1.7
ROX	1.0 ± 1.4	3.0 ± 4.2	95.7 ± 5.5	0.8 ± 1.1	0.8 ± 1.1	98.4 ± 2.0
E1+E2	2.9 ± 3.0	18.6 ± 19.7	80.5 ± 20.0	3.5 ± 1.2	10.8 ± 2.9	84.8 ± 5.4
EE2	6.3 ± 5.3	42.5 ± 38.9	40 – 90*	10.7 ± 13.1	28.6 ± 30.3	40 – 90*

Data expressed as average value of the three SRT (see Table 6.5) plus the standard deviation.
*Sludge adaptation increased the removal efficiency.

The data measured for this substance are very constant (Table II.1), since only 3 values out of 17 had to be dismissed. Moreover, as this compound tends to sorb onto solids, its removal efficiency is more dependant on the concentrations in the sludge phase than those in the liquid.

Tonalide

Tonalide was also significantly removed in both digesters (Table 6.12), with removal efficiencies ranging from 50 to 60% in the mesophilic process and from 70 to 80% in the thermophilic one.

Table 6.12. Summary of Tonalide removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	52	61	62	n.d.
Thermophilic	n.d.	73	82	78

Considering the standard deviations of the results, no significant influence of SRT was observed in none reactor; however, it could be stated that slightly higher removal of AHTN was achieved in the thermophilic process.

Once again, the data confirmation process proved this elimination, because the K_d values obtained from the liquid concentrations (around 10-25 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 25,000-40,000 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

Although the analytical methodology followed was the same, the data obtained for this compound are worse in comparison with Galaxolide. Besides, while the values obtained for the mesophilic process are fairly good (only 2 dismissed out of 8), the results of the thermophilic process are quite bad (Table II.2). Similarly to Galaxolide, the elimination of Tonalide is strongly dependant on its concentration in the sludge. Therefore, only the removal efficiencies calculated with the reliable data on the sludge phase, regardless of the liquid concentrations, were considered.

Carbamazepine

No removal of Carbamazepine was observed in both mesophilic and thermophilic processes (Table 6.13). Only in the last step of operation, a very low elimination was obtained, around 10 and 20% in mesophilic and thermophilic range, respectively.

However, considering the standard deviations of the results, it can be stated that Carbamazepine was not eliminated during anaerobic digestion of sewage sludge independently of temperature and SRT.

Table 6.13. Summary of Carbamazepine removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	0	0	12	n.d.
Thermophilic	n.d.	0	0	22

These results were confirmed by the equal K_d values obtained from the liquid and sludge concentrations (data confirmation process), which were also similar to those obtained in the digested sludge (Carballa *et al.,* 2006).

The data measured for this substance are very constant (Table II.3), since only 3 values out of 17 had to be dismissed, although they have no influence in the final results.

Diazepam

Table 6.14 shows a low removal of Diazepam during the two first operational stages in both digesters (20-30%). However, in the last stage the elimination increased up to 50-60%. A possible explanation for this could be the adaptation of the anaerobic sludge to Diazepam degradation, which would lead to an increase in the removal efficiency from 20-30% to 45-60%, approximately, regardless of the temperature of operation.

Table 6.14. Summary of Diazepam removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	16	20	52	n.d.
Thermophilic	n.d.	30	17	59

Data were confirmed by the process described previously, being the K_d value calculated with the data of the first stages (around 50 $L \cdot kg^{-1}$) similar to that reported by Ternes *et al.* (2004), around 20-40 $L \cdot kg^{-1}$. However, a higher value (around 100 $L \cdot kg^{-1}$) was obtained in the last stage, which indicates that some elimination happened.

This compound has been only determined in the liquid phase (Table II.4), with better data being obtained in the mesophilic reactor than in the thermophilic one.

Ibuprofen

Ibuprofen was quite well removed in both digesters (Table 6.15), with efficiencies ranging from 30 to 60% in the mesophilic process and from 40 to 55% in the thermophilic one.

While no significant influence of SRT was observed in the thermophilic process, the elimination of Ibuprofen increased from 20-35% at 30-d SRT to 60% at 10-d SRT in the mesophilic digester. Similarly to Diazepam, this fact could be explained by the sludge adaptation.

Table 6.15. Summary of Ibuprofen removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	29	39	62	n.d.
Thermophilic	n.d.	41	49	54

Considering the standard deviations of the results, no significant influence of temperature was observed.

This elimination was confirmed by using the data confirmation process, because the K_d values obtained from the liquid concentrations (around 15 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 80 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

The data measured for this substance are very constant (Table II-5), since only 1 value out of 17 had to be dismissed.

Naproxen

Naproxen was very well removed in both digesters (Table 6.16), with removal efficiencies ranging from 80 to 90% in the mesophilic process and from 85 to 95% in the thermophilic one.

Table 6.16. Summary of Naproxen removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	82	88	87	n.d.
Thermophilic	n.d.	94	93	84

Considering the standard deviations of the results, no significant influence of temperature and SRT was observed.

Naproxen degradation was confirmed by means of the data confirmation process, because the K_d values obtained from the liquid concentrations (around 1 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 350-650 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

The data measured for this substance are very constant (Table II-6), since only 1 value out of 17 had to be dismissed, although it does not affect to the conclusions concerning removal.

Diclofenac

Diclofenac showed a similar pattern as Diazepam (Table 6.17). While no (mesophilic range) or very low removal (thermophilic range) occurred during the two first operational stages in both digesters, in the last stage it increased up to 75%. Once again, it could be stated that an adaptation of the anaerobic sludge to Diclofenac degradation occurred, regardless of the temperature of operation.

Table 6.17. Summary of Diclofenac removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	2	0	78	n.d.
Thermophilic	n.d.	31	17	77

Elimination was confirmed by the data confirmation process, because the K_d values calculated from the liquid and sludge concentrations of the first stages were similar (around 100 $L \cdot kg^{-1}$). However, different values were obtained in the last stage, 3 $L \cdot kg^{-1}$ from the liquid and 200 $L \cdot kg^{-1}$ from the sludge, which indicates that some elimination happened. Anyway, taking into account that all these values were in the same range (50-150 $L \cdot kg^{-1}$) as either those calculated for digested sludge (Carballa *et al.,* 2006) or those reported by Ternes *et al.* (2004), no clear conclusions could be derived for this compound.

The data measured for this substance are quite constant (Table II-7), since only 4 values out of 17 had to be dismissed.

Iopromide

The removal of Iopromide was very low in both digesters (Table 6.18), around 10-35% and 15-30% in the mesophilic and thermophilic range, respectively.

A small influence of SRT was observed in the mesophilic process, because the removal efficiency decreased from 35% at 30-d SRT to 15% at 10-d SRT. However, no similar effect could be noticed in the thermophilic range.

Table 6.18. Summary of Iopromide removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	35	8	17	n.d.
Thermophilic	n.d.	n.d.	16	31

Considering the standard deviations of the results, no significant influence of temperature was observed.

This behaviour was proved with the data confirmation process, because the K_d values obtained from the liquid phase (1-5 $L \cdot kg^{-1}$) differ slightly from those calculated with the sludge concentrations (around 25 $L \cdot kg^{-1}$). Even so, these values were very similar to those calculated for digested sludge (Carballa *et al.,* 2006), thus indicating that the elimination should be very low.

The data measured for this substance are quite constant (Table II-8), since only 5 values out of 15 had to be dismissed.

Sulfamethoxazole

Sulfamethoxazole was very well removed in both digesters (Table 6.19), with removal efficiencies >95%.

Table 6.19. Summary of Sulfamethoxazole removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	>99	>99	99	n.d.
Thermophilic	n.d.	>95	>99	99

Considering the standard deviations of the results, no significant influence of temperature and SRT was observed.

This high elimination was checked by means of the data confirmation process, because the K_d values obtained from the liquid concentrations (0.2 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 20,000-30,000 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

The data measured for this substance vary strongly (Table II-9), mainly in the thermophilic digester. However, this variation does not affect the removal efficiencies calculated.

Roxithromycin

Roxithromycin was very well removed in both digesters (Table 6.20), with removal efficiencies higher than 90%.

Table 6.20. Summary of Roxithromycin removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	>99	92	n.d.	n.d.
Thermophilic	n.d.	>99	98	n.d.

Considering the standard deviations of the results, no significant influence of temperature and SRT was observed.

This high elimination was proved with the data confirmation process, because the K_d values obtained from the liquid concentrations differ significantly

from those calculated with the sludge concentrations (around 1,500 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

Similarly to Sulfamethoxazole, the data measured for this substance vary strongly (Table II-10) in both digesters. However, this variation does not affect the removal efficiencies calculated, except for the last stage of operation.

Estrone + 17β-estradiol

The natural estrogens were very well removed in both digesters (Table 6.21), with removal efficiencies of 65-95% in the mesophilic process and 80-90% in the thermophilic one.

Removal efficiency increased in the mesophilic digester from 65% at 30-d SRT to 95% at 10-d SRT, which can be explained by the sludge adaptation. However, no similar effect could be noticed in the thermophilic range.

Considering the standard deviations of the results, no significant influence of temperature was observed.

Table 6.21. Summary of E1+E2 removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	66	n.d.	95	n.d.
Thermophilic	n.d.	81	n.d.	89

This high degradation was assumed with the data confirmation process, because the K_d values obtained from the liquid concentrations (< 10 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 1,000-3,000 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

Not very constant data were obtained for these substances since 8 out of 14 values were dismissed (Table II-11).

17α-ethinylestradiol

EE2 showed a similar pattern than Diclofenac (Table 6.22). While the removal was very low (around 40%) during the two first operational stages in both digesters, it increased up to 90% in the last stage. Once again, it could be

stated that an adaptation of the anaerobic sludge to EE2 degradation occurred, regardless of the temperature of operation.

Table 6.22. Summary of 17α-ethinylestradiol removal efficiencies (%) during conventional operation of the anaerobic digesters. n.d.: no data.

	SRT (d)			
	30	**20**	**10**	**6**
Mesophilic	40	n.d.	92	n.d.
Thermophilic	n.d.-	38	n.d.	91

This fact was also proved in the data confirmation, because the K_d values calculated from the liquid concentrations (<50 $L \cdot kg^{-1}$) were different from those obtained from the sludge concentrations, 200-500 $L \cdot kg^{-1}$ (in the first stages) and 2,000-7,000 $L \cdot kg^{-1}$ (in the last step).

The data measured for this substance are very poor (Table II-12), since only 5 values out of 14 could be considered.

Discussion

Table 6.23 shows a summary of PPCPs removal during mesophilic and thermophilic anaerobic digestion of sewage sludge.

All PPCPs were affected in some extent by the anaerobic digestion process, except Carbamazepine, for which the elimination was absence or very low. The compounds better removed were the antibiotics and Naproxen, with efficiencies around 80-99%. Musks and the natural estrogens were the next substances with high elimination (around 50-95%), followed by Ibuprofen (30-60%) and lastly Iopromide, which was the substance less removed (around 25%). The removal of Diazepam, Diclofenac and 17α-ethinylestradiol increased after sludge adaptation up to 60, 80 and 90%, respectively.

No influence of SRT was observed on PPCPs elimination, except for IBP, IPM and natural estrogens in the mesophilic digester, where higher removal efficiencies were observed at low SRT (sludge adaptation). Similarly, temperature did not affect PPCPs removal, except AHTN, which was better eliminated in the thermophilic digester.

In general, the PPCPs concentrations were quite reliable since most of them fitted the data confirmation process and some conclusions could be dilucidated.

However, for some substances, such as DZP, antibiotics or estrogens, the values measured varied strongly.

Table 6.23. Summary of PPCPs behaviour during mesophilic and thermophilic anaerobic digestion of sewage sludge.

PPCP	Removal (%)	SRT influence	Temperature influence	Method validation
HHCB	60 - 80	(-)	(-)	Good
AHTN	50 - 80	(-)	(+)	M: good T: bad
CBZ	0 - 20	(-)	(-)	Good
DZP	20 - 60	SA	(-)	M: good T: bad
IBP	30 - 60	M (+)	(-)	Good
NPX	80 - 95	(-)	(-)	Good
DCF	0 - 80	SA	(-)	M: good T: medium
IPM	10 - 35	M (+)	(-)	Medium
SMX	> 95	(-)	(-)	Medium
ROX	> 90	(-)	(-)	Medium
E1+E2	65 - 95	M (+)	(-)	Bad
EE2	40 - 90	SA	(-)	Bad

M: Mesophilic T: Thermophilic
SA: Sludge adaptation (-) No influence (+) Influence

As stated in the introduction of this chapter, the information dealing specifically with PPCPs behaviour during anaerobic digestion of sewage sludge is very scarce. The few data available are referred to either estimations from the physico-chemical properties, rough calculations from the mass balances or effects on methanogenesis. Furthermore, the results found are not clear and even contradictory, since some authors (Khan and Ongerth, 2002; Andersen *et al.,* 2003; Stamatelatou *et al.,* 2003) stated that PPCPs exhibit some resistance to anaerobic biodegradation, while others (Van de Plassche and Balk, 1997; Holbrook *et al.,* 2002; Kupper *et al.,* 2004) reported the opposite. Some of this literature, which is mainly focused on musks and estrogens, is next summarized.

Khan and Ongerth (2002) stated that most PPCPs were shown to persist in the aqueous component of digested sludge, exhibiting some resistance to anaerobic degradation. Concentrations in the solids component of digested sludge were extremely low, even compared to those in the aqueous phase of the same sample. This would suggest that once digested, the sludge solids do not retain

their lipophilic properties and that all of the investigated compounds had partitioned fully to the aqueous phase.

Ivashechkin *et al.* (2004) studied the behaviour of Bisphenol A (BPA), as a model compound of endocrine disrupters chemicals (EDCs), during the treatment of sewage sludge. Assuming no considerable degradation of EDCs during anaerobic digestion (Bilitewski *et al.,* 2002), they focused their work on the partitioning of EDCs between the digested sludge and the supernatant. They reported that anaerobically digested sludge possesses enough binding sites and that adsorption will not reach its limit for the environmentally relevant BPA-concentrations. The mass of BPA sorbed per gram of SS decreases with the increasing dry matter content, which is related to the supply of BPA approaching exhaustion. The same occurs with other EDCs (Lai *et al.,* 2000). Transposing these results to the real sludge treatment process, it can be assumed that in the anaerobic digester (dry matter>20 $g \cdot L^{-1}$) over 75% BPA is bound on the solids. In the supernatant after dewatering (dry matter=0.3 $g \cdot L^{-1}$) around 7% BPA should be associated with the solid phase (not taking into account the influence of conditioning agents). They stated that for other EDCs, the output with sludge should be at least as relevant as for BPA, since some of them have even higher partition coefficients.

Holbrook *et al.* (2002) studied the fate of the estrogen receptor agonists during wastewater and biosolids treatment processes and they found that between 51 and 67% of the estrogenic activity contained in the influent wastewater was either biodegraded during the wastewater or biosolids treatment processes or was unavailable to the extraction/detection procedure used. In both aerobic and anaerobic digestion, mass balances revealed an increase in estrogenic activity as treatment progressed and biosolids destruction occurred. In the case of anaerobic digestion, most of the estrogenic activity detected in the biosolids was associated with the suspended solids rather than the liquid phase. However, the value of the liquid fraction did not change as treatment progressed, suggesting that the recycle streams from anaerobically digested biosolids will contribute relatively small amounts of estrogens to the wastewater treatment processes.

Andersen *et al.* (2003) indicated that the natural estrogens are degraded mainly in the denitrifying tank (anaerobic conditions), whereas EE2 was only degraded in the nitrifying tank (aerobic conditions). They also stated that only about 5% of the estrogens are sorbed onto sewage digested sludge. They observed

a clear increase of natural estrogens concentrations in the water and sludge phases by comparing excess and digested sludge. Since the dissolved concentration of the digester liquid was similar to that of the inlet and primary effluent, they assumed that the sorbed E1 and E2 concentrations in the primary sludge are also in the same range as those in the digested sludge. On this assumption, the detected inlet and outlet loads of the digester are about the same, being thus the natural estrogens not degraded appreciably under the methanogenic conditions.

Matsui *et al.* (2000) observed that the E2 concentrations and estrogen activity of the dewatering liquid from the sludge treatment were even more than twice as high as the inflow to the plant. Several reasons explain this fact. First, conjugated compounds originating from primary sludge are expected to be cleaved in the digester; second, the dissolution of particles due to the digestion process may release estrogens by desorption; and third, the E1 to E2 reverse reaction could be shown to take place in an anaerobic environment (E2 has a 5-10 fold higher estrogenic activity that E1).

Johnson and Williams (2004) reported that strictly anaerobic desulphating strains are capable of cleaving E1-3-sulphate and E2-3-sulphate, thus increasing their concentrations. In contrast, Clara *et al.* (2004) and Kreuzinger *et al.* (2004b) indicated that the anaerobic digestion stabilisation accelerates the breakdown of natural estrogens.

Joss *et al.* (2004) indicated that the degradation of natural estrogens (E1 and E2) takes place under all redox conditions (aerobic, anoxic and anaerobic), but at significantly different rates. For E1, an increase by a factor of 3-5 was observed in the transitions from anaerobic to anoxic (nitrate available but no molecular oxygen) and from anoxic to aerobic (O_2 available in solution). The reduction of E1 to E2 and the subsequent removal of E2 could be shown to take place under anaerobic conditions without nitrate. However, EE2 was only removed at a significant rate under aerobic conditions, while in absence of molecular oxygen the fitted values were in the range of the quantification limit. Similar results were obtained by Vader *et al.* (2000).

There are also some indications of AHTN and HHCB degradation during sludge treatment (Blok, 1998). A mass balance calculation for the sludge shows that approximately 40% of AHTN and HHCB are eliminated during sludge digestion (Van de Plassche and Balk, 1997).

Kupper *et al.* (2004) observed that the loads of musks were lower in plants with extended aeration and aerobic sludge stabilisation. Besides, the loads of HHCB-lactone (HHCB metabolite) were higher in these plants that in those using anaerobic sludge stabilisation, which indicates that extended aeration and hygienisation enhance biodegradation of HHCB.

Fountoulakis *et al.* (2004) studied the effect of some pharmaceuticals, such as Carbamazepine, Sulfamethoxazole and Diclofenac, on mesophilic methanogenesis at concentrations ranging from 0 up to 400 $mg \cdot L^{-1}$ and they tried to relate the final effect with the tendency of the compounds to sorb on the anaerobic biomass. The results were that Diclofenac caused severe inhibition at high concentrations (200- 400 $mg \cdot L^{-1}$), moderate inhibition at a concentration of 100 $mg \cdot L^{-1}$ and no inhibition at all at 10 and 50 $mg \cdot L^{-1}$. Carbamazepine caused less inhibition and Sulfamethoxazole seemed not to affect methanogenesis even at high concentrations. They found a direct correlation between the level of the pharmaceuticals inhibition and the affinity to sorb on the anaerobic sludge. But it should be pointed out that at the concentrations levels usually prevailing in STPs, no significant impact of any pharmaceutical is anticipated.

Stamatelatou *et al.* (2003) observed no effect (biogas production rate, volatile fatty acids, pH) of Carbamazepine on the anaerobic process. Besides, its concentration in the digester was constant, indicating that neither sorption nor biodegradation took place.

Ternes *et al.* (2002) observed that Carbamazepine passed through soil under anaerobic conditions into the groundwater. In contrast, Diclofenac was not found in the bank-filtratred water, indicating that under real field conditions this compound can be removed during anaerobic bank filtration. However, whether Diclofenac exhibits special sorption properties or is alternatively biodegraded during anaerobic subsoil-passage was not elucidated.

Kalsch (1999) observed no degradation of diatrizoate under anaerobic conditions. Since the chemical structure of this compound is quite similar to Iopromide, it can be ruled out that IPM is neither degraded under anaerobic conditions.

6.4. Conclusions

A common sludge stabilization process such as anaerobic digestion has been applied to study the removal of selected PPCPs commonly present in sewage:

Galaxolide, Tonalide, Diazepam, Carbamazepine, Ibuprofen, Naproxen, Diclofenac, Iopromide, Sulfamethoxazole, Roxithromycin, 17β-estradiol, Estrone and 17α-ethinylestradiol. Although not all the compounds have the same sorption properties (Ternes *et al.,* 2004), even substances with low K_d values must be considered during sludge stabilization, since the sorbed amount is not only dependent on the distribution coefficient but also on the solids concentration, being this quite high during sludge treatment. Therefore, in this case, the limit of relevance below which the sorption can be neglected is around K_d<1 $L \cdot kgTSS^{-1}$, much lower than that accepted for wastewater treatment (K_d<500 $L \cdot kgTSS^{-1}$).

Although the feeding showed the normal fluctuations related to the STP operation in terms of solids and organic matter content, the operation of both digesters remained stable leading to an effluent of almost constant characteristics (solids and COD concentration). Even if the operative conditions applied in the thermophilic digester were more drastic in terms of OLR respect to the mesophilic ones, the process was stable.

The removal of volatile solids and organic matter ranged from 50 to 70% in both digesters during the whole experimental period. In general, sludge stabilisation increased, i.e. higher solids and COD elimination, when operating at lower OLR, independently of the temperature of operation. However, when both digesters were run at the same SRT, better results were obtained in thermophilic range. Moreover, when comparing the removal efficiencies of the mesophilic digester run at 20 d with those obtained in the thermophilic one with 10-d SRT, they are very similar or even higher in thermophilic range. The same behaviour was observed when comparing the operation at 10 and 6 d, respectively. This fact confirms that the influence of temperature is more important than the effect of the organic load rate. In terms of sludge stabilisation, the compared efficiency of the six operating conditions was the following: mesophilic (30 d) > thermophilic (20 d) > thermophilic (10 d) > mesophilic (20 d) > thermophilic (6 d) > mesophilic (10 d).

Higher concentrations of COD_s and VFA were achieved in the thermophilic digester effluent than in the mesophilic one. This fact confirms that the mesophilic process is superior in terms of liquid effluent quality.

The biogas production rate (GPR) and the specific methane production (SGP), based on VS removed, was higher in the thermophilic process than in the mesophilic one, although both showed similar methane content.

The results proved the higher activity of the thermophilic sludge in comparison with the mesophilic one; the maximum methane production rate and the methane yield of the raw sludge were higher under thermophilic conditions and the stability of the degradation process at greater inlet loads was also demonstrated. Therefore, the use of thermophilic digestion leads to a better utilization of the existing facilities and consequently avoids the digester overloading. The higher degradation efficiency is related to the higher biogas production, thus improving the energetic balance of the process. In contrast, the mesophilic process was superior in terms of effluent quality and process stability.

Concerning PPCPs removal, the conclusions obtained from the anaerobic digestion pilot plant are: i) very high removal (>80%) of Naproxen, Sulfamethoxazole and Roxithromycin; ii) high removal (60-80%) of Galaxolide, Tonalide and natural estrogens; iii) medium removal (30-60%) of Ibuprofen; iv) low elimination (<40%) of Iopromide; and, v) no removal of Carbamazepine (<20%). The elimination of Diazepam, Diclofenac and 17α-ethinylestradiol occurred after sludge adaptation. In general, no influence of SRT and temperature was observed on PPCPs removal.

6.5. References

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Chapter 7

Influence of different sludge pre-treatments on anaerobic digestion operation and PPCPs removal[1,2]

Summary

Many novel treatment technologies, usually representing a pre-treatment prior to the biological degradation process, have been developed in order to improve the recycling and reuse of sewage sludge. Since the hydrolysis is the rate limiting step in the anaerobic digestion of sewage sludge, these pre-treatments are often required to promote solubilisation of organic matter. Among all the methods available, a chemical (alkaline), thermal and oxidative (ozone) treatment have been considered in this study. The behaviour of the 13 PPCPs considered in this work has been studied during the anaerobic digestion of sewage sludge combined with these pre-treatments (advanced operation) in comparison with the conventional process. Two parameters have been analysed: the temperature (mesophilic and thermophilic conditions) and the Sludge Retention Time (SRT). While the thermal and ozonation process led to similar organic matter solubilization (60%), the alkaline treatment increased this value up to 80%. The removal efficiencies of solids and organic matter during anaerobic digestion ranged between 40 and 70% in both digesters, with small influences of SRT, temperature and type of pre-treatment. The removal of NPX, IPM and SMX was not affected by any pre-treatment. Ozonation influenced the elimination of CBZ, HHCB, AHTN and IBP and the alkaline and thermal processes affected ROX and IBP removals, respectively. DZP, DCF and estrogens were removed after sludge adaptation.

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[1]**Carballa, M., Omil, F., Alder, A.C. and Lema, J.M.** (2006). Comparison between the conventional anaerobic digestion (CAD) of sewage sludge and its combination with a chemical or thermal pre-treatment concerning the removal of Pharmaceutical and Personal Care Products (PPCPs). *Wat. Sci. Technol.,* (in press).

[2]**Carballa, M., Manterola, G., Larrea, L., Ternes, T., Omil, F. and Lema, J.M.** (2006). Influence of ozone pre-treatment on anaerobic digestion operation and digested sludge characteristics: removal of pharmaceutical and personal care products. *Chemosphere,* (submitted).

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7.1. Introduction

Due to increasing demands on sewage purification, sludge production is growing and sludge processing and disposal is a problem of major concern in terms of environment, finance and technology. Traditionally, sludge from sewage treatment plants (STPs) was applied on farmland as fertiliser and soil amendment. However, legislation concerning application of sludge on farmland is likely to be tightened, which will limit this disposal path in the future. Therefore, the interest in methods to reduce the volume and mass of biosolids is growing.

Anaerobic digestion is a common process for the treatment of insoluble organic matter or high COD containing effluents. Methanogenesis is usually considered as the rate-limiting step of the overall process. However, when considering particulate substrates as sludges, hydrolysis is the slower step and also controls the kinetics (Eastman and Ferguson, 1981; Pavlostathis and Gosset, 1986). Long retention times around 20-30 days (Parkin and Owen, 1986) are required to reach only moderate efficiencies (30-50% of COD_t or VS) due to the low biodegradability of the sludge solids. Therefore, significant effort has been dedicated in recent years to find ways of improving the performance of anaerobic digestion.

For that purpose, several approaches in the following fields have been considered (Donhanyos *et al.,* 2004):

- Intensification of standard sludge digestion by optimizing the process conditions (reactor feeding, mixing, thickening of input sludge, etc.).
- Increase of process temperature using single or multi-stage thermophilic operation.
- Pre-treatment of input sludge.
- Co-digestion and co-fermentation.

7.1.1. Pre-treatments of sewage sludge

Pre-treatment processes are an additional step in the sewage sludge treatment technology and have been developed to improve subsequent sludge treatment and final output quality. They represent a pre-treatment prior to the biological degradation process, promoting the hydrolysis step.

During the anaerobic treatment of sludge, the process limitation by the rate of the hydrolysis of suspended organic matter is of particular importance. The

composition and biodegradability of the input organic material is one of the important parameters influencing anaerobic digestion. Primary sludge often consists of easily degradable compounds; but in the case of excess activated sludge, a low biogas yield is caused by the low biodegradability of the cell walls and the extra cellular biopolymers formed.

By means of an efficient pre-treatment, cell components and other organic matter are released, thus being the substrate better accessible for the anaerobic bacteria. The cell lysate is the released content of bacteria cells into a bulk liquid after the destruction of cell walls and not only represents better accessible and degradable organic compounds, but also contains some enzymes and co-factors with still remaining activity. The cell lysate accelerate degradation reactions and consequently save energy for biosynthesis. Its presence in the sludge that has to be digested supports anaerobic bacteria growth and methane production (Dohanyos *et al.,* 2000).

The overall objective is to accelerate digestion of input sludge, raise the degree of degradation, optimize the sludge methanogenic potential and thus decrease the amount of sludge to be disposed of. Besides, with increasing sludge solubilization, the supernatant can be used either as a carbon source in the denitrification process or a post-precipitation step may be an interesting option for introducing phosphate recycling, since the phosphate incorporated in the sludge is released during sludge solubilization.

Several disintegration methods have been investigated so far (Müller, 2000):

- Heat treatment especially in the low temperature range from 40 to 150ºC (Shieder *et al.,* 2000; Kepp *et al.,* 1999; Gavala *et al.,* 2003).
- Chemical treatment using acids or alkalis (Rajan *et al.,* 1989; Vigueras, 2001; Abu-Orf *et al.,* 2004).
- Mechanical disintegration using ultrasound, mills and homogenisers (Baier and Schmidheiny, 1997; Kopp *et al.,* 1997; Tiehm *et al.,* 1997; Neis *et al.,* 2000; Lehne *et al.,* 2001).
- Oxidation processes using ozone and hydrogen peroxide (Gilbert, 1983; Song *et al.,* 1992; Scheminski *et al.,* 2000; Weemaes *et al.,* 2000; Goel *et al.,* 2003).
- Biological treatment using enzymes (Nagle *et al.,* 1992; Rintala and Ahring, 1994).

- Combinations, such as thermo-chemical processes (Li and Noike, 1992; Chiu *et al.,* 1997; Delgenes *et al.,* 2000; Dohanyos *et al.,* 2000).

However, the full-scale application depends on the technical conditions and energy demands. In addition, the biodegradability is sometimes not improved and this is attributed to the fact that the soluble molecules generated during the pre-treatment can be refractory and/or inhibitory to anaerobic micro-organisms (Delgenés *et al.*, 2000). The inactivation of enzymes and the formation of some toxic products can also occur. Fractionation of the soluble pre-treated microbial biomass demonstrated that high molecular weight compounds (>100 kDa) are involved in the poor biodegradability and biotoxicity observed.

7.1.2. Chemical pre-treatment

Chemical pre-treatment causes the destruction of complex organic compounds by means of strong mineral acids or alkalis. This process may be used to hydrolyze and decompose lipids, hydrocarbons and proteins into smaller soluble substances, such as aliphatic acids, polysaccharides and amino acids (Chiu *et al.*, 1997).

Alkaline and acid pre-treatments consist of increasing or decreasing the sludge pH, respectively, by the addition of an alkali (sodium hydroxide or lime) or an acid (hydrochloric acid), maintaining this value for a period of time (normally 24 hours). Alkaline treatment solubilizes most of the protein in the sludge whereas the acid process solubilizes the carbohydrate portion of the sludge (Aravinthan *et al.,* 1998).

In this work, an alkaline pre-treatment has been chosen. Although sodium hydroxide (NaOH) was reported to yield greater solubilization efficiency than lime (Rajan *et al.,* 1989), the focus of this work will be lime addition because of its relatively low cost and the ease at which it can be added to an existing treatment plant. Besides, it is the additive proposed for sludge stabilization in the working document on sludge (EU 2000; EU 2004).

The disinfecting capabilities of lime are attributed to its ability to increase temperature, pH and the free ammonia content in the biosolids. This is demonstrated by the chemical reactions that occur when quicklime (CaO) reacts with water.

$$CaO + H_2O \leftrightarrow Ca(OH)_2 + 65.2\ kJ\ (heat)$$

$$Ca(OH)_2 \leftrightarrow Ca^{2+} + 2OH^-\ (pH)$$

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O\ (free\ ammonia)$$

Once selected the additive, two operational parameters are of great importance in alkaline treatment: pH and contact time. Nagle *et al.* (1992) indicated substantial increases in COD solubilization at higher pH. However, the effect of treatment time was only significant during the first 50 min, with less impact from 50 to 240 min. Another factor to be considered is the mixing. An intimate mixing is crucial as it eliminates the potential for pockets of low pH to be created in the biosolids.

In order to increase the solubilization efficiency, the chemical pre-treatment is sometimes performed at high temperature (thermo-chemical treatment). Although this process has been shown very efficient in enhancing sludge digestion (Tanaka *et al.,* 1997), the aggressive reaction conditions often impose special material requirements.

Although sludge solubilization is increased, the methanogenic biodegradability (around 40%) was not affected by chemical or thermo-chemical pre-treatment (Delgenes *et al.,* 2000). The hypothesis proposed by these authors to explain the poor anaerobic biodegradability were: i) that some intramolecular reactions had been induced during the pre-treatment, leading to the formation of soluble refractory compound, and ii) that other molecules such as sodium cation had affected biodegradability performances. They observed an increase of biogas production and the suppression of the inhibition by means of removing high molecular weight compounds (>100 kDa) using partial resin decolorization or acid precipitation.

7.1.3. Thermal pre-treatment

Thermal pre-treatment has gained attention since it is suitable for the improvement of stabilization, dewatering and methane potential of the sludge, the reduction of the numbers of pathogens and its relatively low cost (Li and Noike, 1992; Wang *et al.,* 1997; Gavala *et al.,* 2003).

Thermal hydrolysis refers to a process in which sludge is heated to 130-180ºC during 30-60 minutes at the corresponding water vapour pressure. The process produces a sludge which is partially solubilised and the biological cells

are disintegrated (Machenbach and Odegaard, 2002). This size reduction can lead to a more rapid digestion and an optimal gas production (Pinnekamp, 1987).

While the carbohydrates and the lipids of the sludge are easily degradable, the proteins are protected against enzymatic hydrolysis by the cell wall. Thermal pre-treatment destroy the cell walls and makes proteins accessible for biological degradation. In the case of primary sludge, the thermal pre-treatment does not affect biodegradability, but increases dewatering.

The problems of the application of thermal pre-treatment to a full-scale anaerobic digestion plant are the costs of the process and the quality of the produced material. The costs are related to the energy requirements of the thermal hydrolysis (Müller, 2000).

The quality of the produced material is a result of high pressure and temperature during thermal pre-treatment, which causes that biologically active compounds could be inactivated and some toxic products formed. The formation of problematically biodegradable compounds can be explained by the "Maillard-reaction" (Penaud *et al.,* 2000). Although at lower temperature ranges this effect is less strong, it is suspected that problematically biodegradable compounds are produced in any thermal disintegration process.

This problem can be overcome using a rapid thermal conditioning, which combines high temperatures and pressures with short times (Dohanyos *et al.,* 2004). Due to a short retention time in high pressure and temperature conditions, the cells are disrupted with high efficiency but an inactivation of enzymes does not take place to a high extent.

The most important operational parameters are: the temperature and the treatment time. Delgenes *et al.* (2000) observed that during thermo-chemical pre-treatment (pH 12, 30 min), COD solubilization increased from 50% at 90ºC to 71% at 140ºC. For temperatures higher than 140ºC, no further increase of COD solubilization was observed and above 160ºC it decreased to reach 62.6% at 200ºC. Once again, the methanogenic activity remained in the same level (around 40%) for temperatures below 140ºC, but lower values were obtained at higher temperatures.

Nagle *et al.* (1992) stated that increases in the treatment temperature over room temperature significantly affected COD solubilization with less effect from

80 to 100ºC. They also indicated that the effect of treatment time was most profound during the first 50 min, with less impact form 50-240 min.

7.1.4. Ozonation

Several oxidative treatments have been applied to disintegrate sludge cells. Among the oxidation processes, the treatment using ozone is of special interest because no chemicals are needed and no increase in salt concentration occurs.

Recent reports (Yasui *et al.,* 1996) on full-scale application of ozone treatment to completely eliminate excess sludge production from full-scale activated sludge treatment plants clearly state the role that ozone can play in sludge hydrolysis and the enhancement of biodegradability. In this way, ozonation was also considered as an attractive pre-treatment for solid hydrolysis before anaerobic digestion (Weemaes *et al.,* 2000; Goel *et al.,* 2003).

Ozone is a very reactive oxidizing agent. It reacts with the sludge compounds in two different ways, the direct and the indirect reaction (Staehelin and Hoigné, 1985). Both reactions occur simultaneously. While the indirect reaction is based on the short living hydroxyl radicals, which do not react specifically, the direct reaction rate is lower and depends on the structure of the reactants.

The aim of ozone pre-treatment is to cause the partial oxidation and hydrolysis of the organic matter. A complete oxidation is avoided since it has to remain as a converting (solubilizing) system, and instead larger molecules are cracked into smaller ones, cell-walls of microorganisms are destroyed (Bünning and Hempel, 1996) with the consequent release of intracellular proteins and hardly degradable compounds are transferred into more easily degradable ones.

Therefore, the gas-yield can be increased significantly by ozonation corresponding to the high degree of solubilization. However, the formation of hardly degradable compounds was found as well and degradation processes only performed well after an adaptation of the micro-organisms (Scheminski *et al.,* 2000).

7.1.5. Comparison between alkaline, thermal and ozonation methods

As authors dealing with sludge pre-treatments do not examine the same type of sludge and employ different analytical methods, the comparison of results is often difficult. Usually, it is made with respect to the release of organic matter into solution, the specific energy used and the anaerobic biodegradability of the

pretreated sludge. However, the obtained outcome can sometimes not be entirely explained by disintegration effects and influences of other changes, such as the amount of sludge, sludge properties or the operating schedule of the plant, must be considered.

Müller (2000) compared ozone and thermal treatment, stating that thermal treatment uses more energy, but it is thermal energy which is cheaper than the electrical energy necessary for the other methods. In addition, the consumption of heat energy can be optimised in order to make positive the total energy balance.

Müller *et al.* (2004) compared different mechanical pre-treatments (ball-mill, centrifuge and ultrasonic) with ozone and they observed that the disintegration process carried out with ozone shows the highest specific energy demand, although the achieved degree of disintegration is also the highest.

7.1.6. Secondary effects of pre-treatments

The main problem related to the used of pre-treatments is that sometimes the sludge biodegradability is not improved due to the formation of problematically biodegradable or toxic compounds. Several factors influence this formation, such as the high temperatures during thermal hydrolysis, the introduction of strange ions in the medium during alkaline treatment or the odour-generating compounds formed during the oxidation processes. Nature and quantity of the generation depends on the parameters of reaction.

The negative effect of higher temperatures can be explained by the "Maillard-reaction". In this reaction reduced sugars and aminoacids react to melanoidines, which are difficult to degrade or even inhibitory. Melanoids are brown coloured, nitrogen containing polymeric substances which are similar to humic acids concerning the solubility and the elementary analysis. At temperatures below 100ºC, the generation of melanoids starts at low a level, but it increases with temperature with a strong effect at values above 140ºC, although it depends on the retention time. This formation of hardly degradable materials, the possibility of the formation of dioxins at temperatures of 200ºC (Abendt *et al.,* 1994) and the odour generation (Kuribayashi and Sato, 1993) have limited the use of thermal pre-treatment so far.

Another problem is the dewatering properties of the anaerobic sludge that has been disintegrated prior to digestion. In general, the dewatering characteristics are deteriorated after pre-treatments. Disintegrated sludge needs more flocculant than

untreated sludge and the solid content in the dewatered sludge is sometimes lower (Kopp *et al.,* 1997; Müller *et al.,* 2004).

The pollution of the return process water in terms of COD and nutrients after digested sludge dewatering is increased due to the disintegration processes. Increase in COD and phosphorus is low compared to the increase in ammonia nitrogen, mainly caused by the higher degree of degradation of biomass containing proteins. Especially the ozone treatment leads to a remarkable increase (Müller *et al.,* 2004).

Finally, the reduction of the amount of sludge that has to be disposed of is combined with an increase in the concentrations of pollutants. During pre-treatments, a short term release of bound pollutants, such as heavy metals, can be observed. But after a few minutes, the pollutants are absorbed again by the sludge particles.

7.1.7. Objective

The objective of this chapter is to study the influence of several sludge pre-treatments (alkaline, thermal and ozonation) in the velocity and degree of sludge stabilization by anaerobic digestion. In addition, the effect of these pre-treatments on PPCPs behaviour during sludge digestion was analysed. All the results will be compared to those obtained in the conventional operation described in Chapter 6.

7.2. Materials and methods

7.2.1. Sewage sludge characteristics

Raw sewage sludge used in this work was collected from an urban Sewage Treatment Plant (STP) located in Galicia (NW of Spain). A mixture of primary and secondary sludge (70:30, v/v) was used as feeding and its characteristics were the same as described in Chapter 6 (section 6.2.1 and 6.3.2).

7.2.2. PPCPs

The fate and behaviour of the 13 substances considered in this work have been studied during sludge anaerobic digestion combined with different sludge pre-treatments. The compounds have been spiked to the sludge as described in Chapter 6 (section 6.2.2).

7.2.3. Chemical pre-treatment

The chemical pre-treatment (Fig. 7.1a) was carried out by adding lime (CaO) to the stirred sludge until the pH was over 12, checking this value after 24 hours. The doses used were between 0.05 and 0.2 g lime·g VSS^{-1}.

Since intimate mixing is important to eliminate the creation of potential for pockets of low pH in the sludge, this procedure was performed with a sludge volume of 2 L. After that, the sludge was neutralised with hydrochloric acid prior to be fed in the anaerobic digesters.

(a) (b)

Figure 7.1. Chemical (a) and thermal (b) pre-treatment devices.

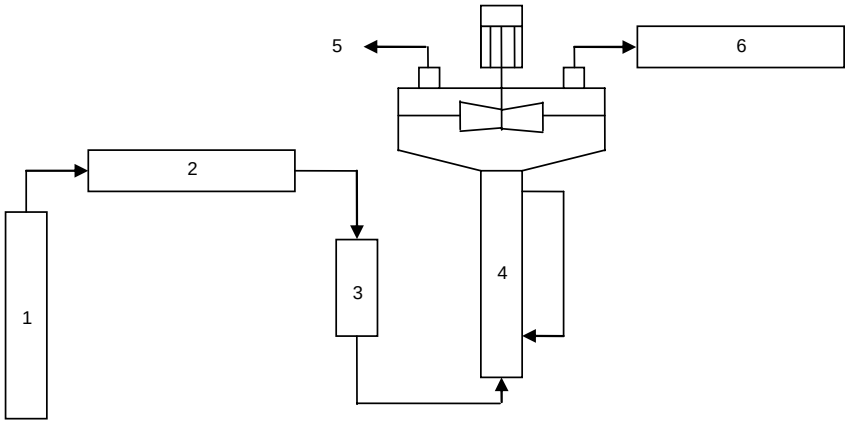
7.2.4. Thermal pre-treatment

The thermal pre-treatment was carried out in an autoclave (Fig. 7.1b) at 130ºC for 60 minutes, followed by a cooling period until room temperature before being stored in the fridge. In this case, a sludge volume of 10 L was used in each assay.

7.2.5. Ozonation

The ozonation of sludge was performed in the lab of Environmental Engineering of CEIT (San Sebastián). The sludge was ozonized in a 10-L bubble column (Figure 7.2) operated in batch and at room temperature (Marañón and Sancho, 2005). The gas flow rate was 240 $L \cdot h^{-1}$ with an ozone concentration of about 20 mg $O_3 \cdot L^{-1}$.The ozone dose was set approximately at 20 mg $O_3 \cdot g\ TSS^{-1}$ in

the reactor. The time needed to add the exact amount of ozone was calculated for each experiment based on the initial TSS of the sludge, being it around 2 h.



1. Oxygen 2. Ozonator 3. Rotameter 4. Ozone column 5. Sampling 6. Tiosulphate

Figure 7.2. Scheme of the batch ozonation unit.

7.2.6. Anaerobic digestion pilot plant

The anaerobic digestion pilot plant used in this work was the same as described in Chapter 6 (section 6.2.3).

During one year, the plat was fed with raw sludge coming from an urban STP. The results of the operation were presented in Chapter 6. Afterwards, the anaerobic digesters were fed with sludge previously treated by different methods (Table 7.1). Firstly, the alkaline treatment was used and two stages of operation were performed in each digester. The mesophilic reactor was operated with a SRT of 20 and 10 days; and the thermophilic one was run at 10 and 6 days. Then, the thermal process was used and the same operational stages have been carried out. Finally, the ozonation pre-treatment was performed. In this case, a single SRT was considered for the mesophilic and thermophilic reactor, 20 and 10 days, respectively.

In each operational stage, once the steady-state was achieved (after a period corresponding to 1 SRT), 1-2 samples of digested sludge were taken for PPCPs analysis. All the samples were taken as 5-day composite samples preserved by refrigeration (4ºC) and with the addition of hydrochloric acid to pH<2 to stop biological activity.

Table 7.1. Operational stages of the mesophilic and thermophilic digester combined with sludge pre-treatments.

	Pre-treatment	Period*	SRT (d)	Duration (months)
Mesophilic digester	Chemical	I)	20	2
		IV)	10	1
	Thermal	II)	20	2
		III)	10	1
	Ozonation	V)	20	2
Thermophilic digester	Chemical	I)	10	2
		IV)	6	1
	Thermal	II)	10	2
		III)	6	1
	Ozonation	V)	10	2

*The periods were named considering the chronological order of performance.

7.2.7. Analytical methods

TS, VS, TSS, VSS, COD, TOC, TC, TN, TKN, VFA and alkalinity were analyzed according to Standard Methods (APHA-AWWA-WPCF, 1999) as described in Chapter 2. The biogas production and composition was monitored as described in Chapter 2.

The soluble content of PPCPs in the digested sludge has been measured for all substances according to the methodology described in section 2.2.1 of Chapter 2. However, the concentrations in sludge phase have been only determined for some of them: musks, anti-inflammatories and estrogens. In addition, some extra measurements of antibiotics, Carbamazepine and Iopromide have been performed in few sludge samples.

7.2.8. Calculations

Pre-treatment effectiveness

The pre-treatment effectiveness has been analyzed in terms of COD and solids solubilization (Eq. 7.1 and 7.2), COD and solids mineralization (Eq. 7.3 and 7.4) and the ratio between soluble and total COD in the pretreated and non-pretreated sludge (Eq. 7.5).

$$COD_{solubilization} = \frac{COD_{s,pret} - COD_{s,non-pret}}{COD_{s,pret}} \times 100 \qquad \text{Eq. 7.1}$$

where:

$COD_{solubilization}$: percentage of COD solubilization (%),

$COD_{s,pret}$: soluble COD in the pretreated feeding ($g \cdot L^{-1}$), and

$COD_{s,non\text{-}pret}$: soluble COD in the non-pretreated feeding ($g \cdot L^{-1}$).

$$VSS_{solubilization} = \frac{VSS_{non-pret} - VSS_{pret}}{VSS_{non-pret}} \times 100 \qquad \text{Eq. 7.2}$$

where:

$VSS_{solubilization}$: percentage of volatile solids solubilization (%),

$VSS_{non\text{-}pret}$: VSS in the non-pretreated feeding ($g \cdot L^{-1}$), and

VSS_{pret}: VSS in the pretreated feeding ($g \cdot L^{-1}$).

$$COD_{mineralization} = \frac{COD_{t,non\text{-}pret} - COD_{t,pret}}{COD_{t,non\text{-}pret}} \times 100 \qquad \text{Eq. 7.3}$$

where:

$COD_{mineralization}$: percentage of COD mineralization (%),

$COD_{t,non\text{-}pret}$: total COD in the non-pretreated feeding ($g \cdot L^{-1}$), and

$COD_{t,pret}$: total COD in the pretreated feeding ($g \cdot L^{-1}$).

$$VS_{mineralization} = \frac{VS_{non-pret} - VS_{pret}}{VS_{non-pret}} \times 100 \qquad \text{Eq. 7.4}$$

where:

$VS_{mineralization}$: percentage of VS mineralization (%),

$VS_{non\text{-}pret}$: VS in the non-pre-treated feeding ($g \cdot L^{-1}$), and

VS_{pret}: VS in the feeding pretreated ($g \cdot L^{-1}$).

$$R = \frac{COD_s}{COD_t} \times 100 \qquad \text{Eq. 7.5}$$

where:

R: ratio between soluble and total COD (%),

COD_s: soluble COD ($g \cdot L^{-1}$), and

COD_t: total COD ($g \cdot L^{-1}$).

PPCPs mass balance

PPCPs mass balance was performed as described in Chapter 6 (section 6.2.5). Annex II shows the PPCPs concentrations in the liquid (supernantant) and solid phase (digested sludge). The values dismissed have been highlighted.

7.3. Results and Discussion

7.3.1. Pre-treatment effectiveness

Table 7.2 shows the main characteristics of the pre-treatments in terms of solids and COD solubilization and mineralization.

Table 7.2. Percentage of solids and COD solubilization and mineralization during alkaline, thermal and ozonation pre-treatment of sewage sludge.

	Solids		**COD**		
	Solubilization	Mineralization	Solubilization	Mineralization	R*
No pret.	-	-	-	-	5 - 8
Alkaline	0 - 13	0	55 - 82	0 - 4	15 - 24
Thermal	0 - 19	0 - 5	55 - 62	0 - 12	11 - 18
Ozonation	8	1	60	0	25

*$R=COD_s/COD_t$

From Table 7.2, it can be observed that the highest COD solubilisation was achieved with the alkaline pre-treatment (55-82%); while the thermal and the ozonation processes led to similar results (around 60%). However, the COD mineralization is very low during the three pre-treatments, being the highest percentage obtained with the thermal process (12%). The ratio between COD_s and COD_t increased from 5-8% in the non-pretreated feeding to 11-25% in the pretreated one.

Solids solubilization and mineralization percentages are below 20 and 5%, respectively, the higher values being obtained with the thermal pre-treatment.

7.3.2. Influence of pre-treatments on anaerobic digestion operation

Once finished the conventional operation of the anaerobic digestion pilot plant, the digesters were fed with sludge previously pre-treated. Several stages of operation depending on the SRT and the type of pre-treatment were performed in each digester (Table 7.1).

A summary of mesophilic and thermophilic digester operation is shown in Table 7.3 and 7.4, respectively.

Digesters performance

Two stages of operation with increasing OLR were performed in the mesophilic (Figure 7.3a) and thermophilic (Figure 7.3b) digesters with the alkaline (period I and IV) and thermal (period II and III) pre-treatments. However, with the ozonation process (period V), only one SRT was considered, 20 d in the mesophilic reactor and 10 d in the thermophilic one.

Despite the variations in OLR_{in}, the operation of both digesters was stable since OLR_{out} and OLR_{biogas} did not change significantly. Only when the SRT was decreased (period III and IV), higher values of OLR_{out} and OLR_{biogas} were achieved.

The OLR_{in} in the mesophilic process (Figure 7.3a) varied from 2-5 $kg \cdot m^{-3} \cdot d^{-1}$ at 20-d SRT to 6-10 $kg \cdot m^{-3} \cdot d^{-1}$ at 10-d SRT. Accordingly, the OLR_{out} and OLR_{biogas} ranged between 1-3 $kg \cdot m^{-3} \cdot d^{-1}$ and 2-4 $kg \cdot m^{-3} \cdot d^{-1}$, respectively. Therefore, the operation of the mesophilic digester was not affected by any pre-treatment.

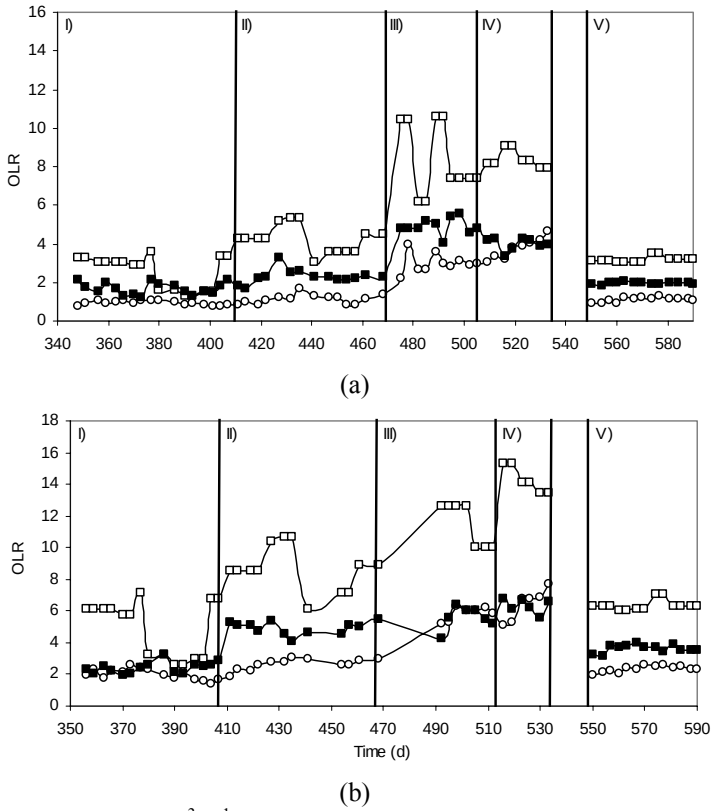
In the thermophilic process, the average OLR_{in} (Figure 7.3b) varied from 4-10 $kg \cdot m^{-3} \cdot d^{-1}$ at 10-d SRT to 8-14 $kg \cdot m^{-3} \cdot d^{-1}$ at 6-d SRT. Accordingly, the OLR_{out} and OLR_{biogas} ranged from 2 to 6 $kg \cdot m^{-3} \cdot d^{-1}$ and from 2-5 to 5-6 $kg \cdot m^{-3} \cdot d^{-1}$, respectively. Similarly to the mesophilic process, the operation of the thermophilic digester was not affected by any pre-treatment.

Table 7.3. Effluent quality and performance of the mesophilic process combined with sludge pre-treatments.

	Chemical		Thermal		Ozonation	Conventional process	
	Operating conditions						
SRT (d)	20	10	20	10	20	20	10
OLR (kg $COD_t \cdot m^{-3} \cdot d^{-1}$)	2.3 ± 1.0	8.1 ± 0.2	4.2 ± 1.0	9.0 ± 1.8	3.4 ± 0.3	2.1 ± 0.2	5.9 ± 1.4
OLR (kg $VS \cdot m^{-3} \cdot d^{-1}$)	1.9 ± 0.5	6.4 ± 1.5	3.1 ± 0.4	5.6 ± 0.9	1.9 ± 0.2	1.7 ± 0.2	4.1 ± 1.3
	Reactor						
TS ($g \cdot L^{-1}$)	39.8 ± 4.0	61.6 ± 0.5	42.8 ± 3.0	51.8 ± 8.4	28.3 ± 1.0	37.3 ± 4.3	40.2 ± 3.3
VS ($g \cdot L^{-1}$)	16.1 ± 1.7	30.9 ± 0.4	18.6 ± 1.5	21.8 ± 1.8	15.6 ± 0.7	12.6 ± 1.2	20.4 ± 2.1
TSS ($g \cdot L^{-1}$)	32.8 ± 4.6	48.5 ± 1.9	40.4 ± 5.5	46.1 ± 9.8	24.6 ± 1.3	33.4 ± 3.2	36.5 ± 3.8
VSS ($g \cdot L^{-1}$)	13.7 ± 1.8	26.1 ± 1.4	17.7 ± 3.1	20.7 ± 2.3	14.4 ± 0.7	12.3 ± 1.2	19.9 ± 2.4
COD_t ($g \cdot L^{-1}$)	18.5 ± 2.4	40.4 ± 1.4	24.8 ± 1.9	30.7 ± 2.9	23.4 ± 1.5	16.4 ± 3.0	30.5 ± 4.6
COD_s ($g \cdot L^{-1}$	1.3 ± 0.2	4.0 ± 1.2	1.7 ± 0.2	4.1 ± 1.1	3.0 ± 0.6	1.8 ± 0.3	3.2 ± 0.8
$N\text{-}NH_4^+$ (g/L)	0.6 ± 0.1	1.3 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	1.4 ± 0.2
pH	7.1 ± 0.1	7.0 ± 0.1	7.3 ± 0.1	7.7 ± 0.2	8.0 ± 0.1	7.8 ± 0.3	8.0 ± 0.3
TA ($g \cdot L^{-1}$)	5.0 ± 1.0	7.5 ± 1.8	9.7 ± 2.1	15.0 ± 2.8	6.2 ± 0.3	4.1 ± 0.6	6.2 ± 0.4
VFA/TA	0.68 ± 0.04	0.49 ± 0.08	0.61 ± 0.07	0.52 ± 0.14	0.24 ± 0.02	0.33 ± 0.10	0.30 ± 0.04
VFA (mg acetic$\cdot L^{-1}$)	3 ± 11	599 ± 890	19 ± 38	69 ± 142	29 ± 47	55 ± 81	182 ± 274
Acetic	3 ± 11	439 ± 657	18 ± 36	19 ± 40	28 ± 45	55 ± 81	133 ± 182
Propionic	0 ± 0	130 ± 175	1 ± 4	50 ± 112	1 ± 3	0 ± 1	40 ± 78
	Biogas						
Daily production ($L \cdot d^{-1}$)	10.6 ± 1.9	25.4 ± 0.5	14.3 ± 3.6	32.2 ± 2.7	12.8 ± 0.6	10.2 ± 1.9	19.4 ± 3.6
GPR ($m^3 \cdot m^{-3} \cdot d^{-1}$)	1.06 ± 0.19	2.54 ± 0.05	1.43 ± 0.36	3.22 ± 0.27	1.28 ± 0.06	1.02 ± 0.19	1.94 ± 0.36
$\%CH_4$	62.0 ± 3.3	63.7 ± 2.1	61.0 ± 3.0	60.4 ± 2.7	61.8 ± 1.0	59.2 ± 3.7	61.5 ± 2.4
$\%CO_2$	32.5 ± 3.0	33.0 ± 2.7	30.5 ± 2.5	30.9 ± 3.2	35.2 ± 1.4	33.7 ± 2.1	31.6 ± 2.3
SGP (m^3 $CH_4 \cdot kg$ VS_{rem}^{-1})	0.49 ± 0.10	0.53 ± 0.26	0.43 ± 0.09	0.60 ± 0.15	0.68 ± 0.09	0.54 ± 0.16	0.58 ± 0.27

Table 7.4. Effluent quality and performance of the thermophilic process combined with sludge pre-treatments.

	Chemical		Thermal		Ozonation	Conventional process	
	Operating conditions						
SRT (d)	10	6	10	6	10	10	6
OLR (kg $COD_t \cdot m^{-3} \cdot d^{-1}$)	3.6 ± 1.3	13.8 ± 0.4	8.7 ± 1.8	10.9 ± 1.5	6.6 ± 0.5	4.3 ± 0.5	12.8 ± 0.5
OLR (kg $VS \cdot m^{-3} \cdot d^{-1}$)	3.7 ± 1.1	11.3 ± 3.0	6.1 ± 0.8	8.0 ± 0.7	3.8 ± 0.4	3.4 ± 0.4	9.2 ± 0.4
	Reactor						
TS ($g \cdot L^{-1}$)	48.4 ± 7.5	71.7 ± 8.7	56.8 ± 4.5	48.6 ± 4.8	31.1 ± 2.3	41.6 ± 6.1	45.5 ± 4.8
VS ($g \cdot L^{-1}$)	18.3 ± 2.6	31.2 ± 2.7	22.5 ± 3.0	23.5 ± 1.1	16.6 ± 1.2	13.2 ± 1.7	21.2 ± 2.4
TSS ($g \cdot L^{-1}$)	41.3 ± 10.6	53.9 ± 4.6	55.6 ± 5.7	42.9 ± 1.3	26.6 ± 1.1	41.5 ± 4.8	39.7 ± 2.8
VSS ($g \cdot L^{-1}$)	15.0 ± 3.2	24.3 ± 1.6	20.7 ± 2.0	22.3 ± 1.5	14.5 ± 0.5	12.0 ± 1.2	19.5 ± 1.6
COD_t ($g \cdot L^{-1}$)	19.7 ± 3.7	41.3 ± 2.6	28.1 ± 1.8	35.9 ± 1.4	24.8 ± 1.4	15.0 ± 2.1	31.3 ± 3.0
COD_s ($g \cdot L^{-1}$	2.6 ± 0.3	8.7 ± 3.1	4.3 ± 0.4	6.8 ± 0.7	8.3 ± 0.7	3.0 ± 0.3	8.5 ± 1.0
$N\text{-}NH_4^+$ (g/L)	0.8 ± 0.1	1.6 ± 0.1	0.9 ± 0.2	1.2 ± 0.1	1.3 ± 0.1	0.6 ± 0.1	1.5 ± 0.1
pH	7.3 ± 0.1	7.2 ± 0.2	7.7 ± 0.2	8.1 ± 0.2	8.3 ± 0.1	7.7 ± 0.1	8.3 ± 0.2
TA ($g \cdot L^{-1}$)	4.8 ± 1.8	5.1 ± 0.4	10.3 ± 4.0	9.4 ± 2.3	6.3 ± 0.3	4.3 ± 1.0	6.2 ± 0.3
VFA/TA	0.70 ± 0.04	0.74 ± 0.08	0.61 ± 0.05	0.38 ± 0.04	0.24 ± 0.04	0.48 ± 0.11	0.39 ± 0.08
VFA (mg acetic$\cdot L^{-1}$)	181 ± 171	1817 ± 1662	349 ± 478	305 ± 271	284 ± 189	439 ± 324	1,065 ± 559
Acetic	16 ± 38	978 ± 957	119 ± 202	50 ± 63	126 ± 64	143 ± 193	201 ± 144
Propionic	124 ± 88	599 ± 463	204 ± 298	252 ± 243	151 ± 154	262 ± 237	711 ± 390
	Biogas						
Daily production ($L \cdot d^{-1}$)	16.3 ± 1.5	41.1 ± 2.8	35.0 ± 1.8	37.3 ± 2.4	23.4 ± 0.4	20.2 ± 2.8	37.3 ± 5.9
GPR ($m^3 \cdot m^{-3} \cdot d^{-1}$)	1.63 ± 0.15	4.11 ± 0.28	3.50 ± 0.18	3.73 ± 0.24	2.34 ± 0.04	2.02 ± 0.28	3.73 ± 0.59
$\%CH_4$	62.3 ± 3.7	63.9 ± 2.1	59.4 ± 4.4	65.9 ± 5.5	65.2 ± 2.20	58.3 ± 3.0	66.6 ± 3.1
$\%CO_2$	32.6 ± 4.8	33.6 ± 2.0	35.1 ± 5.4	31.0 ± 6.1	32.7 ± 2.4	34.1 ± 3.7	31.3 ± 3.4
SGP (m^3 $CH_4 \cdot kg$ VS_{rem}^{-1})	0.37 ± 0.04	0.49 ± 0.26	0.54 ± 0.13	0.59 ± 0.12	0.68 ± 0.11	0.65 ± 0.24	0.54 ± 0.09



(a)

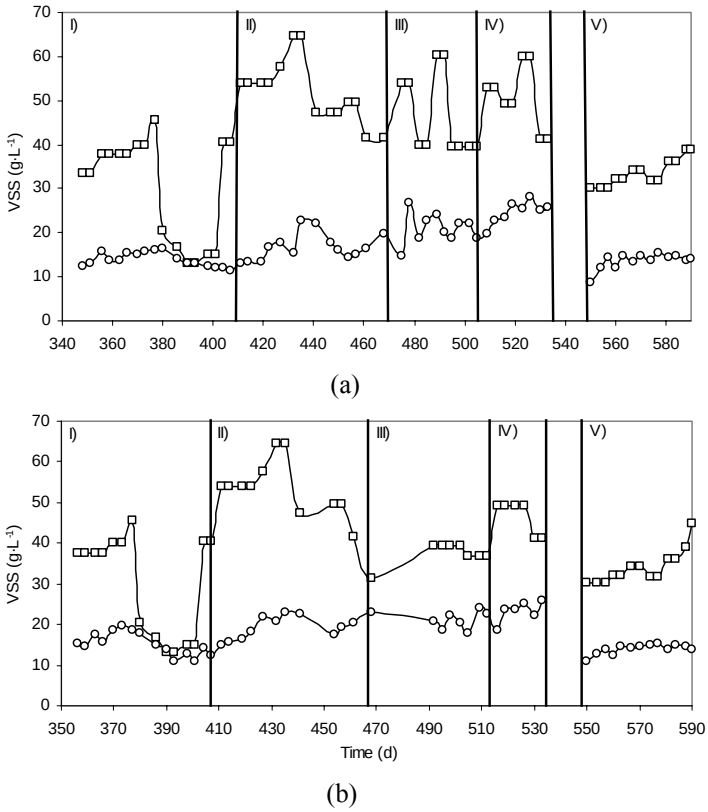
(b)

Figure 7.3. OLR ($kg \cdot m^{-3} \cdot d^{-1}$) at the inlet (□), outlet (o) and in the biogas (■) of mesophilic (a) and thermophilic (b) digester during the experimental period. See Table 7.1 for operational conditions of periods I to V.

Solids reduction

Figure 7.4 shows the VSS concentrations in the feeding and digested sludge of mesophilic (a) and thermophilic (b) reactor, respectively.

The feeding showed the normal fluctuations related with the STP operation, with VSS concentrations of 40-65 $g \cdot L^{-1}$ during alkaline (except the last days of period I with values of 20 $g \cdot L^{-1}$) and thermal pre-treatments, and lower values in the ozonation process (30-40 $g \cdot L^{-1}$). However, the VSS concentration in the effluent remains constant in both digesters, between 10 and 20 $g \cdot L^{-1}$. These values are in the range of those reported (Table 6.1) for digested sludge (Govin *et al.,* 1991; Zabranská *et al.,* 2000).



(a)

(b)

Figure 7.4. VSS concentrations in the feeding (□) and digested sludge (o) of mesophilic (a) and thermophilic (b) digester. See Table 7.1 for operational conditions of periods I to V.

Table 7.5 shows the average VSS removal efficiencies obtained in each stage of operation in comparison with those obtained in the conventional process. During advanced operation, the solids elimination ranged from 55 to 70% in the mesophilic range, whereas in the thermophilic digester it varied from 40 to 60%. These values are in the same range as those reported in literature (Table 6.2) for mesophilic (27-62%) and thermophilic (44-56%) digesters.

Independently of the type of operation (conventional or with pre-treatment), the solids removal decreased in both digesters when they were run at lower SRT. Alkaline and thermal pre-treatments slightly enhanced solids elimination in the mesophilic process regardless of the SRT; however, no effect was observed with the ozonation process. In the thermophilic digester, no influence of any pre-

treatment was obtained except the thermal hydrolysis at 6-d SRT, which led to lower solids removal (40%).

Table 7.5. Average removal efficiencies (%) of VSS during mesophilic and thermophilic anaerobic digestion combined with alkaline, thermal and ozonation pre-treatment of sewage sludge. n.d.: no data.

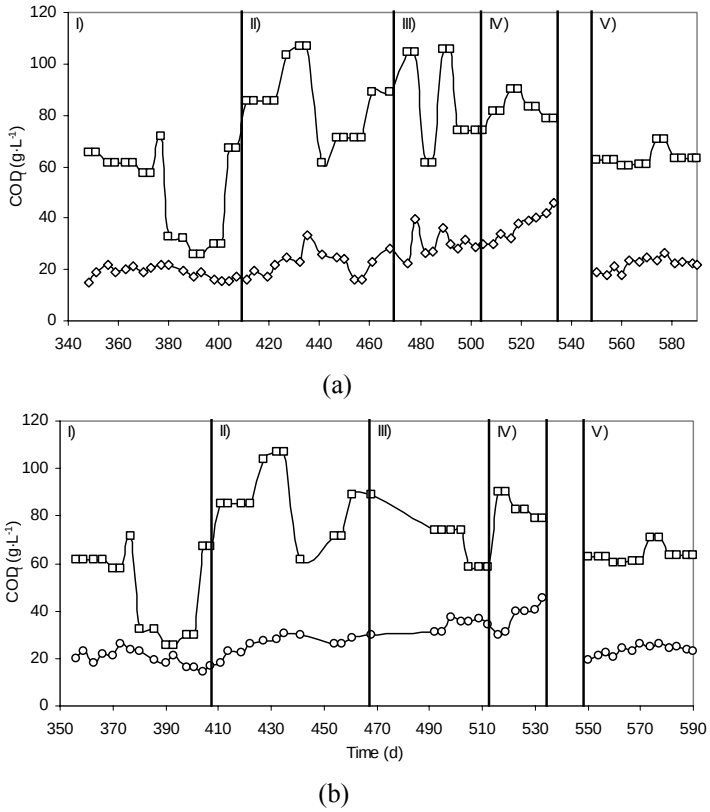
Mesophilic digester				
SRT (d)	**Conventional**	**Alkaline**	**Thermal**	**Ozonation**
20	61	66	69	60
10	50	61	56	n.d.
Thermophilic digester				
10	61	59	61	59
6	53	53	39	n.d.

When both digesters were run at the same SRT, during conventional operation the solids removal was higher in the thermophilic process. However, similar results were achieved in both reactors when they were operated with pretreated sludge.

COD reduction

Figure 7.5 shows the total COD concentrations in the feeding and digested sludge of mesophilic (a) and thermophilic (b) reactor, respectively. Once again, the feeding showed the normal fluctuations related with the STP operation (60-110 $g \cdot L^{-1}$), except the last days of period I with values around 30 $g \cdot L^{-1}$. However, the COD_t concentrations in the effluent remained constant in both digesters, around 20-40 $g \cdot L^{-1}$. These values are in the range of those reported in literature (Table 6.1) for digested sludge (Govin *et al.,* 1991; Tapana and Pagilla, 2000; Zabranská *et al.,* 2000).

Table 7.6 shows the average COD_t removal efficiencies obtained in each stage of operation in comparison with those obtained in the conventional process. During advanced operation, the elimination of total COD ranged from 50 to 75% in the mesophilic range, whereas in the thermophilic digester it varied from 45 to 70%. Independently of the type of operation (conventional or advanced), the removal of organic matter decreased in both digesters when they were run at lower SRT.



(a)

(b)

Figure 7.5. COD_t concentrations in the feeding (□) and digested sludge (o) of mesophilic (a) and thermophilic (b) digester. See Table 7.1 for operational conditions of periods I to V.

Alkaline and thermal pre-treatments slightly enhanced COD_t elimination in the mesophilic process at 20-d SRT; however, no effect was observed with the ozonation process. At 10-d SRT, only the thermal process led to higher efficiencies.

In the thermophilic digester, no influence of any pre-treatment was obtained at 10-d SRT; however, the COD_t removal decreased with both alkaline and thermal processes at 6-d SRT.

When both digesters were run at the same SRT, higher removal efficiencies were achieved in the thermophilic process, regardless of the type of operation.

Table 7.6. Average removal efficiencies (%) of total COD during mesophilic and thermophilic anaerobic digestion combined with alkaline, thermal and ozonation pre-treatment of sewage sludge. n.d.: no data.

Mesophilic digester				
SRT (d)	**Conventional**	**Alkaline**	**Thermal**	**Ozonation**
20	62	70	75	66
10	51	50	64	n.d.
Thermophilic digester				
10	65	59	69	64
6	56	49	43	n.d.

While the COD_s level (Figure 7.6) in the feeding varied strongly, from 5 to 20 $g \cdot L^{-1}$, due to type of sludge and the pre-treatment used, the concentrations in both digesters remained constant, with higher values in the thermophilic reactor (around 5 $g \cdot L^{-1}$) than in the mesophilic one (around 2 $g \cdot L^{-1}$). These values are in the same range as those reported in literature (Song *et al.,* 2004).

However, when the SRT was decreased (period III and VI), more fluctuations were observed in both digesters and higher concentrations were obtained, mainly in the thermophilic process (up to 15 $g \cdot L^{-1}$). Finally, with the ozonation process (period V), the COD_s concentrations in the digested sludge remained constant again.

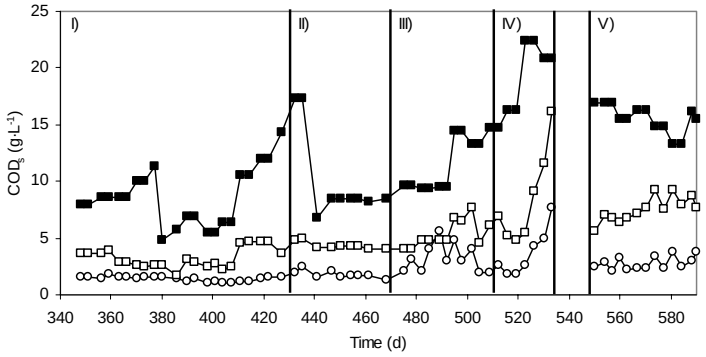


Figure 7.6. COD_s concentrations in the feeding (■) and digested sludge of mesophilic (o) and thermophilic (□) digester.

Table 7.7 shows the average COD_s removal efficiencies obtained in each stage of operation in comparison with those obtained in the conventional process.

Table 7.7. Average removal efficiencies (%) of soluble COD during mesophilic and thermophilic anaerobic digestion combined with alkaline, thermal and ozonation pre-treatment of sewage sludge. n.d.: no data.

Mesophilic digester				
SRT (d)	**Conventional**	**Alkaline**	**Thermal**	**Ozonation**
20	65	82	81	80
10	57	82	74	n.d.
Thermophilic digester				
10	56	64	60	46
6	16	60	51	n.d.

During advanced operation, the elimination of soluble COD ranged from 75 to 80% in the mesophilic range, whereas lower efficiencies were obtained in the thermophilic digester, from 45 to 65%. The removal of COD_s decreased in both digesters when they were run at lower SRT, except with alkaline treatment, with which similar results were obtained.

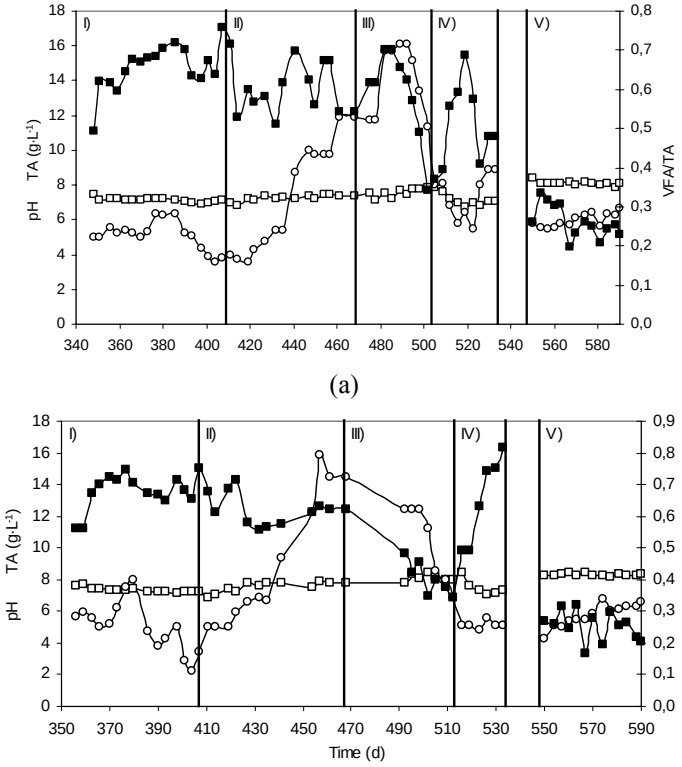
The three pre-treatments tested enhanced COD_s elimination in the mesophilic range, from 60 to 80%. However, in the thermophilic process the results obtained were similar at 10-d SRT and better in the advanced operation at 6-d SRT.

When both digesters were run at the same SRT, higher removal efficiencies were achieved in the mesophilic process, regardless of the type of operation.

VFA, alkalinity and pH

The pH value remained essentially constant during the experimental period in both digesters (Figure 7.7). The pH of the thermophilic process (Figure 7.7b) was slightly higher (7.5-8.5) than that of the mesophilic one (Figure 7.7a), which ranged between 7.0 and 8.0, approximately.

The alkalinity level in the mesophilic digester (Figure 7.7a) remained constant (around 5 $g \cdot L^{-1}$) until day 440, when it started increasing up to 16 $g \cdot L^{-1}$, recovering the initial levels at the end of the experiment. The same pattern was observed in the thermophilic reactor, with slightly higher values, that explained the also higher pH value of this process. The increased alkalinity, and thus pH, in the thermophilic digester is in agreement with previous studies (Yu *et al.,* 2002; Song *et al.,* 2004).



(a)

(b)

Figure 7.7. pH (□), TA (o) and VFA/TA (■) ratio in the mesophilic (a) and thermophilic (b) digester. See Table 7.1 for operational conditions of periods I to V.

The alkalinity in anaerobic digestion is related with ammonia nitrogen and VFA concentrations. Figures 7.8 and 7.9 show their concentrations during the experimental period, respectively.

The ammonia nitrogen concentrations were higher in the thermophilic digestion process (600-1,700 $mg \cdot L^{-1}$) than those of the mesophilic one (500-1,500 $mg \cdot L^{-1}$). This indicates that the activity for the degradation of nitrogenous organic compounds under the thermophilic conditions was higher than that under mesophilic conditions (Sánchez *et al.,* 2000). The maximum values of ammonia nitrogen concentration in both mesophilic (1,500 $mg \cdot L^{-1}$) and thermophilic (1,700 $mg \cdot L^{-1}$) reactors exceed the threshold level (1,000 $mg \cdot L^{-1}$) considered as inhibitory for methane production (Koster and Lettinga, 1988). However, it is also reported that methanogenic bacteria can acclimate to ammonia nitrogen

concentrations up to 3,100 mg·L^{-1}, with little effect in the methane production (Fujishima *et al.,* 2000).

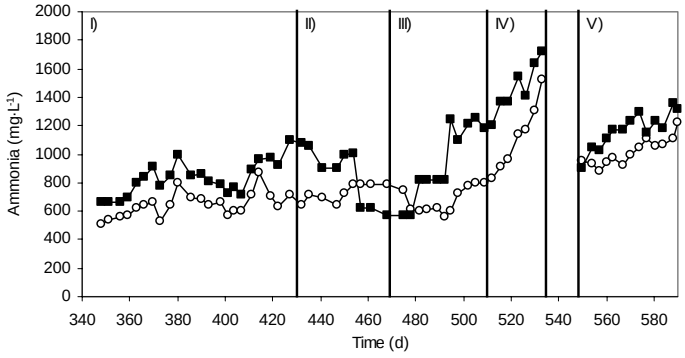


Figure 7.8. Ammonia nitrogen concentrations in the mesophilic (o) and thermophilic (■) digester.

The increase in ammonia nitrogen content between day 480 and 535 (Figure 7.8) explains the also higher TA obtained (Figure 7.7) in these days.

The VFA levels in the thermophilic process were generally higher than those in the mesophilic one (Figure 7.9), which is consistent with the COD_s data (Figure 7.6). This fact shows that the mesophilic digestion was superior to the thermophilic one in terms of the effluent quality.

The VFA concentrations remained below 1 g HAc·L^{-1} in both digesters, except in the last days of period IV, where higher values were obtained, up to 3.8 g·L^{-1} in the thermophilic digester and 2.5 g·L^{-1} in the mesophilic one. The main component of the VFA in the mesophilic process was acetic (Table 7.3), but in the thermophilic process it was propionic (Table 7.4).

The VFA content of the COD_s was below 0.5% (2% maximum) and below 3% (maximum 7%) for the mesophilic and thermophilic digestion process, respectively. However, when the digesters were run at low SRT with the thermal pre-treatment (period IV), the values increased up to 3% (8% maximum) in the mesophilic process and up to 9% (18% maximum) in the thermophilic one. All these values are lower than those reported by Song *et al.* (2004), 22.7% for the mesophilic reactor and 30.3% for the thermophilic one.

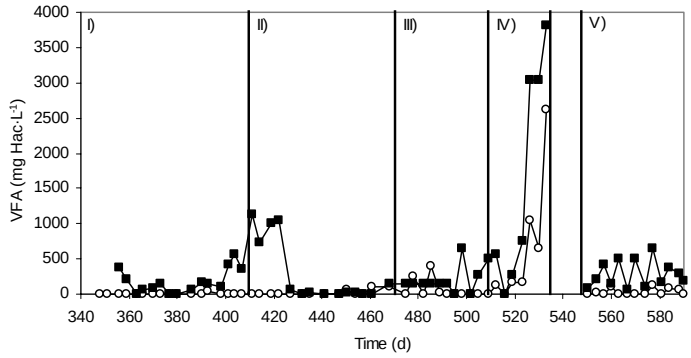


Figure 7.9. VFA concentrations in the mesophilic (o) and thermophilic (■) digester.

The VFA/TA ratio (Figure 7.7) varied strongly in both digesters during alkaline and thermal pre-treatments (periods I-IV), around 0.3-0.8; however, it remained constant (0.2-0.3) during ozonation process (period V). This fact indicates that, although the VFA levels were higher in the thermophilic digester, the buffering capabilities were similar in both processes.

An increase of VFA/TA values was observed in both digesters at the end of period IV, probably as a result of the decrease in the TA (Figure 7.7) and the higher VFA concentrations observed (Figure 7.9).

Gas production and methane content

Daily biogas production varied from 10.6 to 32.2 $L \cdot d^{-1}$ in the mesophilic process (Table 7.3), and from 16.3 to 41.1 $L \cdot d^{-1}$ in the thermophilic one (Table 7.4). Therefore, the Gas Production Rate (GPR) of the thermophilic process (1.6-4.1 $m^3 \cdot m^{-3} \cdot d^{-1}$) is higher than that of the mesophilic one (1.1-3.2 $m^3 \cdot m^{-3} \cdot d^{-1}$). These values are higher than those obtained in the conventional operation (Chapter 6).

When the pilot plant was operated with the high SRT, the highest biogas productions were obtained with the thermal hydrolysis, followed by those obtained with the ozonation process and lastly, the alkaline treatment, independently of the temperature (mesophilic vs. thermophilic conditions). However, at low SRT, while the same pattern was obtained in the mesophilic digester, the values obtained with the alkaline treatment were higher than those achieved with the thermal hydrolysis in the thermophilic process.

The average methane content of the biogas remained constant in both digesters during the experimental period (60-65%). Figure 7.10 shows the Specific Gas Production (SGP), based on the VS removed, in both digesters during the experimental period. The values were very similar in both digesters, ranging from 300 to 800 L CH_4·kg VS_{rem}^{-1}. Only between days 380 and 400, higher values were obtained due to the decrease in the amount of VS removed (Figure 7.4). Dismissing these values in the calculation of the average SGP for each experimental period, the results (Tables 7.3 and 7.4) indicated the highest SGP being achieved with the ozonation process, followed by alkaline treatment and lastly, the thermal hydrolysis. However, the SGP values with the thermal hydrolysis were higher than those with the alkaline pre-treatment when the digesters were run at low SRT.

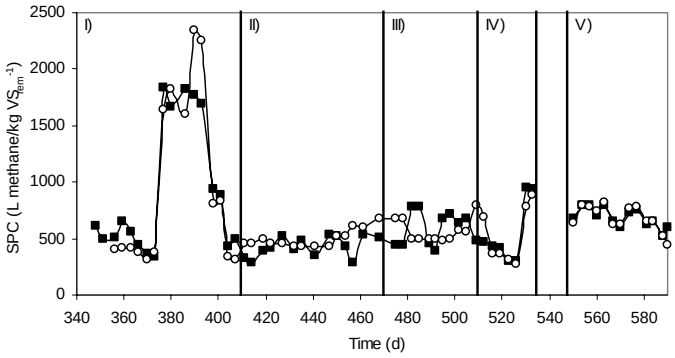


Figure 7.10. Specific methane production in the mesophilic (■) and thermophilic (o) digester.

Discussion

The sludge feeding composition was not constant and dependant on the operation of the sludge train in the sewage treatment plant. Average solids concentration in the feed was kept around the optimum value 50 g·L^{-1} (Killilea *et al.,* 2000) established in order to obtain an efficient solids removal in the digesters without causing mechanical problems with pumps, heat exchangers and mixing units.

Despite the variation of the feeding characteristics, the operation of both reactors was stable except with the thermal treatment at low SRT (period IV). In this period, an increase of VFA concentrations led to a decrease in the TA values

and consequently the ratio VFA/TA increased. Even if the operative conditions applied in the thermophilic digester were more drastic in terms of OLR respect to the mesophilic ones, no significant differences were observed between the performances of both processes, as can be seen from the values of the stability parameters reported (Figure 7.7).

Although the VFA content observed in both digesters in period IV was high (Figure 7.9), the buffering capacity of the digested sludge was enough to maintain the pH in the neutral range (Figure 7.7).

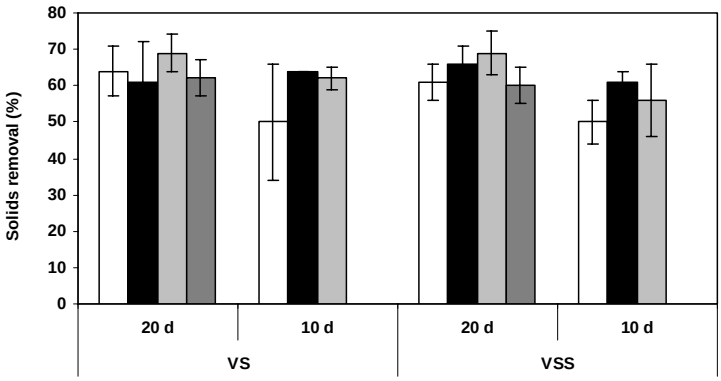
The difference between the volatile solids content in the thermophilic and mesophilic digested sludge was not significant (Figure 7.4), as expected from the also similar specific methane production in both digesters (Figure 7.10).

To sum up, Figures 7.11 and 7.12 show the removal efficiencies of volatile solids and COD, respectively, in the mesophilic and thermophilic digester during the experimental period.

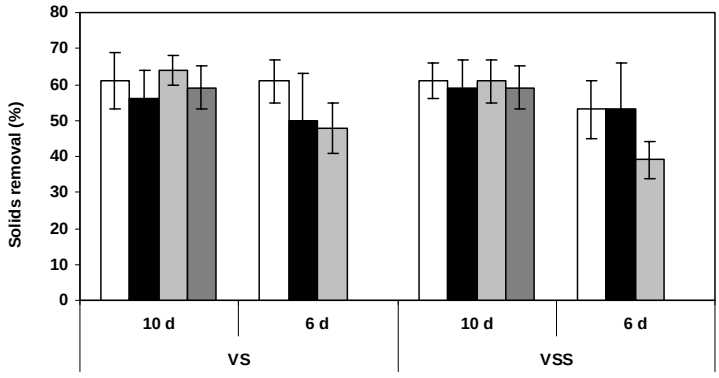
In general, the higher OLR applied the lower solids and COD removal efficiencies obtained under both mesophilic and thermophilic conditions. Besides, no significant differences (less than 15%) were observed between the three pre-treatments tested.

Similar solids elimination was obtained in the conventional and advanced operation of thermophilic digester (Figure 7.11b) when it was run at high SRT (10 d). However, the results obtained in the conventional process were better than those achieved with the pre-treatments when it was operated at low SRT (6 d). In the mesophilic digester (Figure 7.11a), advance operation lead to similar or higher solids elimination than the conventional process, regardless of the SRT.

When both digesters were operated at the same SRT, the operation at high temperature (thermophilic conditions) raises the solids removal in the conventional process. However, no effect was observed when the pre-treatments were used.



(a)



(b)

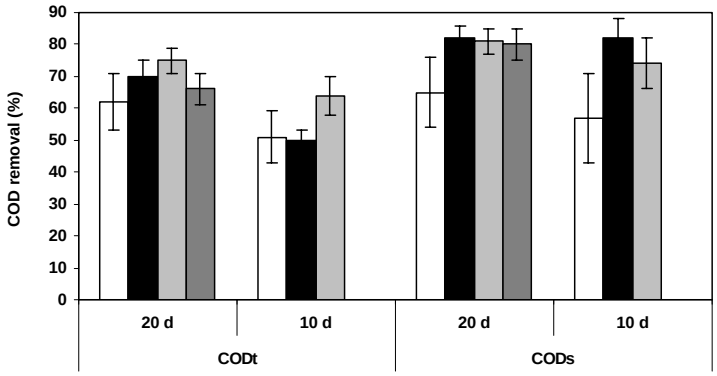
Figure 7.11. Volatile solids removal (%) in the mesophilic (a) and thermophilic (b) digester. □ Conventional; ■ Alkaline; ■ Thermal; ■ Ozonation.

Higher COD elimination was obtained in the advanced operation of mesophilic digester (Figure 7.12a), independently of the SRT. However, no significant differences were observed in the thermophilic process except for the COD_s elimination at 6-d SRT, which was improved by the use of the pre-treatments.

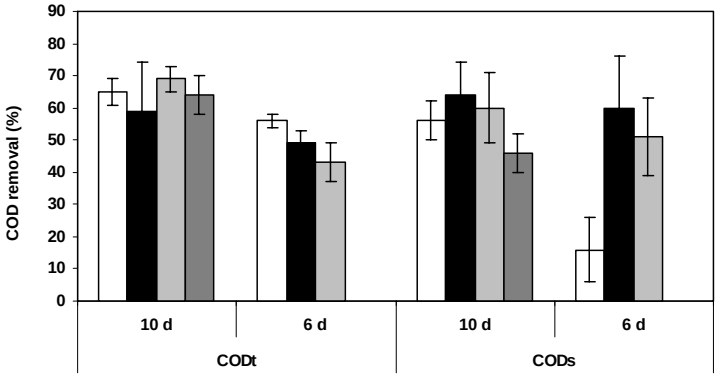
When both digesters were operated at the same SRT, the thermophilic process led to higher removal efficiencies of total COD, whereas the elimination of soluble COD was better in the mesophilic range.

Therefore, it seems possible to state that the conventional thermophilic anaerobic digestion process leads to a higher degree of stabilization of the

digested sludge compared to the conventional mesophilic process. However, when applying sludge pre-treatments, these differences decrease and the results are more similar.



(a)



(b)

Figure 7.12. COD removal (%) in the mesophilic (a) and thermophilic (b) digester. □ Conventional; ■ Alkaline; ■ Thermal; ■ Ozonation.

Operational problems

The operational problems found were the same as those described in section 6.3.2 of Chapter 6. In addition, during sludge ozonation a serious foaming appeared at the end of the experiment, making very difficult the process.

It should be also pointed out that during thermal hydrolysis, the sludge was not stirred. Thus, an extra period of 10-15 min was considered in order to assure that all the sludge content was at the selected temperature (130ºC).

7.3.3. Overall efficiencies of the advanced operation

In the previous section, the influence of different sludge pre-treatments on the anaerobic digestion operation was described. Here, the overall performance of the advanced operation (pre-treatment + anaerobic digestion) is evaluated in terms of solids and COD removal. The only difference is the reference used for the removal efficiencies calculations. While in the section before it was the pretreated feeding, here it was the non-pretreated one.

Figure 7.13 shows the solids and COD removal in the mesophilic and thermophilic digester during advanced operation with alkaline (a, b), thermal (c, d) and ozone (e, f) pre-treatment in comparison with those obtained in the single anaerobic digestion process.

Slightly higher VS removal efficiencies were obtained in the anaerobic digestion process compared to the advanced operation when the alkaline (Figure 7.13a) and thermal (Figure 7.13c) pre-treatments were applied in both digesters. The reason is that the VS concentrations in the pretreated feeding were higher (40-65 $g \cdot L^{-1}$) than in the non-pretreated one (35-55 $g \cdot L^{-1}$), due to the lime addition in the alkaline process and to a concentration effect caused by the water vaporization in the thermal treatment (130ºC, 60 min). With ozonation, no significant differences were observed between the anaerobic digestion and the advanced operation (Figure 7.13 e).

In contrast, the VSS removal efficiencies were higher in the advanced operation than in the anaerobic digestion, except with the thermal treatment (Figure 7.13c), regardless of temperature of operation. The reason is that the VSS concentrations in the pretreated feeding were lower than those in the non-pretreated one due to the solids solubilization. In the case of the thermal process, the solubilization is compensated with the concentration effect, leading to similar or higher VSS content in the pretreated feeding.

Concerning the organic matter, the elimination of total COD in the advanced operation and anaerobic process was similar for the alkaline (Figure 7.13b) and ozone treatments (Figure 7.13f) and slightly higher for the thermal process (Figure 7.13d). Once again, the concentration effect explains the results achieved with the thermal pre-treatment. However, the soluble COD removal was much higher in the anaerobic process than in the overall advanced operation, mainly in the thermophilic digester, independently of the type of pre-treatment. This fact is explained by the solubilization effect of the pre-treatments.

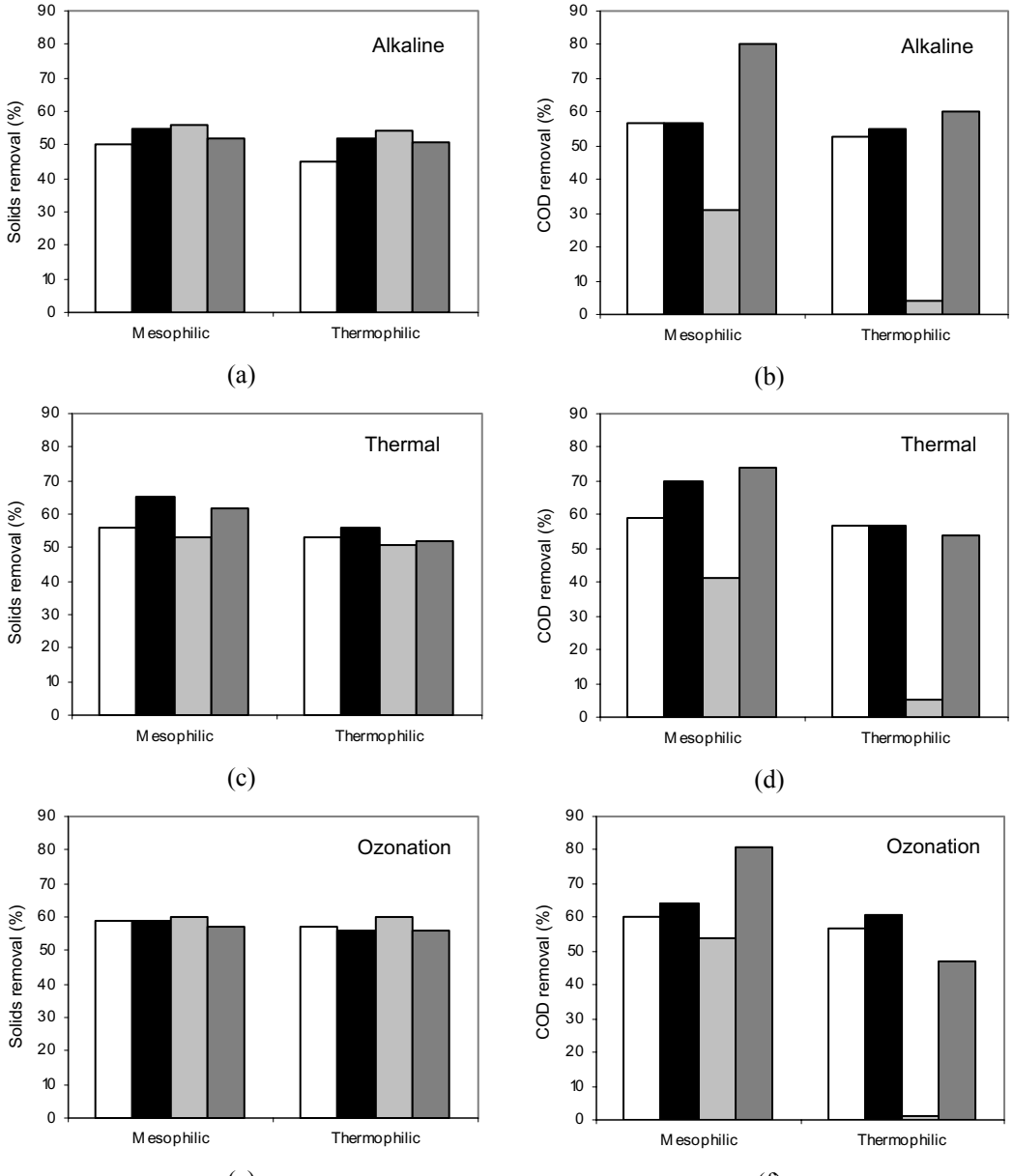


Figure 7.13. Solids and COD removal (%) during advanced operation (AO) with alkaline (a, b), thermal (c, d) and ozone (e, f) pre-treatments compared to the single anaerobic digestion process (AD). VS_{AO}, $COD_{t,AO}$ (□); VS_{AD}, $COD_{t,AD}$ (■); VSS_{AO}, $COD_{s,AO}$ (■); VSS_{AD}, $COD_{s,AD}$ (■).

7.3.4. Fate of PPCPs during sludge anaerobic digestion combined with pre-treatments

For each stage of digesters operation, the mass balance of PPCPs was performed following the method described in section 6.2.5. The results obtained are shown in the following sections.

Background concentration

For those compounds detected in the STP considered, the background concentration in the raw sludge was determined. The parameters used in the calculations were the same as indicated in Chapter 6 (Table 6.5) and the results obtained for each operational period are shown in Table 7.8.

The musks were the substances with the highest concentrations in the raw sludge, around 1,000-2,000 $\mu g \cdot L^{-1}$ for Galaxolide and 350-650 $\mu g \cdot L^{-1}$ for Tonalide. The concentrations of Ibuprofen, Naproxen, Iopromide and Sulfamethoxazole were similar, ranging from 7 to 16 $\mu g \cdot L^{-1}$; E2 content was between 1 and 2 $\mu g \cdot L^{-1}$ and the levels of the other compounds detected, Carbamazepine, E1 and EE2, were below 0.5 $\mu g \cdot L^{-1}$.

Table 7.8. Background content of PPCPs in the raw sludge of anaerobic digestion combined with alkaline, thermal and ozonation. See Table 7.1 for periods description.

	Alkaline		**Thermal**		**Ozonation**
Period	I	II	III	IV	V
TSS_P ($g \cdot L^{-1}$)	50 - 80	60 - 120	95 - 120	15 - 25	50 - 55
TSS_B ($g \cdot L^{-1}$)	25 - 35	25 - 35	60 - 70	20 - 35	15 - 20
			C_{raw} ($\mu g \cdot L^{-1}$)		
HHCB	1,267 ± 396	1,759 ± 775	1,957 ± 321	1,269 ± 127	1,024 ± 64
AHTN	452 ± 138	607 ± 256	653 ± 111	448 ± 23	359 ± 23
CBZ	0.48 ± 0.07	0.58 ± 0.15	0.62 ± 0.06	0.48 ± 0.03	0.44 ± 0.01
IBP	12 ± 3	15 ± 5	16 ± 2	12 ± 1	10
NPX	11 ± 2	13 ± 4	14 ± 2	11	9
IPM	10 ± 1	11 ± 1	11 ± 1	10	10
SMX	8 ± 2	12 ± 5	13 ± 2	8 ± 1	7
E1	0.12 ± 0.04	0.16 ± 0.07	0.17 ± 0.03	0.12 ± 0.01	0.10 ± 0.01
E2	1.18 ± 0.37	1.65 ± 0.73	1.84 ± 0.30	1.19 ± 0.13	0.96 ± 0.06
EE2	0.24 ± 0.07	0.31 ± 0.13	0.33 ± 0.06	0.23 ± 0.01	0.19 ± 0.01

Inlet concentration

The total inlet concentration (Table 7.9) is the sum of the background (Table 7.8) and the spike (Chapter 6, Table 6.2).

Table 7.9. Spiked and total inlet concentrations (in $\mu g \cdot L^{-1}$) of PPCPs in the feeding of the anaerobic digesters during advanced treatment.

PPCP	Spike	C_{in}		
		Alkaline	**Thermal**	**Ozonation**
HHCB	400	1,913 ± 577	2,013 ± 444	1,424 ± 64
AHTN	200	730 ± 190	751 ± 135	559 ± 23
CBZ	20	≈ 20	≈ 20	≈ 20
DZP	20	20	20	20
IBP	10	23 ± 4	24 ± 3	20
NPX	10	22 ± 3	22 ± 2	19
DCF	10	10	10	10
IPM	40	50 ± 1	50 ± 1	50
SMX	40	50 ± 4	51 ± 3	47
ROX	40	40	40	40
E1	4	≈ 4	≈ 4	≈ 4
E2	8	≈ 9	≈ 9	≈ 9
EE2	4	≈ 4	≈ 4	≈ 4

The C_{in} for the PPCPs considered ranged between 4 and 50 $\mu g \cdot L^{-1}$, with the exception of Galaxolide and Tonalide, which showed higher values, around 1,500-2,000 and 550-750 $\mu g \cdot L^{-1}$, respectively.

The contribution of the background concentration was not significant for Carbamazepine, E1 and EE2, and it was lower than 20% for IPM, SMX and E2. However, the concentrations of Ibuprofen and Naproxen in the raw sludge were in the same range as the spike (around 10 $\mu g \cdot L^{-1}$); while for Galaxolide and Tonalide they were much higher, between 3-5 and 2-3 times, respectively.

Outlet concentration

The PPCPs concentrations measured in the liquid (supernatant) and solid phase (digested sludge) are shown in Annex II.

A statistical selection of data has been carried out following the criteria explained in section 6.2.5. The values dismissed have been highlighted.

Mass balance results

Tables 7.10 and 7.11 show the results of PPCPs mass balance in each experimental period of the mesophilic and thermophilic process, respectively. The error was calculated as the standard deviation when the number of data was higher than two or as the average error when only two values were available.

Similarly to the conventional operation, the combined concentrations of E1 and E2 were considered in the mass balance calculations.

PPCPs removal

The main mechanisms involved in PPCPs removal during anaerobic digestion are sorption and biodegradation, since volatilization and photodegradation are negligible.

From Tables 7.10 and 7.11, it can be observed that those compounds with high sorption affinity, such as musks, Diclofenac and estrogens, are mainly present associated to solids. For the other substances, the distribution between the liquid and solid phase is more equal.

Next, the results obtained for each single substance during the advanced operation will be discussed, considering the following factors:

- Type of pre-treatment: alkaline versus thermal versus ozonation.
- Type of operation: conventional versus advanced.
- SRT: high versus low.
- Temperature: mesophilic versus thermophilic.
- Data confirmation process.
- Data selection.

Galaxolide

Galaxolide was significantly removed in both digesters (Table 7.12), with removal efficiencies ranging from 50 to 85% in the mesophilic process and from 50 to 70% in the thermophilic one.

Table 7.10. Mass flux of PPCPs (μg·d^{-1}) and removal efficiency (%) in the mesophilic digester during alkaline, thermal and ozonation pre-treatments.

Mass flux of PPCPs (μg·d^{-1})			**Alkaline**		**Thermal**		**Ozonation**
			SRT 20 d	***SRT 10 d***	***SRT 20 d***	***SRT 10 d***	***SRT 20 d***
HHCB	***Inlet***		834	2,159	1,178	1,669	712
	Outlet	***Dissolved***	0.5 ± 0.0	0.9	0.8 ± 0.4	1.3	0.3 ± 0.0
		Sorbed	266 ± 19	621	313 ± 41	871	101 ± 4
	Removal (%)		68.0 ± 1.7	71.2	73.4 ± 2.5	47.7	85.8 ± 0.5
AHTN	***Inlet***		326	807	427	648	280
	Outlet	***Dissolved***	0.2 ± 0.0	0.5	0.4 ± 0.2	-	0.1 ± 0.0
		Sorbed	145 ± 14	335	161 ± 26	-	49 ± 8
	Removal (%)		55.4 ± 3.0	58.5	62.1 ± 4.3	-	82.5 ± 2.1
CBZ	***Inlet***		10	21	10	20	10
	Outlet	***Dissolved***	3.0 ± 0.6	5.9	3.8 ± 0.5	-	3.6 ± 0.8
		Sorbed	7.2 ± 1.3	14	6.7 ± 0.4	-	4.8 ± 0.3
	Removal (%)		0.5 ± 4.4	3.2	0	-	17.4 ± 3.9
DZP	***Inlet***		10	20	10	20	10
	Outlet	***Dissolved***	1.5 ± 0.2	2.6	2.0 ± 1.0	2.3	3.1 ± 0.7
		Sorbed	1.5 ± 0.0	4.0	2.5 ± 1.1	3.4	2.4 ± 0.6
	Removal (%)		69.8 ± 1.7	67.1	54.6 ± 14.9	71.7	44.5 ± 8.8
IBP	***Inlet***		11	25	13	22	10
	Outlet	***Dissolved***	5.1 ± 0.0	6.7	3.0 ± 1.1	6.2	4.5 ± 0.1
		Sorbed	4.0 ± 0.1	3.9	2.7 ± 0.2	5.5	3.5 ± 1.2
	Removal (%)		16.3 ± 1.0	57.9	57.4 ± 5.1	46.3	20.7 ± 9.5
NPX	***Inlet***		10	23	12	21	9
	Outlet	***Dissolved***	0.9 ± 0.0	2.0	0.6 ± 0.1	1.3	-
		Sorbed	0.5 ± 0.1	1.5	0.6 ± 0.0	1.4	-
	Removal (%)		86.6 ± 0.6	85.1	89.8 ± 0.3	87.0	-

Table 7.10. Mass flux of PPCPs (μg·d^{-1}) and removal efficiency (%) in the mesophilic digester during alkaline, thermal and ozonation pre-treatments. *Cont.*

Mass flux of PPCPs (μg·d^{-1})			Alkaline		Thermal		Ozonation
			SRT 20 d	*SRT 10 d*	*SRT 20 d*	*SRT 10 d*	*SRT 20 d*
DCF	***Inlet***		5	10	5	10	5
	Outlet	***Dissolved***	1.7 ± 0.1	1.7	1.9 ± 0.8	2.1	0.3
		Sorbed	3.1 ± 0.2	1.5	2.6 ± 0.3	3.7	1.2
	Removal (%)		3.9 ± 0.7	68.9	11.0 ± 6.3	42.1	70.4
IPM	***Inlet***		25	51	25	50	25
	Outlet	***Dissolved***	14.0 ± 3.1	29.5	13.3 ± 1.5	25.5	14.5 ± 3.0
		Sorbed	8.6 ± 1.5	15.3	3.1 ± 0.2	12.6	5.8 ± 2.0
	Removal (%)		10.0 ± 4.6	11.8	35.5 ± 3.8	23.9	18.2 ± 3.1
SMX	***Inlet***		24	52	26	48	23
	Outlet	***Dissolved***	0.1 ± 0.0	0.2	0.3 ± 0.2	0.3	0.1
		Sorbed	0.1 ± 0.0	0.3	0.2 ± 0.0	0.3	0.2
	Removal (%)		99.1 ± 0.1	99.1	98.3 ± 0.5	98.8	98.9
ROX	***Inlet***		20	40	20	40	-
	Outlet	***Dissolved***	-	2.5	0.6 ± 0.8	0.1	-
		Sorbed	-	9.9	3.7 ± 0.2	0.3	-
	Removal (%)		-	69.0	78.8 ± 1.9	99.1	-
E1+E2	***Inlet***		7	14	7	13	7
	Outlet	***Dissolved***	0.5 ± 0.2	0.1	0.3 ± 0.1	0.0	0.1 ± 0.0
		Sorbed	3.8 ± 1.0	0.8	3.9 ± 0.5	0.5	1.1 ± 0.1
	Removal (%)		34.9 ± 7.9	93.5	40.7 ± 6.1	96.4	81.6 ± 0.4
EE2	***Inlet***		2	4	2	4	2
	Outlet	***Dissolved***	0.1	0.0	0.1 ± 0.0	0.1	0.1
		Sorbed	2.0	0.8	2.1 ± 0.5	3.2	0.2
	Removal (%)		0	81.2	0	21.5	85.9

Table 7.11. Mass flux of PPCPs (μg·d^{-1}) and removal efficiency (%) in the thermophilic digester during alkaline, thermal and ozonation pre-treatments.

Mass flux of PPCPs (μg·d^{-1})			Alkaline		Thermal		Ozonation
			SRT 10 d	***SRT 6 d***	***SRT 10 d***	***SRT 6 d***	***SRT 10 d***
HHCB	***Inlet***		1,667	3,670	2,357	2,838	1,424
	Outlet	***Dissolved***	1.7 ± 0.1	1.4	1.9 ± 0.1	2.4	2.1 ± 0.2
		Sorbed	577 ± 11	1,072	649 ± 117	1,429	446 ± 48
	Removal (%)		65.3 ± 0.5	70.8	72.4 ± 3.5	49.5	68.5 ± 2.4
AHTN	***Inlet***		652	1,372	853	1,101	559
	Outlet	***Dissolved***	0.8 ± 0.0	0.7	0.9 ± 0.1	-	1.4 ± 0.1
		Sorbed	294 ± 17	504	294 ± 59	-	382 ± 21
	Removal (%)		54.8± 1.9	63.2	65.4 ± 4.9	-	31.5 ± 2.7
CBZ	***Inlet***		20	35	21	35	20
	Outlet	***Dissolved***	8.6 ± 2.5	20.2	8.9 ± 0.1	13.4	4.7 ± 0.8
		Sorbed	11.6 ± 3.5	35.3	18.3 ± 0.3	18.6	3.9 ± 0.0
	Removal (%)		0	0	0	7.9	57.8 ± 2.9
DZP	***Inlet***		20	34	20	34	20
	Outlet	***Dissolved***	5.3 ± 0.8	8.3	5.7 ± 0.2	8.0	5.2 ± 0.5
		Sorbed	7.0 ± 1.1	14.2	9.9 ± 0.5	10.8	4.3 ± 0.4
	Removal (%)		38.8 ± 1.1	33.7	22.1 ± 2.6	44.6	52.5 ± 3.3
IBP	***Inlet***		22	43	26	37	20
	Outlet	***Dissolved***	-	11.1	4.5 ± 1.2	10.7	6.5 ± 1.9
		Sorbed	-	11.9	10.5 ± 0.6	13.1	7.4 ± 0.6
	Removal (%)		-	46.2	42.9 ± 1.5	35.6	30.8 ± 8.7
NPX	***Inlet***		21	40	24	35	19
	Outlet	***Dissolved***	1.4 ± 0.1	1.8	0.5 ± 0.2	2.3	-
		Sorbed	1.3 ± 0.4	2.7	1.7 ± 0.0	2.2	-
	Removal (%)		87.2 ± 0.8	88.5	91.1 ± 0.5	87.2	-

Table 7.11. Mass flux of PPCPs (μg·d^{-1}) and removal efficiency (%) in the thermophilic digester during alkaline, thermal and ozonation pre-treatments. *Cont.*

Mass flux of PPCPs (μg·d^{-1})			Alkaline		Thermal		Ozonation
			SRT 10 d	***SRT 6 d***	***SRT 10 d***	***SRT 6 d***	***SRT 10 d***
DCF	***Inlet***		10	17	10	17	10
	Outlet	***Dissolved***	4.4 ± 0.2	1.9	1.8 ± 0.3	1.8	0.5
		Sorbed	6.9 ± 0.6	3.7	8.0 ± 2.6	4.4	2.7
	Removal (%)		0	67.1	0	63.7	68.4
IPM	***Inlet***		50	86	51	85	50
	Outlet	***Dissolved***	-	36.1	24.0	32.2	-
		Sorbed	-	33.1	12.7	23.4	-
	Removal (%)		-	19.9	28.0	34.6	-
SMX	***Inlet***		48	88	53	82	47
	Outlet	***Dissolved***	0.4 ± 0.3	0.3	0.7 ± 0.9	0.9	0.1
		Sorbed	0.3 ± 0.1	0.2	0.4 ± 0.0	0.6	0.4
	Removal (%)		98.6 ± 0.6	99.4	97.7 ± 1.1	98.2	98.9
ROX	***Inlet***		40	68	40	68	-
	Outlet	***Dissolved***	-	-	-	1.1	-
		Sorbed	-	-	-	1.3	-
	Removal (%)		-	-	-	96.5	-
E1+E2	***Inlet***		13	23	14	23	13
	Outlet	***Dissolved***	1.3 ± 0.3	0.3	0.3 ± 0.1	0.2	0.2 ± 0.1
		Sorbed	11.3 ± 2.5	2.0	6.5 ± 0.8	2.3	1.2 ± 0.1
	Removal (%)		5.2 ± 16.8	90.1	51.5 ± 4.1	88.7	88.8 ± 0.7
EE2	***Inlet***		4	7	4	7	4
	Outlet	***Dissolved***	0.5 ± 0.1	0.1	0.1 ± 0.1	0.1	0.1
		Sorbed	5.3 ± 0.5	0.7	2.0 ± 0.7	2.6	0.5
	Removal (%)		0	89.0	50.6 ± 12.1	62.8	84.9

Table 7.12. Summary of Galaxolide removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	68	73	86	65
	10	71	48	n.d.	69
Thermophilic	10	65	72	69	76
	6	71	50	n.d.	80

Comparing the values obtained with each pre-treatment, the results are different depending on the SRT:

- At high SRT, no significant differences were observed between the three pre-treatments in thermophilic range (65-70%); however, the ozonation treatment enhanced HHCB removal in the mesophilic digester (up to 85%).
- At low SRT, higher removal efficiencies were achieved with the alkaline pre-treatment (around 70%) compared to the thermal process (around 50%) in both digesters.

In the same way, comparing the values obtained with the pre-treatments and those achieved in the conventional process (Chapter 6), the results are different depending on the SRT:

- At high SRT, no significant differences were observed in thermophilic range; however, the ozonation treatment enhanced HHCB removal in the mesophilic digester (from 65 to 85%).
- At low SRT, similar removal efficiencies were achieved with the alkaline pre-treatment (70-80%), while the thermal process deteriorated the elimination (from 70-80% to 50%).

While no influence of the SRT was observed in the alkaline treatment, the HHCB elimination decreased in both digesters with the thermal process when operating at low SRT, from 70 to 50% approximately.

When both digesters were operated at the same SRT, similar removal was obtained with the alkaline treatment; however, the thermophilic process led to better results (around 70%) than the mesophilic one (around 50%) with the thermal treatment.

These results were proved with the data confirmation process, because the K_d values obtained from the liquid concentrations (around 15 $L \cdot kg^{-1}$) differ

significantly from those calculated with the sludge concentrations (around 30,000 - 45,000 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

In addition, the data measured for this substance is very good (Table II-1), since only 2 values out of 16 had to be dismissed. Moreover, as this compound tends to sorb onto solids, its removal efficiency is more dependant on the concentrations in the sludge phase than those in the liquid. Therefore, the data dismissed did not affect the removal efficiency calculated.

Tonalide

Tonalide was significantly removed in both digesters (Table 7.13), with removal efficiencies ranging from 55 to 85% in the mesophilic process and from 30 to 65% in the thermophilic one.

Table 7.13. Summary of Tonalide removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	55	62	83	61
	10	59	n.d.	n.d.	62
Thermophilic	10	55	65	32	82
	6	63	n.d.	n.d.	78

Comparing the values obtained with each pre-treatment, similar results were obtained with the alkaline and thermal processes in both digesters (55-65%); however, while under mesophilic conditions the ozonation treatment led to better results (around 80%), in the thermophilic digester the elimination decreased to 30%, approximately.

In the same way, comparing the values obtained with the pre-treatments and those achieved in the conventional process (Chapter 6), the results are different depending on the temperature (mesophilic or thermophilic):

- In the mesophilic digester, no differences were observed with the alkaline and thermal pre-treatment (55-60%) and the conventional operation; however, the ozonation process enhanced AHTN removal up to 83%.
- In the thermophilic digester, the results obtained in the conventional process (around 80%) were better than those achieved with any pre-treatment (30-65%).

Neither influence of the SRT nor of the temperature was observed on AHTN removal during advanced operation.

These results were proved with the data confirmation process, because the K_d values obtained from the liquid concentrations (around 15-20 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 20,000 - 35,000 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

In addition, the data measured for this substance is very good (Table II-2), since only 4 values out of 16 had to be dismissed. Moreover, as this compound tends to sorb onto solids, its removal efficiency is more dependant on the concentrations in the sludge phase than those in the liquid. Therefore, only two of the data dismissed affected the removal efficiency calculated.

Carbamazepine

Carbamazepine was not removed in any digester (Table 7.14) except with the ozonation process, around 20 and 60% in the mesophilic and thermophilic reactor, respectively.

Table 7.14. Summary of Carbamazepine removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	0	0	17	0
	10	3	n.d.	n.d.	12
Thermophilic	10	0	0	58	0
	6	0	8	n.d.	22

A similar value (around 50%) was obtained in the mesophilic digester with the thermal treatment at 10-d SRT, but it was not considered as reliable since it does not correspond with the other results achieved with the thermal process.

However, the results obtained with the ozonation process could be possible since CBZ reacts quite fast with ozone with an apparent second-order rate constant of $3 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ (Huber *et al.,* 2003).

Checking these values in the data confirmation process, it could be observed that while, in general, similar K_d values were obtained from the liquid and sludge phase (30-70 $L \cdot kg^{-1}$), the ozonation process in the thermophilic digester led to different values, 9 and 135 $L \cdot kg^{-1}$, respectively. Besides, these K_d values do not

correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006), indicating that some elimination occurs.

The data measured for this substance is very good (Table II-3), since only 1 value out of 16 had to be dismissed. Moreover, as this compound tends to remain in the liquid phase, its removal efficiency is more dependant on the concentrations in the liquid than those in the sludge. Therefore, the value dismissed did not affect the removal efficiency calculated.

Diazepam

Diazepam was partly removed in both digesters (Table 7.15), with removal efficiencies ranging from 45 to 70% in the mesophilic process and from 20 to 55% in the thermophilic one.

Table 7.15. Summary of Diazepam removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	70	55	45	20
	10	67	72	n.d.	52
Thermophilic	10	39	22	53	17
	6	34	45	n.d.	59

Comparing the values obtained with each pre-treatment, the results are different depending on the SRT:

- At high SRT, while the alkaline treatment led to the highest DZP elimination in the mesophilic range (around 70%), in the thermophilic one, the best results were achieved with the ozonation process (around 55%).
- At low SRT, similar removal efficiencies were achieved with the alkaline and thermal pre-treatment in both mesophilic (around 70%) and thermophilic digester (around 40%).

In the mesophilic digester, higher removal efficiencies were obtained in the advanced operation with any pre-treatment than those achieved in the conventional process, independently of the SRT. However, in the thermophilic reactor, the elimination of DZP was higher with the pre-treatments when operating at 10-d SRT, but lower at 6-d SRT. Anyway, it should be considered that the removal of this substance occurred after sludge adaptation. Therefore, the

improvement in the efficiency is more likely an effect of operation time than pre-treatment.

No significant influence of the SRT on DZP removal was observed during advanced operation with the alkaline treatment; however, with the thermal process, the elimination increased when operating both digesters at lower SRT.

When both digesters were operated at the same SRT, the mesophilic conditions led to higher removal efficiencies than the thermophilic ones, regardless of the type of operation.

For this compound, the data confirmation process could be only done with the data of the liquid phase since measurements in the sludge were not performed. The results confirm the elimination obtained because the K_d value calculated (around 70-130 $L \cdot kg^{-1}$) is different than that reported by Ternes *et al.* (2004), around 20-40 $L \cdot kg^{-1}$.

In addition, the data measured for this substance is very good (Table II-4), since all values fitted the process.

Ibuprofen

Ibuprofen was partly removed in both digesters (Table 7.16), with removal efficiencies ranging from 15 to 60% in the mesophilic process and from 30 to 45% in the thermophilic one.

Table 7.16. Summary of Ibuprofen removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	16	57	21	39
	10	58	46	n.d.	62
Thermophilic	10	n.d.	43	31	49
	6	46	36	n.d.	54

Comparing the values obtained with each pre-treatment, the results are different depending on the SRT:

- At high SRT, the highest removal efficiencies were obtained with the thermal process in both digesters, 57 and 43% in the mesophilic and thermophilic reactor, respectively.

- At low SRT, no significant differences were observed between thermal and alkaline treatments in any digester.

In the same way, comparing the values obtained with the pre-treatments and those achieved in the conventional process (Chapter 6), the results are different depending on the temperature (mesophilic or thermophilic) and the SRT:

- At high SRT, only the thermal process increased the IBP removal (up to 57%) in the mesophilic digester. The other pre-treatments led to similar or lower efficiencies than the conventional operation.
- At low SRT, while similar results were obtained in the conventional and alkaline treatment, the thermal hydrolysis decreased the IBP elimination to 50% in the mesophilic digester and to 35% in the thermophilic one.

No significant influence of the SRT on IBP removal was observed during advanced operation in both digesters, except the alkaline treatment in mesophilic conditions, which led to higher removal efficiencies at 10-d SRT (around 60%) than at 20-d SRT (around 15%).

When both digesters were operated at the same SRT, only the results with the thermal treatment can be compared and they were similar, thus not affecting the temperature IBP removal during this process.

These results were proved with the data confirmation process, because the K_d values obtained from the liquid concentrations (around 15 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 55-75 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

In addition, the data measured for this substance is very good (Table II-5), since only 2 values out of 16 had to be dismissed.

Naproxen

Naproxen was very well removed in both digesters (Table 7.17), with removal efficiencies higher than 85%.

There are no results of this compound during ozonation treatment because the data obtained did not fit the data confirmation process.

The elimination of NPX was not influenced by the type of pre-treatment, type of operation, SRT and temperature.

Table 7.17. Summary of Naproxen removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	87	90	n.d.	88
	10	85	87	n.d.	87
Thermophilic	10	87	91	n.d.	93
	6	89	87	n.d.	84

These results were proved with the data confirmation process, because the K_d values obtained from the liquid concentrations (around 1 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 350-430 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

In addition, the data measured for this substance is very good (Table II-6), since only 1 value out of 12 had to be dismissed, without affecting the removal efficiency calculated.

Diclofenac

From the results of Table 7.18, two periods could be differentiated concerning DCF elimination: a first period in which there was no removal (alkaline and thermal processes at high SRT), and a second one with removal efficiencies up to 70% (alkaline and thermal processes at low SRT and ozonation). Taking into account the chronological order in which the different experiments were performed (Table 7.1), the first period (no removal) corresponds with the first experiments and the second period with the last ones. Therefore, it can be concluded that the elimination of Diclofenac occurred after sludge adaptation.

Table 7.18. Summary of Diclofenac removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	4	11	70	0
	10	69	42	n.d.	78
Thermophilic	10	0	2	68	17
	6	67	64	n.d.	77

These results were proved with the data confirmation process, because for the first experiments, the K_d values obtained from the liquid concentrations (around

40 $L \cdot kg^{-1}$) were similar to those calculated with the sludge concentrations (around 55 $L \cdot kg^{-1}$). Besides, these values correspond to the K_d values calculated for digested sludge (Carballa *et al.,* 2006). However, in the last experiments, different values were obtained from the liquid (10 $L \cdot kg^{-1}$) and the sludge (400 $L \cdot kg^{-1}$) phase, indicating that some removal occurs.

Although the data measured for this substance varied strongly (Table II-7), considering the two periods mentioned before, all the data fitted the confirmation process.

Iopromide

Iopromide was partly removed in both digesters (Table 7.19), with removal efficiencies ranging from 10 to 35% in the mesophilic process and from 20 to 35% in the thermophilic one.

Table 7.19. Summary of Iopromide removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	10	36	18	8
	10	12	24	n.d.	17
Thermophilic	10	-	28	n.d.	16
	6	20	35	n.d.	31

Comparing the values obtained with each pre-treatment, the results were slightly better with the thermal treatment (25-35%) than with the alkaline and ozonation processes (10-20%) in both digesters.

In general, higher removal efficiencies were obtained in the advanced operation when both digesters were run at high SRT compared to the conventional process; whereas the operation at low SRT led to similar results.

Neither SRT nor temperature affected IPM removal during advanced operation.

These results were proved with the data confirmation process, because the K_d values obtained from the liquid concentrations (around 5-10 $L \cdot kg^{-1}$) were quite similar to those calculated with the sludge concentrations (around 20 $L \cdot kg^{-1}$). Besides, these values correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

The data measured for this substance in the mesophilic digester was very good, since all values fitted the data confirmation process. However, the data of the thermophilic process was worst (Table II-8) and 6 values out of 8 had to be dismissed.

Sulfamethoxazole

Sulfamethoxazole was very well removed in both digesters (Table 7.20), with removal efficiencies higher than 98%.

Table 7.20. Summary of Sulfamethoxazole removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	99	98	99	99
	10	99	99	n.d.	99
Thermophilic	10	99	98	99	99
	6	99	98	n.d.	99

The elimination of SMX was not influenced by the type of pre-treatment, type of operation, SRT and temperature.

These results were proved with the data confirmation process, because the K_d values obtained from the liquid concentrations (< 1 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 4,500-5,500 $L \cdot kg^{-1}$). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

In addition, the data measured for this substance is very good (Table II-9), since only 4 values out of 16 had to be dismissed without affecting the removal efficiency calculated.

Roxithromycin

Roxithromycin was significantly removed in both digesters (Table 7.21), with removal efficiencies ranging from 70 to 90% in the mesophilic process and higher than 95% in the thermophilic one.

From the results available, it can be concluded that the elimination of ROX was not influenced by the type of pre-treatment, type of operation, SRT and temperature. Only the alkaline treatment in mesophilic range led to lower removal efficiencies (around 70%).

Table 7.21. Summary of Roxithromycin removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	n.d.	79	n.d.	92
	10	69	99	n.d.	n.d.
Thermophilic	10	n.d.	n.d.	n.d.	98
	6	n.d.	97	n.d.	n.d.

These results were proved with the data confirmation process, because the K_d values obtained from the liquid concentrations (around 200 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (400 and 1,500 $L \cdot kg^{-1}$ for mesophilic and thermophilic range, respectively). Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

The data measured for this substance varied strongly (Table II-10), leading to very different removal efficiencies. This fact explained that most of the data had to be dismissed, mainly those of the thermophilic reactor.

Estrone + 17β-estradiol

The natural estrogens were very well removed in both digesters (Table 7.22), with removal efficiencies higher than 80%, except with the alkaline and thermal pre-treatments at high SRT (around 35-50%). Similarly to Diclofenac, considering the chronological order of experiments performance, this behaviour can be explained by the sludge adaptation. Therefore, no significant influence of type of pre-treatment, temperature and type of operation was observed on the elimination of natural estrogens.

Table 7.22. Summary of Estrone and 17β-estradiol removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	35	41	82	n.d.
	10	94	96	n.d.	95
Thermophilic	10	n.d.	52	89	n.d.
	6	90	89	n.d.	89

These results were proved with the data confirmation process, because the K_d values obtained from the liquid concentrations (< 30 $L \cdot kg^{-1}$) differ significantly from those calculated with the sludge concentrations (around 1,500 $L \cdot kg^{-1}$).

Besides, these values do not correspond with the K_d values calculated for digested sludge (Carballa *et al.,* 2006).

The data measured for these substances is quite good (Table II-11), since only 5 values out of 16 had to be dismissed.

17α-ethinylestradiol

Similarly to Diclofenac, two periods could be differentiated concerning EE2 elimination (Table 7.23): a first period in which there was no removal, and a second one with removal efficiencies up to 90%. Once again, these two periods correspond with the chronological order in which the different experiments were performed (Table 7.1), thus being concluded that the elimination of EE2 occurred after sludge adaptation.

In the mesophilic digester, the elimination started with the thermal pre-treatment at 10-d SRT (period III) and the removal efficiencies increased up to 85% with the ozone treatment (period V). However, in the thermophilic reactor, the removal started in period II (thermal pre-treatment at 10-d SRT) and again rose up to 85% with the ozonation process.

Table 7.23. Summary of 17α-ethinylestradiol removal efficiencies (%) during advanced operation compared to the conventional process. n.d.: no data.

	SRT (d)	Alkaline	Thermal	Ozonation	Conventional
Mesophilic	20	0	0	86	n.d.
	10	81	22	n.d.	92
Thermophilic	10	0	51	85	-
	6	89	63	n.d.	91

Considering that EE2 removal occurred after sludge adaptation with efficiencies between 80 and 90%, no influence of type of pre-treatment, temperature, SRT and type of operation was observed.

The data confirmation process indicates that some elimination should occur, since the K_d values obtained from the liquid concentrations (<70 $L \cdot kg^{-1}$) were different to those calculated with the sludge concentrations (350-550 $L \cdot kg^{-1}$).

The data measured for this substance varied strongly (Table II-12), which leads to many values to be dismissed. In order to get some conclusions for this compound, a refinement of EE2 results was performed in the following way. Those concentrations (in liquid or sludge) which led to wrong K_d values were not

considered in the mass balances and instead the concentration calculated from the right K_d value was used. The results are shown in Table II-13 and led to the conclusions explained before.

Discussion

Table 7.24 shows a summary of PPCPs removal during mesophilic and thermophilic anaerobic digestion of sewage sludge.

All PPCPs were affected in some extent by the anaerobic digestion process, except Carbamazepine, which was only removed with the ozonation process in the thermophilic digester (60%). However, this result is quite strange since in the mesophilic range the elimination with the ozone treatment was below 20%.

The elimination of Naproxen (>85%), Iopromide (10-30%) and Sulfamethoxazole (>99%) was not affected by the operational conditions (temperature, SRT and type of operation) of the anaerobic digestion pilot plant.

Similarly, the removal of Galaxolide (65-75%) and Roxithromycin (>95%) in the thermophilic reactor was not influenced by the SRT and the type of operation (conventional versus advanced). However, small effects were observed in the mesophilic range. Galaxolide was better removed with the ozonation process (from 65 to 85%) and the alkaline treatment decreased the elimination of Roxithromycin (from 90 to 70%).

The ozonation treatment affects the elimination of Tonalide in both digesters, but in different way; while better results were obtained in the mesophilic digester (from 60 to 80%), in the thermophilic reactor the removal decreased to 30%.

The removal of Ibuprofen is influenced by the ozonation (in both digesters) and the thermal (only in mesophilic) treatments, but with contrary effects. While ozonation decreased the removal of IBP to 20-30%, the thermal process increased it up to 55%.

Finally, the removal of Diazepam, Diclofenac and estrogens was affected by sludge adaptation since higher efficiencies were achieved in the last experiments.

The elimination of Diazepam was higher in the mesophilic digester (45-70%) than in the thermophilic one (20-60%). Diclofenac removal started with the thermal pre-treatment at low SRT (period III) in both digesters (40-60%), increasing up to 70-80% in the last experiments.

Table 7.24. Summary of PPCPs removal (%) during anaerobic digestion of sewage sludge.

	Mesophilic digester				**Thermophilic digester**			
	Conventional	**Alkaline**	**Thermal**	**Ozonation**	**Conventional**	**Alkaline**	**Thermal**	**Ozonation**
HHCB	65	69	65	86	74	67	65	69
AHTN	57	56	62	83	79	58	66	32
CBZ	0	0	0	17	0	0	0	58
DZP	18 - 52	69	60	45	20 - 59	37	30	53
IBP	41	30	54	21	47	46	41	31
NPX	86	86	89	-	91	88	90	-
DCF	0 - 78	4 - 69	11 - 42	70	26 - 77	0 - 67	2 - 64	68
IPM	23	11	32	18	20	20	31	-
SMX	99	99	99	99	99	99	98	99
ROX	96	69	86	-	99	-	97	-
E1+E2	76	35 - 94	41 - 96	82	84	90	52 - 89	89
EE2	41 - 92	0 - 81	0 - 22	86	38 - 91	0 - 89	51 - 63	85

The elimination of natural estrogens increased from 35-50% to 90-95% with no differences between both digesters, and 17α-ethinylestradiol started being removed with the thermal pre-treatment at low SRT in the mesophilic reactor (20%) and at high SRT in the thermophilic one (50%). In both digesters, the removal efficiencies increased up to 90% at the end of the experiments.

As stated in Chapter 6, the information dealing specifically with PPCPs behaviour during anaerobic digestion of sewage sludge is very scarce (a summary of the literature found was made at the end of section 6.3.3), but it was even more difficult/impossible to find data of these compounds during the advanced treatments described in this chapter.

7.4. Conclusions

Three common sludge pre-treatments, alkaline, thermal and ozonation, have been used in order to improve the sludge stabilization by anaerobic digestion. The use of these pre-treatments leads to COD solubilization percentages between 55 and 80%, the highest values being obtained with the alkaline process. Therefore, the biogas productions and soluble organic matter removal efficiencies during anaerobic digestion of pretreated sludge are higher.

However, the elimination of solids and total COD stays in the same range, with small differences depending on the SRT or type of operation. While in the mesophilic digester, the sludge pre-treatments led to slightly higher removal efficiencies of solids and COD_t, in the thermophilic reactor the results obtained in the conventional and advanced operation were similar. In general, lower removal efficiencies of solids and organic matter were obtained in both digesters when they were run at higher OLR.

The conventional thermophilic anaerobic digestion process usually led to higher degree of stabilization of the digested sludge compared to the conventional mesophilic process. However, when applying sludge pre-treatments, these differences decrease and the results were more similar.

Concerning PPCPs, the elimination of Naproxen, Iopromide and Sulfamethoxazole was not affected by the sludge pre-treatments. In contrast, Carbamazepine was only removed when the ozone process was applied under thermophilic conditions. For the other substances, small influences were observed:

- Thermal treatment affected positively Ibuprofen elimination in mesophilic conditions.
- Ozonation process affected positively the removal of musks in the mesophilic digester and negatively the elimination of Tonalide (in thermophilic range) and Ibuprofen (in both digesters).

Finally, the removal of Diazepam, Diclofenac and estrogens was more related with sludge adaptation than with the operational conditions in the digesters.

7.5. References

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Chapter 8

Influence of different pre-treatments on digested sludge characteristics: suitability for final disposal[1]

Summary

The debate on sludge recycling and disposal has recently been a target of growing interest. This is due to the fact that some concern was expressed about the potential risks of the agricultural use of sludge for health and the environment, which lead to revisions in government policy and regulations. Therefore, many novel treatment processes have been proposed in order to make the recycling and reuse of sewage sludge possible. The main objective is to improve the sludge quality in terms of pathogens, dewatering properties, heavy metals and organic pollutants. In this work, the use of some advanced anaerobic digestion treatments (with alkaline, thermal and ozone pre-treatments) has been assessed in order to make the sludge suitable for land application according the current legislation. All the pre-treatments proved to be efficient to reach the requirements proposed in the Working Document on Sludge (EU, 2000).

[1] **Carballa, M., Omil, F. and Lema, J.M.** (2006). Influence of different pre-treatments on digested sludge characteristics: suitability for final disposal. *In preparation.*

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8.1. Introduction

Sewage sludge is defined in Article 2a of the Sewage Sludge Directive 86/278/EEC as "i) residual sludge from sewage plants treating domestic or urban wastewaters and from other sewage plants treating wastewaters of a composition similar to domestic and urban wastewaters; ii) residual sludge from septic tanks and other similar installations for the treatment of sewage; iii) residual sludge from sewage plants other than those referred to in i) and ii)".

The successful strategy for water protection by biological wastewater treatment (EU Directive 271/91) resulted in a sludge production of about 20 to 40 kg dry matter per population equivalent and year. Sewage sludge is not produced for any purpose but represents a by-product of fulfilling a legal requirement. The quantity of sludge production is only little influenced by the treatment efficiency, but it is not the case for sludge quality which depends on wastewater and sludge treatment technologies.

In 1999, the EU-15 produced about 7.2 million tonnes of sewage sludge from Sewage Treatment Plants (STPs) (EU, 2004) and the expected production for the year 2005 is 8.3 million tonnes (Magoarou, 2000). The production in Spain was approximately 800,000 tonnes in 1998 and it is expected to increase up to 1.5 million tonnes by the end of 2005 (PNLD, 2001).

Sewage sludge treatment and disposal is important as it accounts for approximately half of the costs that a wastewater treatment facility must bear. Traditionally, the management options for sludge are landfilled (regulated by the Landfill Directive 1999/31/EC), incinerated (regulated by the Waste Incineration Directive 2000/76/EC) and landspread (regulation on going). Latest information (EU, 2004) on disposal and recovery of sludge in the EU indicates that 45% is recycled to land (largely in agriculture), 18% is landfilled, 17% is incinerated and 1% is disposed of to surface water (despite this being prohibited since 1 January 1999). The use of 19% of sludge is not specified. In Spain, estimations for year 2005 indicate that 54% would be recycled to land (largely in agriculture), 34% would be landfilled, 7% incinerated and 5% would be disposed into the sea (PNLD, 2001).

Agricultural use of sewage sludge can be seen as the option with the lowest loss of valuable compounds of the sludge, especially phosphorus and nitrogen, and the lowest increase of entropy. However, the application of sludge on soils can pose certain environmental problems, mainly related to an excessive or

unbalanced supply of nutrients, the introduction of pollutants, such as heavy metals and organic compounds, and the spreading of human, animal or plant pathogens. Therefore, the use of sludge shall be carried out in such a way as to minimise the risk of negative effects to human, animal and plant health; the quality of surface and groundwater; the long-term quality of the soil; and the biodiversity of the microorganisms living in the soil.

In that sense, the current legislation related to sludge application on land (EU Directive 86/278) is under revision (Working Document on Sludge, 3rd draft, 2000; Draft Discussion Document, 2004) in order to ensure the long term beneficial use of sludge. In the mentioned document, different types of treatment (conventional and advanced) are proposed as well as the limit values for several hazardous substances (heavy metals, pathogens and organic compounds). Besides, the conditions for sludge use on land are established.

In the following sections, the different parameters affecting final sludge disposal are discussed.

8.1.1. Organic matter and soil depletion

Organic matter and soil characteristics (fertility, structure, erodibility) are related. Any soil needs the correct content of organic matter in order to be productive, not absolutely a high content in all cases. In addition, climatic conditions have to be considered when estimating minimum or optimum soil organic matter levels in terms of self-sustaining soil productivity and fertility (from the agronomic standpoint). It has been sometimes proposed that a level of organic matter ranging between 2.5 and 3% in soil is the bare minimum for long term use of agricultural soils (EU, 2004).

8.1.2. Fertilisation properties

Fertilisation is considered here from the viewpoint of nutrients supply, such as nitrogen and phosphorus, which are needed for an appropriated growth of commercial crops.

The nutrient content of sludge varies sharply depending on the wastewater type and the treatment it has undergone. It is generally rich in nitrogen (1-6%) in the liquid phase, thus the sludge losing the “soluble nitrogen” during dewatering or lime treatment. The phosphorus content is 1-2%, being it present in the form of

organic phosphates or in combination with iron, aluminium, magnesium and calcium forming insoluble precipitates (mineral phosphorus).

Fertilisation should be in line with inherent soil properties and requirements in order to avoid over-fertilisation and soil saturation, which can lead to a euthrophication risk. However, it should be considered that nitrogen and phosphorus are mainly present in the sludge in an organic form, i.e. with no leaching potential until it is mineralised.

8.1.3. Heavy metals content

Potentially toxic elements, such as heavy metals, are also removed with the solids during primary and secondary sedimentation stages of conventional wastewater treatment. Metal removal during primary sedimentation is a physical process, dependent on the settlement of precipitated, insoluble metal or the association of metals with setteable particulate matter. During secondary wastewater treatment, metal removal is dependent on the uptake of metals by the microbial biomass and the precipitation/entrapment on the sludge in the form of insoluble sulphides and hydroxides (Kalyuzhnyi and Gladchenko, 2004). In total, approximately 65-80% of Zn, Cu, Cd, Pb, Cr and Hg are transferred from raw sewage to the sludge (Lester, 1981). Only Ni showed the less reduction (40%).

Table 8.1 shows the heavy metals content in several types of sludge. It can be observed that the concentrations vary strongly with the higher levels being obtained for iron, zinc and chromium.

Some heavy metals may have the effect of impairing the natural mechanisms through which soil microbes reproduce and therefore deplete the bio-potential of the soil eco-system. Moreover, if the concentration is high enough, heavy metals can penetrate the natural barriers in plant roots and end up in the edible part of vegetables. Some heavy metals can then accumulate in animal and human organs and cause poisoning effects, induce cancer or produce mutagenic changes.

Therefore, maximum permissible concentrations of heavy metals in the sludge for use on land have been established in several countries (Table 8.2). It can be observed that Sweden, Holland and Denmark have more stringent restrictions than the current legislation (Directive 86/278) and even than the values proposed in the Working Document on Sludge (EU, 2000).

Table 8.1. Heavy metals content ($mg \cdot kg^{-1}$) in different types of sludge.

Sludge	Cu	Ni	Cr	Fe	Zn	Pb	Cd	Hg	Reference
PS	-	-	-	350[a]	-	-	-	-	Ghyoot *et al.*, 1997
PS	226	8	16	-	322	37	0.6	0.7	LeBlanc *et al.,* 2004
BS	274	314	837	-	4,371	186	1.3	8	Mamais *et al.,* 2000
SS	330	36	73	-	811	104	2	1.5	EU, 2004
SS	655	155	551	-	2,054	455	13	5	Smith *et al.*, 1989
Non-St-S	139-153	24-26	60-62	5,431-5,519	447-469	86-88	1.1-1.2	-	Fuentes *et al.,* 2004
LS	287	102	-	-	766	75	7	8	Jiménez *et al.,* 2000
St-S	157	41	573	-	2,147	73	3	0.4	Wates *et al.,* 1997
Bs	215	80	14	-	213	32	3	1	LeBlanc *et al.,* 2004
PDS	302	67	886	-	2,752	283	1.6	4.1	Mamais *et al.,* 2000
DS	327	228	981	-	4,140	264	1.6	7.0	Mamais *et al.,* 2000
DS	300-500	25-100	50-500	-	1,500-2,000	100-400	2-8	2-8	Pollak, 2001
DS	-	-	-	13,500–22,400	-	-	-	-	Ghyoot *et al.*, 1997
DS	327-347	27-31	3,739-3,879	24,663-25,753	834-908	164-170	18-19	-	Fuentes *et al.,* 2004
DS	255	622	663	72,200	2,823	57	-	-	Xiang *et al.,* 2000
DS	415	45	-	25,917	1,242	53	3	-	Ito *et al.,* 1999

[a]Concentration in $mg \cdot L^{-1}$.

PS: Primary Sludge; BS: Biological Sludge;SS: Sewage Sludge; Non-St-S: Non-stabilised Sludge; LS: Lime stabilised; St-S: Stabilised Sludge; Bs: Biosolids; PDS: Primary Digested Sludge; DS: Digested Sludge.

Table 8.2. Limit values for heavy metals ($mg \cdot kg^{-1}$) in sludge for use on land.

	Directive 86/278/EC	**Spain**	**Germany**	**Sweden**	**Holland**
Cd	20 – 40	20 – 40	5 – 10	2	1,25
Cu	1,000 – 1,750	1,000 – 1,750	800	600	75
Ni	300 - 400	300 – 400	200	50	38
Pb	750 – 1,200	750 – 1,200	1,000	100	225
Zn	2,500 – 4,000	2,500 – 4,000	2,00	800	300
Hg	16 – 25	16 – 25	6 – 10	2.5	0.75
Cr	-	1,000 – 1,500	900	100	75
	Denmark	**Canada**	**USA**	**South African**	***Proposed***
Cd	0.8	20 – 34	39	15.7	10
Cu	1,000	850 – 2,700	1,500	50.5	1,000
Ni	30	180 – 420	420	200	300
Pb	120	500 – 2,000	300	50.5	750
Zn	4,000	1,850 – 4,200	2,800	353.5	2,500
Hg	0.8	5 - 13	17	10	10
Cr	100	1,100 – 2,800	1,200	1,750	1,000

These limit values force the removal of heavy metals from sewage sludge prior to land application (Sreekrishnan *et al.,* 1993). Three possible routes are identified to improve the sludge quality in terms of heavy metals (Rulkens, 2004):

- Source control (prevention of the discharge of pollutants to the sewer).
- Removal of colloidal and suspended particles as a first treatment step.
- Removal of heavy metals from the sludge by chemical leaching with inorganic and organic acids or complexing agents or by microbiological leaching (Marchioretto *et al.,* 2002).

8.1.4. Pathogens content

Like other urban wastes, sewage sludge may contain different kinds of pathogens that are infectious for different species of animals and plants as well as for humans. Therefore, the hygienic properties of sludge are of essential importance in sludge handling and disposal.

Tables 8.3 and 8.4 show the pathogens content in the raw and digested sludge, respectively, and Table 8.5 indicates the average removal efficiencies obtained during anaerobic digestion of sewage sludge.

Table 8.3. Pathogens content in raw sludge (in MPN·gTS^{-1}, except Salmonella in MPN·4gTS^{-1}).

Sludge	*T. col.*	*F. col.*	*E. coli*	*F. streptoc.*	*Cl. perfr.*	*Salmonella*	*Shigella*	Reference
M	-	10^8	-	-	-	1–12	-	Tapana *et al.*, 2000
M	10^5-10^9	10^5–10^8	-	-	-	-	-	Han *et al.*, 1997
M	All groups of bacteria: 10^8–10^9 CFU·g TS^{-1}							Dohnayos *et al.*, 2004
PB	-	10^2–10^5	-	-	-	1-10	-	Watanabe *et al.*, 1997
M	-	-	10^5-10^6	-	-	20-40	-	Horan *et al.*, 2004[a]

[a]Salmonella seftenberg (CFU·g TS^{-1}). M: Mixture; PB: Primary and Biological.

Table 8.4. Pathogens content in digested sludge (in MPN·gTS^{-1}, except Salmonella in MPN·4gTS^{-1}).

Conditions	*T. col.*	*F. col.*	*E. coli*	*F. streptoc.*	*Cl. perfr.*	*Salmonella*	*Shigella*	Reference
37ºC, SRT 15 d	-	10^6-10^7	-	-	-	< 1	-	Tapana *et al.*, 2000
35ºC, SRT 24-40 d	10^7	10^6-10^7	-	-	-	-	-	Han *et al.*, 1997
	Mesophilic: 10^4-10^5; Thermophilic: 10^3-10^4							Dohnayos *et al.*, 2004
35ºC, SRT 10-30 d	-	10^2-10^4	-	-	-	1.8-30	-	Watanabe *et al.*, 1997
55ºC, SRT 10-20 d	-	10^0	-	-	-	1.8	-	Watanabe *et al.*, 1997

Table 8.5. Removal efficiencies (%) of pathogens during anaerobic digestion of sewage sludge.

Conditions	*T. col.*	*F. col.*	*E. coli*	*F. Strept.*	*Cl. perfr.*	*Salmonella*	*Shigella*	Reference
37ºC, SRT 15 d	-	1-2 log	-	-	-	-	-	Tapana *et al.*, 2000
35ºC, SRT 24-40 d	1 log	1 log	-	-	-	-	-	Han *et al.*, 1997
	Mesophilic: 3–5 log; Thermophilic: 4–6 log							Dohnayos *et al.*, 2004
Meso-Thermo	67–99	-	-	-	-	-	-	Song *et al.*, 2004
Chemical	-	-	-	-	3–8 log	-	-	Abu-Orf *et al.*, 2004
35ºC, SRT 12 d	-	-	1.5-1.7 log	-	-	1.9-2.2 log	-	Horan *et al.*, 2004[a]

[a]Salmonella seftenberg (CFU·g TS^{-1})

The land usage of sewage sludge is severely limited by the density level of pathogenic microorganism (Table 8.6). The United States Environmental Protection Agency regulates the pathogens content of land-applied biosolids. It classifies biosolids as either class A or class B based on the reduction that is achieved in terms of pathogens (US EPA, 1993). A class A product contain little levels of pathogens, thus being land applied without any restrictions at the site. A class B product has less stringent standards for treatment and contains small but acceptable amounts of bacteria. Class B requirements ensure that pathogens have been reduced to levels that protect public health and the environment and include certain restrictions related to their end use.

Table 8.6. Pathogens requirements for sludge use on land.

	US EPA 40 CFR 503		**Working document on Sludge**	
	Class A	Class B	**Conventional treatment**	**Advanced treatment**
Salmonella spp (MPN·4 g TS^{-1})	< 3	-	-	No presence in 50 g 4 log reduction
E. coli (CFU·g TS^{-1})	-	-	2 log reduction $<10^3$	6 log reduction $<5 \cdot 10^2$
Cl. perfringens (MPN·g TS^{-1})		-	$<3 \cdot 10^3$	-
F. coliform (MPN·g TS^{-1})	$< 10^3$	$< 2 \cdot 10^6$	-	-
Enterovirus (MPN·4 g TS^{-1})	< 1	-	-	-
Helminth eggs (ova·4 g TS^{-1})	< 1	-	-	-

Therefore, it is important to minimise the potential transmission of pathogens by waste through effective treatment processes and then matching efficiency of pathogen removal to operational restriction on application practices and land use. For solid wastes, the most important factor influencing pathogen die-off is the couple time-temperature during the treatment process (Chu *et al.,* 1999). In general, mesophilic conditions lead to Class B biosolids (Carrington *et al.,* 1991; Horan *et al.,* 2004), while to achieve a Class A product, the thermophilic range is needed (Watanabe *et al.,* 1997; Huyard *et al.,* 2000; Dohanyos *et al.,* 2004). In addition, the use of pre-treatment methods can improve the microbial reduction (Jepsen *et al.,* 1997; Jean *et al.,* 2000).

8.1.5. Dewatering properties

The move to greater emphasis on the disposal of wastewater sludge through routes such as incineration and the added cost of landfill emplacement puts high demands on the sludge dewatering technology.

The greater organic loading of wastewater streams has created a higher stress on the treatment processes that often reduces the organic removal efficiency. Higher levels of volatile organics in the sludge reduce its dewaterability substantially, increasing the water content and the volume of the final waste. Increasing dewatering efficiency at higher volatile solids content requires the use of organic polymers to enhance flocculation, which increases both the disposal costs and the organic loading of the final waste.

The selection of conditioning chemicals for digested sludge has been a trial and error process that requires testing of sludge samples in the laboratory or modification of the process conditions and evaluating performance. What is lacking is the ability to predict the effects of digestion processes on conditioning chemical requirements. Conditioning requirements vary widely and there seems to be no reasonable explanation for the variations. Novak and Park (2004) showed that the polymer conditioning requirements are linked to volatile solids destruction. As the VS destruction increases, the polymer conditioning requirements increase in proportion to it.

Therefore, the improvement of the dewatering technology has recently received research interest. Sludge characterization for dewatering performance has largely been achieved using techniques such as Capillary Suction Time (CST) and Specific Resistance to Filtration (SRF) measurements. Although they are good comparative techniques, the results do not produce a characterisation parameter that is independent of the starting solids concentration and the applied pressure (Dentel, 1997; Novak *et al.,* 1999). In addition, they do not provide data on the expected extent of filtration, the maximum solids achievable or the role of chemical additives (Scales *et al.,* 2004).

8.1.6. Linear Alkylbenzene Sulfonates

There are some organic compounds that are not easily broken down during waste treatment and tend to accumulate, thus being the source of concern due to their eco-toxicity, the eco-toxicity of the products resulting from their degradation or to their potential for bio-accumulation.

Among them, surfactants form a group of chemicals with considerable environmental relevance due to a combination of their inherent environmental properties, their very large production volume and their widespread use. They are essential ingredients in most household laundry products, domestic and industrial cleaners, as well as in personal care and cosmetic products. They are typically discharged into the environment through the sewage treatment infrastructure or directly in situations where no treatment systems are available.

Linear Alkylbenzene Sulfonates (LAS), belonging to the anionic surfactants group, occupy maximum production. In Europe, during 1996 and 1997, it was approximately 400,000 ton (ERASM, 1999). LAS are characterised as readily biodegradable under aerobic conditions (ERASM, 1999), but poorly biodegradable under anaerobic conditions (Steber, 1991; Federle and Schwab, 1992). However, under oxygen-limited conditions, LAS mineralize even if the rate is not as rapid as that observed under aerobic conditions. Once biodegradation has been initiated in aerobic or oxygen-limited conditions, the intermediates can continue to biodegrade anaerobically. In addition, their physico-chemical properties indicate a significant partitioning between the aqueous and the solid phase, thus sorbing onto sludge and passing into anaerobic digesters (Field *et al.,* 1995).

LAS data for stabilised sludge have been obtained in several countries and range from <500 $mg \cdot kg^{-1}$ to a maximum value of 30,000 $mg \cdot kg^{-1}$ (Berna *et al.*, 1989) depending on the STP operating conditions and water hardness. Aerobic stabilised sludge always has a LAS content lower than 500 $mg \cdot kg^{-1}$, whereas anaerobic stabilised sludge has a LAS concentrations typically in the range 5,000-10,000 $mg \cdot kg^{-1}$ (McAvoy *et al.,* 1994; Waters and Feijtel, 1995). The Working Document on Sludge (EU, 2000) proposes a limit value for LAS of 2,600 $mg \cdot kg^{-1}$.

Therefore, sludge can still contain considerable amounts of LAS when they are applied to soils (Table 8.7). But, when judging potential risk, the rapid biodegradation under aerobic conditions after application of the sludge to soils has to be taken into account.

Recently in literature, while their behaviour under aerobic conditions is well documented and confirmed (Berna *et al.,* 1991; Romano and Ranzani, 1992; Holt *et al.,* 1995; Prats *et al.,* 1997), there are uncertainties about their biodegradation under anaerobic conditions. The few recent observations of LAS being degraded under anaerobic conditions (Sanz *et al.,* 1999; Denger and Cook, 1999; Haggesen

et al., 2002; Mogensen and Ahring, 2002; Angelidaki *et al.*, 2004) do seem to conflict with previous publications which indicate no degradation (Giger *et al.*, 1989; Steber, 1991; Federle and Schwab, 1992; Knudsen *et al.*, 1997; ERASM, 1999).

Table 8.7. LAS content ($mg \cdot kg^{-1}$) in digested sludge.

Type of sludge	LAS	Reference
Anaerobic digested	2,900–11,900	McEvoy and Giger, 1985
Anaerobic digested	4,200	Giger *et al.*, 1987
Anaerobic digested	9,300–18,800	Holt and Bernstein, 1992
Anaerobic digested	6,660	Sedlak and Booman., 1986
Anaerobic digested	4,660	Rappaport and Eckhoff, 1990
Anaerobic digested	6,000–9,400	Waters and Feijtel, 1995
Anaerobic digested	1,600–18,800	Jones and Northcott, 2000
Sewage sludge	2.3-17.5	Riu *et al.*, 2001
Anaerobic digested	1,800-1,900	Knudsen *et al.*, 2000

8.1.7. Objective

In this chapter, several digested sludge characteristics (organic matter, nutrients, pathogens, heavy metals, LAS and dewatering) have been analysed in order to dilucidate its final disposal according to the legal requirements.

8.2. Materials and methods

8.2.1. Sewage sludge

Raw sewage sludge used in this work was collected from an urban Sewage Treatment Plant (STP) located in Santiago de Compostela (NW of Spain). A mixture (70:30, v/v) of primary and secondary sludge was used as feeding of the anaerobic digestion pilot plant and its characteristics were indicated in Chapter 6 (sections 6.2.1 and 6.3.2).

During conventional operation, this raw sludge was digested under mesophilic and thermophilic conditions at several SRT (Chapter 6). The operational conditions of the digesters were indicated in Table 6.4.

During advanced operation, the raw sludge was pretreated using an alkaline, thermal and ozone treatment prior to be fed in the anaerobic digesters (Chapter 7). The characteristics of the mesophilic and thermophilic digester operation were indicated in Tables 7.3 and 7.4, respectively.

8.2.2. pH, total carbon, total nitrogen and organic matter content

Sample preparation

The lyophilised sludge was crushed and sieved (< 2 mm), being the fraction lower than 2 mm used for the pH determination. Afterwards, it was grinded in an Agata mill and the fraction obtained was used for carbon, nitrogen, phosphorus, cations and heavy metals determination.

pH

10 g of sludge were mixed with 25 mL of deionised water (water-sludge ratio 1:2.5) and stirred. After 10 minutes, the pH of the liquid phase was measured with a pH-meter.

Total carbon and total nitrogen

The total carbon and total nitrogen were determined in an elemental analyzer. 0.1 g of sludge was burnt and the resulting carbon dioxide and nitrous oxides were measured. Calibration between area and concentrations was made with commercial standards.

Organic matter content

The organic matter content (OM) was determined from the total carbon concentration (TC), using the following equation:

$$OM\ (\%) = 1.72 \times TC\ (\%) \qquad \text{Eq. 8.1}$$

8.2.3. Total P, Hg, Ca, Mg, K, Cu, Ni, Cr, Fe, Zn, Pb and Cd

Sample extraction

0.5 g of sludge was extracted in a microwave with 6 mL of nitric acid (HNO_3) and 2 mL of hydrochloric acid (HCl). The ramp of temperatures in the microwave oven was the following:

- 5 min until 80ºC
- 5 min until 120ºC
- 10 min until 160ºC
- 10 min until 180ºC
- 20 min until 200ºC

After the extraction, the 8 mL of solvents were collected and filled up to 50 mL with deionised water. Aliquots of this extracted solution were later used for the different determinations.

Total phosphorus

Total phosphorus (P) determination is based in the formation of a blue-coloured complex of P which is measured by a colorimetric method.

Reactives:

- Solution A: 12.5 g of ammonium molibdate are dissolved in 100 mL of deionised water. 0.305 g of antimonium tartrate and 125 mL of concentrated sulphuric acid are added and the final volume is filled up to 250 mL with deionised water.
- Solution B: 1.76 g of ascorbic acid are added to 20 mL of solution A and the final volume is filled up to 200 mL with deionised water. This solution must be prepared when used.

5 mL of the extracted solution were mixed with 5 mL of solution B. After one hour, the absorbance of the sample was measured at 680 nm. An 8-point calibration line in the concentration range of 0–5 ppm was used for the calculations.

Mercury

Mercury (Hg) is determined in a mercury equipment. 10-20 mg of sludge was burnt in a combustion furnace at 1,100ºC for 5 minutes. The Hg is stem from the sludge and transported by an air current to an amalgam in which it is retained. In a second phase, the amalgam is heated to remove the mercury which is transported to the measure cell. Calibration between absorbance and Hg concentration was made with commercial standards.

Ca, Mg, K, Cu, Ni, Cr, Fe, Zn, Pb and Cd

These cations and heavy metals were determined by Atomic Absorption Spectrophotometry (AAS) using a hollow cathode lamp, except potassium, for which the Atomic Emission Spectrophotometry (AES) was used.

In the AAS, the extracted solution is sprayed into the flame of the instrument by an air/acetylene mixture and atomised. Light of a suitable wavelength for a particular element is shone through the flame and some of this light is absorbed

by the atoms of the sample. The amount of light absorbed is proportional to the concentration of the element in the solution and hence in the original sample. Measurements are made separately for each element of interest according to the suitable wavelength (Table 8.8).

Table 8.8. Specific wavelengths of selected cations and heavy metals.

	λ (nm)		λ (nm)
Ca	422.7	**K**	766.5
Cd	228.8	**Mg**	285.2
Cr	357.9	**Ni**	232.0
Cu	324.8	**Pb**	283.3
Fe	248.3	**Zn**	213.9

The AES is often used to determine the concentration of alkali and alkaline earth metals, such as potassium. These metals have characteristics that make them reach excited states easily even at low concentrations. The main components of an atomic emission spectrophotometer are similar to that of an atomic absorption spectrophotometer; however, a hollow cathode lamp is not used in the AES. Instead, the flame that is used to atomize the sample is also used as the source. The energy emitted by the metal is proportional to its concentration in the solution.

Prior to the Fe, Mg and Ca determinations, 9 mL of the extracted solution were mixed with 1 mL of lanthanum oxide (586.4 g Ln_2O_3 + 250 mL of concentrated HCl filled up to 1 L with deionised water) in order to avoid interferences during the measurements.

For each compound, an 8-point calibration line is used in the calculations.

8.2.4. Pathogens content

Sample preparation

50 g of sludge were mixed in a sterile vessel with 450 mL of sterile phosphate buffer (SPBD) and stirred for 1 o 2 min at low speed (8000 rpm). From these homogenous samples, decimal dilutions (APHA-AWWA-WPCF, 1999) were prepared for enumeration of *Total coliforms*, *Fecal streptococcus*, *Escherichia coli* and *Clostridium perfringens*.

Total coliforms

The *coliform* group consists of several genera of bacteria belonging to the family *Enterobacteriaceae* and is defined as all facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 h at 35ºC.

The standard test for the *coliform* group was carried out by the multiple-tube fermentation technique (APHA-AWWA-WPCF, 1999), with the results being reported in terms of the Most Probable Number (MPN) of organisms present. 3-7 series of 3 tubes per test tube rack were used and the method includes 2 phases:

- Presumptive phase: appropriate graduated quantities of sample were inoculated in tubes containing Lauryl tryptose broth and incubated at 35ºC for 48 h. The positive tubes were those showing turbidity and gas production and they were subjected to the confirmed phase.
- Confirmed phase: the positive tubes from presumptive phase were inoculated in brilliant green lactose bile broth and incubated at 35ºC for 48 h. Once again, the positive tubes show turbidity and gas production. The MPN value is calculated from the number of positive brilliant green lactose bile tubes.

Fecal streptococcus

The *F. streptococcus* group consists of a number of species of the genus *Streptococcus*. They all give a positive reaction with Lancefield's group D antisera and have been isolated from the feces of warm-blooded animals. The *fecal streptococci* have been used with *fecal coliforms* to differentiate human fecal contamination from that of other warm-blooded animals.

Similarly to *T. coliforms*, the multiple-tube technique was used for enumeration of *F. streptococcus* (APHA-AWWA-WPCF, 1999) using 3-7 series of 3 tubes per test tube rack, thus the results being reported in terms of MPN.

- Presumptive phase: appropriate graduated quantities of sample were inoculated in tubes containing azide dextrose broth and incubated at 35ºC for 48 h. The positive tubes were those showing turbidity and they were subjected to the confirmed phase.
- Confirmed phase: the positive tubes from presumptive phase were inoculated in Enterocossel broth + agar and incubated at 35ºC. At the end

of 24 h, the plates were examined for colony growth (black colour). If no colonies are present, the tubes were reincubated and checked again at the end of 48 h. The MPN value is calculated from the number of positive tubes.

Escherichia coli

E. coli, the most common member of the genus *Escherichia,* is a common bacterium that normally inhabits the intestinal tracts of humans and animals, but can cause infection in other parts of the body, especially the urinary tract. It is a Gram-negative, rod-shaped bacterium propelled by long, rapidly rotating flagella.

The most probable number of *E. coli* was obtained according to the Spanish Official method (BOE, 1987) using three tubes per test tube rack. The method includes one presumptive and two confirmed phases:

- Presumptive phase: appropriate graduated quantities of sample were inoculated in tubes containing Lauryl tryptose broth and incubated at 35ºC for 48 h. The positive tubes were those showing turbidity and gas production and they were subjected to the first confirmed phase.
- 1st confirmed phase: the positive tubes from presumptive phase were inoculated in EC broth and incubated at 44ºC for 24 h. The positive tubes were those showing turbidity and gas production and they were subjected to the second confirmed phase.
- 2nd confirmed phase: the positive tubes from 1st confirmed phase were inoculated in tryptone water and incubated at 44ºC for 24 h. At the end of this period, 3-4 drops of indol (Kovac's reactive) were added to each tube. The positive tubes were those showing a rose ring. The MPN value is calculated from the number of positive indol tubes.

Clostridium perfringens

Sulphite-reducing bacteria have been used as indicators of fecal pollution for many years. *Cl. perfringens*, one of the members of the group, is highly specific for fecal pollution and the most reliable indicator for viruses and protozoan in treated drinking water.

Cl. perfringens counts were obtained by pour plate method inoculating 1 mL aliquots from series of ten-fold dilutions in Tryptose Sulfite Cycloserine Agar Base (Merck) supplemented with D-cycloserine and Fluorocult TSC agar

supplement (Merck). All plates were incubated at 46ºC for 24 h in a WA 6200 anaerobic cabinet (Heraeus, Germany). All black colonies on this media emitting light blue fluorescent after exposure to UV lamp were counted as *Cl. perfringens* (Araujo et al. 2001).

Salmonella spp

Salmonella spp is a genus of rod-shaped, gram-negative bacteria that are a common cause of food poisoning. They are ubiquitous in the environment and can be detected at low concentrations in most surface waters.

The presence of *Salmonella spp* was determined following the General Qualitative Isolation and Identification Procedures for Salmonella described in Standard Methods (APHA-AWWA-WPCF, 1999). The method comprises four phases:

- Pre-enrichment: appropriate graduated quantities of sample were inoculated in buffered peptone water and incubated at 35ºC for 24 h.
- Enrichment: appropriate volumes from the pre-enrichment step were inoculated in two broths:
    - Rappaport-Vassiliadis: incubation at 42ºC for 24 h.
    - Tetrathionate broth: incubation at 35ºC for 24 h.
- Isolation: each tube from the enrichment step is streaked in two plates, xylose lysine desoxycholate agar (XLD) and Agar Hektoen (AH), and incubated at 35ºC for 24 h. After this period, the typical *Salmonella spp* colonies appeared (black-centered red colonies). 5 colonies in total per sample were chosen for the confirmation phase.
- Confirmation: five biochemical tests were used to confirm the presence of *Salmonella spp*. Firstly, the selected colonies from XLD and AH are grown on Tryptone Soy Agar (TSA) at 35ºC for 24 h.
    - Oxidase test with colonies from TSA. The change of the colony colour after one drop of oxidase reactive means positive. *Salmonella spp* is oxidase (-).
    - Triple Sugar Iron Agar (TSI) with colonies from XLD and AH. The positive tubes lead to an alkaline (red) reaction in the surface and an acid (yellow) reaction in the bottom, with or without H_2S production (black).

- Lysine Iron Agar (LIA) with colonies from XLD and AH. The positive tubes lead to an alkaline (red) reaction in the bottom with H_2S production (black).
- Urea broth with colonies from XLD and AH. The positive tubes change the colour from yellow to red. *Salmonella spp* is urea (-).
- Kit API 20E o rapid API 20E with colonies from TSA. This test is used when the previous ones indicate presence of *Salmonella spp.*

Shigella spp

Shigella spp is a genus of the Enterobacteriaceae, nearly genetically identical to *E. coli* and closely related to *Salmonella spp*. It is the causative agent of shygellosis, a debilitating diarrheal disease.

Similarly to *Salmonella spp,* the presence of *Shigella spp* was determined following the General Qualitative Isolation and Identification Procedures for Salmonella described in Standard Methods (APHA-AWWA-WPCF, 2001). The method comprises three phases:

- Pre-enrichment: appropriate graduated quantities of sample were inoculated in Tryptone Soy Broth (TSB) and incubated at 35ºC for 8 h. Then, the Shigella broth is added and incubated at 35ºC for 16 h (24 h in total).
- Isolation: each tube from the enrichment step is streaked in two plates, Salmonella-Shigella agar (SS) and xylose lysine desoxycholate agar (XLD), and incubated at 35ºC for 24 h. After this period, the typical *Shigella spp* colonies appeared (colorless and transparent). 5 colonies in total per sample were chosen for the confirmation phase.
- Confirmation: four tests were used to confirm the presence of *Shigella spp.* Firstly, the selected colonies from XLD and AH are grown on Tryptone Soy Agar (TSA) at 35ºC for 24 h.
    - Oxidase test with colonies from TSA. The change of the colony colour after one drop of oxidase reactive means positive. *Shigella spp* is oxidase (-).

- Triple Sugar Iron Agar (TSI) with colonies from XLD and AH. The positive tubes lead to an alkaline (red) reaction in the surface and an acid (yellow) reaction in the bottom, without H_2S production (black).
- Lysine Iron Agar (LIA) with colonies from XLD and AH. The positive tubes lead to an alkaline (purple) reaction in the surface and an acid reaction (yellow) in the bottom without H_2S production (black).
- Kit API 20E o rapid API 20E with colonies from TSA. This test is used when the previous ones indicate presence of *Shigella spp.*

8.2.5. Dewatering properties

The dewatering properties were studied by determining the Specific Resistance to Filtration (SRF) and the compressibility coefficient (s).

Specific Resistance to Filtration (SRF)

The SRF test, know as the Buchner funnel test, is one of the most commonly employed test for the evaluation of sewage sludge dewaterability and filterability. The SRF test measures the resistance of sludge to filtration or dewatering. The filtering was performed using a 9-cm diameter Whatman #1 filter paper at applied vacuum pressures of 150, 450 and 650 mbar. The volume of filtrate (30 mL) collected was recorded as a function of time. SRF was determined using a plot of filtration time/filtrate volume (t/V) versus filtrate volume (V). Using the slope of the line (b), SRF is calculated from the following formula:

$$SRF = \left(\frac{2A^2P}{\mu C} \right) b \qquad \text{Eq. 8.2}$$

where:

SRF: specific resistance to filtration ($m \cdot kg^{-1}$),

P: pressure of filtration ($N \cdot m^{-2}$),

μ: viscosity of filtrate ($N \cdot s \cdot m^{-2}$),

C: weight of dry solids per volume of filtrate ($kg \cdot m^{-3}$), and

A: area of the filter paper (m^2).

Compressibility coefficient

The compressibility coefficient (s) is an empirical measure of the effect of the pressure differential across the sludge cake on its permeability. It is determined from analysis of specific resistance data obtained at various pressure differentials, from the following equation:

$$SRF = SRF_0 P^S \qquad \text{Eq. 8.3}$$

where:

SRF_0: specific resistance to filtration ($m \cdot kg^{-1}$), and

P: pressure of filtration ($N \cdot m^{-2}$).

8.2.6. Linear Alkylbenzene Sulphonates (LAS)

0.5 g of freeze-dried sludge sample was twice extracted with 10 mL of methanol. 25 µg of C8 was spiked to the first extraction slurry as surrogate standard. For each extraction step, the slurry was ultrasonicated for 15 min. Then, it was centrifuged at 1,500 rpm for 10 min and the supernatant was collected. The 2 solvent fractions were finally combined and an aliquot of 2 mL was used for the purification step (Solid Phase Extraction, SPE).

The SPE was performed in Strata SAX cartridges (55 µm, 70 A, 500 mg/3 mL, 8B-S008-HBJ), previously preconditioned by flushing 10 mL methanol and 10 mL water Lichrosolv (Merck). Then, the cartridges were eluted with 2 mL of a hydrochloric acid solution (2 N) in methanol (8.3 mL HCl 37%/50 mL MeOH). After evaporation to dryness, the residue was solved in 1 mL of the mobile phase (10 g $LiClO_4$ dissolved in 1 L of methanol/H_2O Lichrosolv, 80:20). Finally, the determination was carried out by HPLC (Hypersil BDS C8, 150 x 2 mm, 5µm), coupled to fluorescent (226-296 nm) and UV (220 nm) detection.

8.2.7. Calculations

Removal efficiencies for pathogens, heavy metals and LAS were calculated taking into account the concentration in the non-pretreated feeding (C_{in}) and the concentration in the digested sludge (C_{out}), relating the percentage to the initial concentration.

8.3. Results and Discussion

8.3.1. pH, organic matter and nutrients

Table 8.9 shows the pH, organic matter (OM) and nutrients (N, P) content in the raw sludge as well as after alkaline, thermal and ozone treatment.

Table 8.9. pH, organic matter and nutrients content in the non-pretreated and pretreated sludge.

	Raw	Alkaline	Thermal	Ozonation
pH	5.5 – 5.8	6.3 – 9.0	5.4 – 5.5	5.8
OM (%)	38.7 – 67.2	43.0 – 52.2	56.9 – 58.3	64.1
C (%)	22.5 – 39.1	25.0 – 30.3	33.1 – 33.9	37.3
N (%)	1.6 – 3.9	2.3 – 3.0	3.6 – 4.1	3.0
P (%)	1.6 – 3.4	1.7 – 2.3	2.6 – 2.7	2.6
C/N (%)	6.2 – 14.5	10.3 – 11.1	8.1 – 9.5	12.3

The average pH was around 5.5, except when the alkaline pre-treatment was used (up to 9.0). The carbon, nitrogen and phosphorus concentrations remained in the same level, 25-40%, 2-4% and 2-3%, respectively. The ratio C/N was between 6 and 15.

Therefore, it could be concluded that the pre-treatments did not affect the agronomic value of the sludge.

Table 8.10 shows the pH, organic matter and nutrients content in the digested sludge from conventional and advanced operation in mesophilic and thermophilic range.

The pH of the digested sludge remained in the neutral range in both digesters, between 5.4 and 6.8. The carbon content (10-30%), and consequently the organic matter (20-50%), in the mesophilic digested sludge were similar to that in the thermophilic digested sludge. Higher concentrations were obtained when the digesters were run at low SRT.

The concentrations of nutrients ranged from 2 to 4% for nitrogen and from 1 to 5% for phosphorus in both digesters. Once again, higher concentrations were obtained when the digesters were run at low SRT, except with the alkaline pre-treatment in both digesters (nitrogen) and with the thermal process in the thermophilic range (phosphorus). The C/N ratio varied between 5-9 and 6-15% in the mesophilic and thermophilic digested sludge, respectively.

Table 8.10. pH, organic matter and nutrients content in the digested sludge after conventional and advanced anaerobic treatment.

	SRT	Mesophilic digester				SRT	Thermophilic digester			
	(d)	Conv.	Alkaline	Thermal	Ozone	(d)	Conv.	Alkaline	Thermal	Ozone
pH	20	6.7	6.0	6.0	6.8	10	6.0	6.3	6.2	6.2
	10	5.8	5.4	6.4	-	6	5.5	5.6	6.4	-
OM (%)	20	31.5	18.8	33.7	50.2	10	23.6	27.1	29.6	40.1
	10	43.0	41.0	45.1	-	6	43.9	39.5	45.3	-
C (%)	20	18.3	11.0	19.6	29.2	10	13.7	15.8	17.2	23.3
	10	25.0	23.8	26.2	-	6	25.5	23.0	26.4	-
N (%)	20	2.1	3.4	2.3	4.1	10	1.4	2.8	1.6	2.8
	10	3.1	2.8	3.5	-	6	1.7	2.2	3.0	-
P (%)	20	2.1	2.6	3.4	5.3	10	0.9	2.0	4.8	4.5
	10	2.8	2.7	3.7	-	6	2.2	2.2	3.6	-
C/N (%)	20	8.7	5.5	8.5	7.1	10	10.2	5.6	11.1	9.5
	10	8.1	8.6	7.5	-	6	15.2	10.5	8.9	-

8.3.2. Cations and heavy metals content

Table 8.11 shows the cations (Ca, Mg and K) and heavy metals (Cu, Ni, Cr, Fe, Zn, Pb, Cd and Hg) content in the raw sludge as well as after alkaline, thermal and ozone treatment.

The content of Mg and K in the pretreated and non-pretreated sludge was similar, 0.3-1.2% (MgO) and 0.1-2.0% (K_2O), respectively. However, the concentration of CaO increased after alkaline pre-treatment from 1-6% to 19-24%.

The content of the heavy metals in the sludge was not affected by the sludge pre-treatments, being the concentrations measured below the limit values proposed in the Working Document on Sludge (EU 2000; EU 2004): 191-331 $mg \cdot kg^{-1}$ (Cu), 19-132 $mg \cdot kg^{-1}$ (Ni), 73-301 $mg \cdot kg^{-1}$ (Cr), 810-24,380 $mg \cdot kg^{-1}$ (Fe), 350-1,120 $mg \cdot kg^{-1}$ (Zn), 54-115 $mg \cdot kg^{-1}$ (Pb), 1-3 $mg \cdot kg^{-1}$ (Cd) and 0.7-1.7 $mg \cdot kg^{-1}$ (Hg).

Table 8.11. Cations (%) and heavy metals ($mg \cdot kg^{-1}$) content in the non-pretreated and pretreated sludge.

	Raw	Alkaline	Thermal	Ozone	Limit values
CaO	0.9 – 6.2	19.4 – 23.5	1.0 – 2.1	1.5	-
MgO	0.5 – 1.2	0.4 – 0.6	0.5 – 1.1	0.3	-
K_2O	0.1 – 2.0	0.8 – 0.9	0.9 – 1.0	0.7	-
Cu	275 – 331	191 – 282	238 – 305	315	1,000
Ni	32 – 64	28 – 52	28 – 132	19	300
Cr	122 – 244	73 – 78	177 – 301	165	1,000
Fe	4,730 – 24,380	810 – 5,290	10,500 – 17,300	6,470	-
Zn	500 – 880	350 – 370	600 – 810	1,120	2,500
Pb	71 – 105	54 – 70	81 -115	88	750
Cd	1 – 3	1 – 2	1	1	10
Hg	1.5 – 1.7	1.0 – 1.1	0.7 – 1.5	0.9	10

Table 8.12 shows the cations and heavy metals concentrations in the digested sludge after conventional (without pre-treatment) and advanced (with pre-treatment) operation.

The concentrations of CaO, MgO and K_2O in the digested sludge ranged from 1-22%, 0.5-1.5% and 0.8-2.2%, respectively, with no significant influences of temperature, SRT and type of operation.

Table 8.12. Cations (%) and heavy metals content ($mg \cdot kg^{-1}$) in the digested sludge after conventional and advanced anaerobic treatment.

	SRT	Mesophilic digester				SRT	Thermophilic digester			
	(d)	Conv.	Alkaline	Thermal	Ozone	(d)	Conv.	Alkaline	Thermal	Ozone
CaO	20	1.6	19.8	12.1	4.6	10	0.9	20.5	21.3	3.1
	10	15.9	18.4	21.6	-	6	10.7	16.1	12.1	-
MgO	20	1.5	0.8	1.3	0.5	10	1.1	0.8	0.6	0.5
	10	0.6	0.7	0.6	-	6	0.6	0.6	0.7	-
K_2O	20	2.2	1.3	1.0	1.2	10	1.5	1.1	1.3	1.3
	10	1.3	1.2	0.8	-	6	1.3	1.1	1.2	-
Cu	20	299	297	207	680	10	201	225	170	575
	10	371	373	355	-	6	298	298	364	-
Ni	20	70	92	116	62	10	152	60	48	55
	10	44	49	60	-	6	33	34	43	-
Cr	20	180	181	253	318	10	163	99	122	282
	10	193	190	227	-	6	153	147	255	-
Fe	20	25,370	10,650	8,680	15,700	10	21,400	9,220	4,370	11,880
	10	15,700	17,700	15,700	-	6	10,500	12,900	21,800	-
Zn	20	790	670	560	2,400	10	540	470	360	2,100
	10	790	790	1,010	-	6	790	560	1,150	-
Pb	20	113	99	105	167	10	76	74	66	156
	10	122	120	117	-	6	122	95	120	-
Cd	20	1	2.0	1.0	2.0	10	1.0	2.0	1.0	2.0
	10	<1	1.0	1.0	-	6	1.0	1.0	1.0	-
Hg	20	1.3	1.0	1.4	1.5	10	1.6	1.2	1.0	1.1
	10	1.6	1.9	1.8	-	6	1.3	1.1	2.2	-

The concentrations of all metals in the digested sludge were far below the current limit values established by Directive 86/278/EEC (Table 8.2), and also below the limit values proposed in the Working Document on Sludge (EU, 2000). This fact indicates that all the operational conditions tested lead to a digested sludge suitable for land application.

Although the metal concentrations in the digested sludge (Table 8.12) were in the same range than those measured in the feeding (Table 8.11), having into account that there was an average solids removal efficiency of 50%, this means that around 50% of heavy metals was removed during anaerobic digestion. Different factors could be responsible for this behaviour, such as:

- Precipitation as inorganic salts (phosphates, sulphides, carbonates), which would accumulate in the bottom of the digesters.
- Increase in the solubility due to changes in the structure (temperature) or in the oxidation state (ozonation) which would lead to the irreversible formation of more stable molecules.

8.3.3. Pathogens content

Table 8.13 shows the pathogens content in the raw sludge as well as after alkaline, thermal and ozone treatment.

Table 8.13. Pathogens content in the non-pretreated and pretreated sludge.

	Raw	**Alkaline**	**Thermal**	**Ozonation**
T. coliforms **(MPN·g TS^{-1})**	$2.4\cdot10^{5}$-$2.4\cdot10^{7}$	$3.0\cdot10^{1}$-$2.4\cdot10^{5}$	$2.4\cdot10^{1}$-$1.1\cdot10^{2}$	$4.6\cdot10^{3}$
E. coli **(MPN·g TS^{-1})**	$4.6\cdot10^{3}$-$1.1\cdot10^{6}$	$3.0\cdot10^{1}$-$9.3\cdot10^{1}$	$3.0\cdot10^{-1}$-$1.5\cdot10^{0}$	$1.1\cdot10^{-3}$
F. streptococ. **(MPN·g TS^{-1})**	$1.1\cdot10^{2}$-$2.4\cdot10^{7}$	$3.0\cdot10^{1}$-$2.4\cdot10^{5}$	$4.6\cdot10^{2}$-$1.1\cdot10^{4}$	$2.4\cdot10^{6}$
Cl. perfringens **(CFU·g TS^{-1})**	$8.4\cdot10^{4}$-$8.1\cdot10^{6}$	$8.0\cdot10^{2}$-$3.0\cdot10^{5}$	$1.0\cdot10^{1}$	$2.3\cdot10^{5}$
Salmonella spp **(PA·50 g TS^{-1})**	Presence	Absence	Absence	Presence
Shigella spp **(PA·50 g TS^{-1})**	Absence	Absence	Absence	Absence

PA: Presence/Absence.

While *Salmonella spp* was present in the raw feeding, *Shigella spp* was not detected in any sludge sample. Alkaline and thermal processes led to the elimination of *Salmonella spp*. However, it was still present in the ozonized sludge.

All the pathogens considered were significantly removed (>85%) during sludge pre-treatments, with the exception of *F. streptococcus* during ozonation process (around 63%).

Taking into account US EPA requirements for Class A and B sludge (Table 8.6), it can be concluded that the three pre-treatments lead to a Class B product, but only the thermal process ensures a Class A sludge. Concerning the new requirements established in the Working Document on Sludge, the three processes fulfill the limit values for *E. coli*, even those established for an advanced treatment ($<5 \cdot 10^2$ CFU·g TS^{-1}). However, only with the alkaline and the thermal treatments *Salmonella spp* was not present, and *Cl. perfringens* was reduced to levels lower than $3 \cdot 10^3$ MPN·g TS^{-1} with the thermal process exclusively.

Tables 8.14 and 8.15 show the pathogens content and the removal efficiencies, respectively, in the digested sludge after conventional (without pre-treatment) and advanced (with pre-treatment) operation.

According to the feeding, *Shigella spp* was not present in any digested sludge sample. *Salmonella spp* was inactivated during anaerobic digestion treatment, except in the mesophilic digester at 10-d SRT during conventional and advanced operation with the alkaline pre-treatment.

Total coliforms were very well removed (>98%) in both digesters regardless of SRT and type of operation. Only in the conventional operation of the mesophilic digester at 10-d SRT, the elimination was lower (74%). Han *et al.* (1997) did not observe any influence of SRT on the coliform destruction, indicating that coliform destruction is mainly function of temperature than SRT.

Escherichia coli was also very well removed (>90%) in both digesters regardless of SRT and type of operation.

Table 8.14. Pathogens content in the digested sludge after conventional and advanced anaerobic treatment.

	SRT	Mesophilic digester				SRT	Thermophilic digester			
	(d)	Conv.	Alkaline	Thermal	Ozone	(d)	Conv.	Alkaline	Thermal	Ozone
T. coliforms	20	$1.1 \cdot 10^{2}$	$2.4 \cdot 10^{4}$	$1.1 \cdot 10^{4}$	$4.6 \cdot 10^{1}$	10	$9.0 \cdot 10^{-2}$	$1.5 \cdot 10^{0}$	$4.3 \cdot 10^{-1}$	$2.4 \cdot 10^{0}$
(MPN·g TS^{-1})	10	$1.1 \cdot 10^{5}$	$2.4 \cdot 10^{5}$	$1.1 \cdot 10^{4}$	-	6	$1.1 \cdot 10^{1}$	$7.5 \cdot 10^{-1}$	$1.1 \cdot 10^{1}$	-
E. coli	20	$4.6 \cdot 10^{1}$	$1.1 \cdot 10^{4}$	$1.1 \cdot 10^{4}$	$4.3 \cdot 10^{0}$	10	$3.0 \cdot 10^{-2}$	$3.0 \cdot 10^{-2}$	$1.5 \cdot 10^{-1}$	$4.0 \cdot 10^{-2}$
(MPN·g TS^{-1})	10	$4.6 \cdot 10^{4}$	$1.2 \cdot 10^{4}$	$1.1 \cdot 10^{4}$	-	6	$3.0 \cdot 10^{-2}$	$3.0 \cdot 10^{-2}$	$4.0 \cdot 10^{-2}$	-
F. streptoc.	20	$1.1 \cdot 10^{2}$	$2.4 \cdot 10^{3}$	$1.1 \cdot 10^{4}$	$1.1 \cdot 10^{4}$	10	$1.1 \cdot 10^{1}$	$2.1 \cdot 10^{-1}$	$2.4 \cdot 10^{1}$	$2.4 \cdot 10^{0}$
(MPN·g TS^{-1})	10	$2.4 \cdot 10^{3}$	$4.6 \cdot 10^{5}$	$2.4 \cdot 10^{4}$	-	6	$1.1 \cdot 10^{1}$	$2.4 \cdot 10^{0}$	$2.4 \cdot 10^{0}$	-
Cl. perfring.	20	$1.6 \cdot 10^{5}$	$1.8 \cdot 10^{5}$	$1.2 \cdot 10^{4}$	$3.2 \cdot 10^{5}$	10	$9.3 \cdot 10^{4}$	$2.7 \cdot 10^{4}$	$1.7 \cdot 10^{3}$	$2.0 \cdot 10^{5}$
(CFU·g TS^{-1})	10	$3.1 \cdot 10^{5}$	$5.6 \cdot 10^{4}$	$2.0 \cdot 10^{4}$	-	6	$3.8 \cdot 10^{5}$	$4.9 \cdot 10^{3}$	$4.5 \cdot 10^{2}$	-
Salmonella spp	20	A	A	A	A	10	A	A	A	A
(PA·50g TS^{-1})	10	P	P	A	-	6	A	A	A	-
Shigella spp	20	A	A	A	A	10	A	A	A	A
(PA·50g TS^{-1})	10	A	A	A	-	6	A	A	A	-

A: Absence; P: Presence.

Table 8.15. Removal efficiencies (%) of pathogens during conventional and advanced anaerobic treatment.

	SRT	Mesophilic digester				SRT	Thermophilic digester			
	(d)	Conv.	Alkaline	Thermal	Ozone	(d)	Conv.	Alkaline	Thermal	Ozone
T. col.	20	100	99.9	99.9	100	10	100	100	100	100
	10	74.0	98.8	99.2	-	6	100	100	100	-
E. coli	20	100	94.0	98.6	100	10	100	100	100	100
	10	89.1	99.4	-	-	6	100	100	100	-
F. strep.	20	36.6	100	99.9	99.9	10	93.4	100	100	100
	10	98.8	89.3	59.8	-	6	100	100	100	-
Cl. perfr.	20	27.6	72.3	98.7	90.1	10	56.2	94.5	99.7	93.3
	10	0	99.6	98.7	-	6	0	100	100	-
Salmonella spp	20	A	A	A	A	10	A	A	A	A
	10	P	P	A	-	6	A	A	A	-
Shigella spp	20	-	-	-	-	10	-	-	-	-
	10	-	-	-	-	6	-	-	-	-

A: Absence; P: Presence.

Fecal streptococcus was also very well removed (>90%) in both digesters regardless of SRT and type of operation. Only in the mesophilic reactor during conventional operation at 20-d SRT and advanced operation with the thermal pre-treatment at 10-d SRT, the removal efficiencies were lower, 37 and 60%, respectively.

No elimination of *Clostridium perfringens* was observed in both digesters during conventional operation at low SRT. At high SRT, the removal efficiencies were higher in the thermophilic range (56%) than in the mesophilic one (28%). Advanced operation improved the inactivation of this micro-organisms in both digesters (>70%) regardless of SRT and type of pre-treatment. Abu-Orf *et al.* (2004) indicated an inactivation of *Cl. perfringens* during chemical conditioning of digested sludge with lime and fly ash attributing the effectiveness to the elevated pH and free ammonia, since the spores are highly resistant to high temperatures. They pointed out to the storage period and temperature as the limiting factors in achieving a Class A product.

As reported in literature (Oles *et al.*, 1997; Nielsen and Petersen, 2000), thermophilic conditions leads to a better sanitizing effect than mesophilic ones, since it fulfilled the US EPA limit values for Class A sludge as well as the requirements proposed in the Working Document on Sludge to be considered as an advanced treatment.

8.3.4. Dewatering properties

Table 8.16 shows the Specific Resistance to Filtration (SRF) and the compressibility coefficient (s) of the raw sludge as well as after alkaline, thermal and ozone treatment.

Table 8.16. Dewatering properties of the non-pretreated and pretreated sludge.

	SRF $\cdot 10^{-14}$ (m·kg^{-1})			s
	150 mbar	**450 mbar**	**650 mbar**	
Raw sludge	5.3 – 7.9	1.8 – 4.3	0.1 - 3.9	0.6 – 4.7
Alkaline	6.0 – 10.6	4.1 – 7.7	0.7 – 3.4	0.5 – 3.1
Thermal	1.6 – 5.3	0.8 – 3.8	0.3 – 3.0	1.8 – 2.1
Ozonation	26.3	6.0	3.0	2.5

As expected, lower vacuum levels (650 mbar), which means less pressure drop through the sludge cake, lead to lower specific resistances, regardless of type of sludge (pretreated or non-pretreated).

At lower vacuum pressures (150 and 450 mbar), the alkaline and ozone treatments increased the SRF of the pretreated sludge (6-26·10^{14} m·kg^{-1}) compared to the non-pretreated sludge (5-8·10^{14} m·kg^{-1}), whereas the thermal treatment led to lower values (2-5·10^{14} m·kg^{-1}). However, the values obtained at 650 mbar with and without pre-treatment were similar (0.1-4.0·10^{14} m·kg^{-1}). The compressibility coefficient values of the pretreated and non-pretreated sludge were in the same range, around 0.5-4.7.

Tables 8.17 and 8.18 show the dewatering properties of the mesophilic and thermophilic digested sludge, respectively, after conventional and advanced operation.

Table 8.17. Dewatering properties of the mesophilic digested sludge.

	SRT	**SRF ·10^{14} (m·kg^{-1})**			**s**
	(d)	150 mbar	450 mbar	650 mbar	
Conventional	20	1.8	1.6	1.1	0.6
	10	23.2	15.1	13.2	0.7
Alkaline	20	0.7	0.6	0.2	1.2
	10	9.3	7.2	3.6	1.1
Thermal	20	9.1	6.7	6.4	0.4
	10	5.5	5.0	3.6	0.5
Ozonation	20	81.5	18.4	9.9	2.4

Table 8.18. Dewatering properties of the thermophilic digested sludge.

	SRT	**SRF ·10^{14} (m·kg^{-1})**			**s**
	(d)	150 mbar	450 mbar	650 mbar	
Conventional	10	4.7	3.8	3.5	0.4
	6	17.4	15.4	13.0	0.3
Alkaline	10	0.2	0.2	0.1	2.4
	6	5.1	4.8	4.5	0.2
Thermal	10	3.7	3.1	2.6	0.4
	6	9.8	7.9	7.3	0.3
Ozonation	10	89.6	19.6	10.9	2.4

Similarly to the raw sludge, higher vacuum pressures led to lower specific resistances, regardless of temperature, SRT, type of pre-treatment and type of operation.

Alkaline and thermal (except in mesophilic range at 20-d SRT) processes decreased the SRF of the digested sludge in both digesters, regardless of SRT. In

contrast, the ozone treatment deteriorated the dewatering properties of both mesophilic (Table 8.17) and thermophilic (Table 8.18) digested sludge.

The compressibility coefficient values were higher after ozone and alkaline (except in thermophilic range at 6-d SRT) treatments in both digesters, which indicates a greater influence of P_{vacuum} on SRF; however, the thermal process led to similar values in thermophilic range and lower values in the mesophilic one.

It was also found in literature that the disintegration of sewage sludge has a strong influence on the conditioning and dewatering characteristics. In particular, the polymer demand of the sludge increases (Scheminski *et al.,* 2000; Müller *et al.,* 2004) and the solid content in the dewatered sludge is sometimes lower (Müller, 2000; Kopp *et al.,* 1997).

Comparing the three pretreatments, when the digesters were operated at high SRT, the SRF values were higher with ozonation, followed by the thermal process, thus the best results being obtained with the alkaline treatment. At low SRT, while the thermal treatment led to lower SRF under thermophilic conditions (Table 8.18), in the mesophilic range (Table 8.17) the best results were obtained with the alkaline process. The influence of the vacuum pressure (s) was higher with the ozone pre-treatment, followed by the alkaline process and lastly the thermal treatment. These results are in accordance with literature since Lin *et al.* (1997) indicated an improvement in dewaterability after anaerobic digestion of sludge pretreated with NaOH and the negative effect of ozonation is widely reported (Weemaes *et al.,* 2000; Ahn *et al.,* 2002; Battimelli *et al.,* 2003; Boehler and Siegrist, 2003).

The SRF values increased at lower SRT, except with the thermal treatment in the mesophilic digester. However, the compressibility coefficient was not affected by SRT, except with the alkaline process in the thermophilic range, with lower values being achieved at low SRT.

When both digesters were operated at the same SRT, the thermophilic digested sludge had better dewatering properties with lesser dependence of the vacuum pressure (except with the alkaline process) than the mesophilic one, independently of type of operation. In the case of ozonation, although the SRT in the thermophilic reactor was half than that in the mesophilic one, similar dewatering properties were observed in both digested sludges. The better performance of thermophilic digestion in terms of sludge dewaterability has been

also reported in literature (Garber *et al.,* 1975; Tapana and Pagilla, 2000; Bivins and Novak, 2001).

8.3.5. Linear Alkylbenzene Sulfonates

Table 8.19 shows the LAS concentrations in the raw sludge as well as after alkaline, thermal and ozone treatment.

Table 8.19. LAS concentrations ($mg \cdot kg^{-1}$) in the non-pretreated and pretreated sludge.

	C_{10}	C_{11}	C_{12}	C_{13}	C_T
Raw	1.2 – 3.0	31.1 – 66.4	59.2 – 112.3	59.8 – 89.7	151 – 271
Alkaline	0.3 – 3.8	7.7 – 74.7	14.1 – 124.7	13.4 – 108.4	36 – 312
Thermal	1.1 – 4.8	33.4 – 72.0	65.4 – 95.4	70.5 – 81.6	170 – 254
Ozone	3.3	52.0	87.3	82.4	225

The LAS content in the sludge was not affected by any pre-treatment because their concentrations remained in the same level as those in the non-pretreated sludge, 0.3-4.8 $mg \cdot kg^{-1}$ of C_{10}, 7.7-74.7 $mg \cdot kg^{-1}$ of C_{11}, 14.1-124.7 $mg \cdot kg^{-1}$ of C_{12} and 13.4-108.4 $mg \cdot kg^{-1}$ of C_{13}.

Table 8.20 and Figure 8.1 show the content of the different LAS homologues and the removal efficiencies, respectively, in the digested sludge after conventional (without pre-treatment) and advanced (with pre-treatment) operation.

The LAS content of the digested sludge of this work (90-550 $mg \cdot kg^{-1}$) was much lower than the typical values obtained in literature (Table 8.7) and almost one order lower than the limit value proposed in the Working Document on Sludge (2,600 $mg \cdot kg^{-1}$). Besides, these concentrations do not reach the threshold value (15,000 $mg \cdot kg^{-1}$) found as inhibitory for biogas formation (Battersby and Wilson, 1989).

The removal efficiencies of the different LAS homologues were similar in both digesters during all the experiments, ranging from 10 to 70%. Only when the thermal treatment was applied at low SRT, the elimination was very low (< 10%).

In full-scale anaerobic digesters, Prats *et al.* (1997) found a removal of 18%, Giger *et al.* (1987) found 20-30% removal and Osburn (1986) reported 0-35%. However, it is not clear to which process (binding, humification, co-metabolism and anaerobic desulphonation) can be ascribed.

Table 8.20. LAS content ($mg \cdot kg^{-1}$) in the digested sludge after conventional and advanced anaerobic treatment.

	SRT	Mesophilic digester				SRT	Thermophilic digester			
	(d)	Conv.	Alkaline	Thermal	Ozone	(d)	Conv.	Alkaline	Thermal	Ozone
C_{10}	20	0.8	1.1	1.5	2.4	10	0.8	0.9	1.3	23.1
	10	4.3	3.9	5.7	-	6	3.6	2.8	6.1	-
C_{11}	20	22.2	25.4	43.8	29.1	10	20.8	19.7	37.1	135.5
	10	82.8	82.3	86.8	-	6	68.5	49.6	91.2	-
C_{12}	20	43.7	56.7	89.3	47.5	10	46.5	33.7	74.9	201.5
	10	129.2	128.8	115.8	-	6	108.5	79.0	120.3	-
C_{13}	20	47.5	55.6	106.0	45.1	10	39.7	32.4	81.4	191.1
	10	106.4	107.9	98.1	-	6	88.3	65.7	105.1	-
C_T	20	114.2	138.8	240.6	124.2	10	107.8	86.7	194.6	551.2
	10	322.7	322.9	306.4	-	6	268.8	197.1	322.7	-

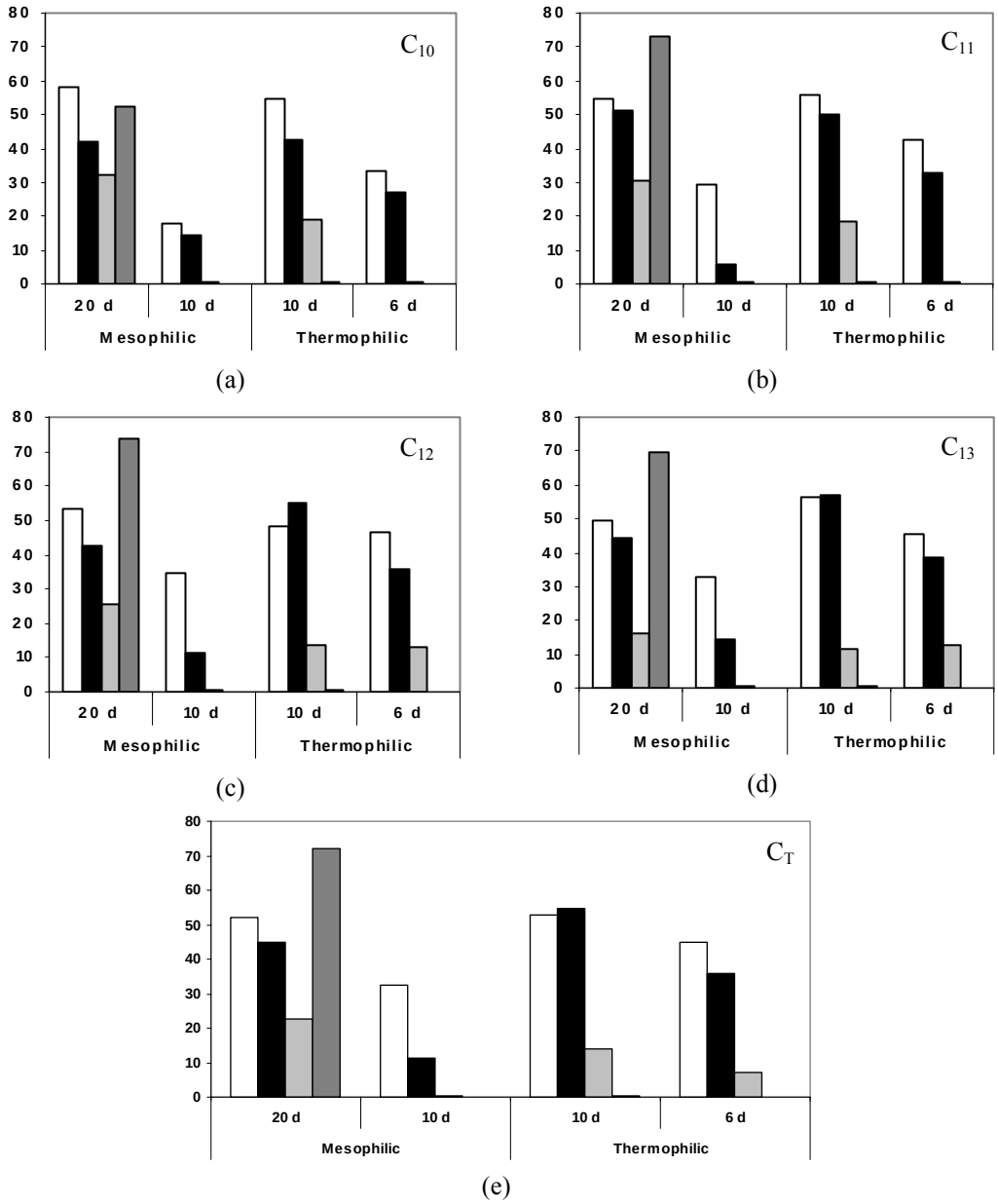

Figure 8.1. Removal efficiencies (%) of C_{10} (a), C_{11} (b), C_{12} (c), C_{13} (d) and C_T (e) during conventional (□), alkaline (■), thermal (■) and ozone (■) treatment.

With the exception of the ozone treatment in the mesophilic digester, the conventional operation led to higher or similar elimination of LAS than the advanced operation. Comparing the three pre-treatments, the best results were achieved with the ozone process in the mesophilic digester, while in the thermophilic one, it was the alkaline treatment which led to the highest removal. Higher elimination was obtained with the alkaline process compared to the thermal treatment in both digesters, independently of SRT.

Lower SRT decreased LAS elimination from 50 to 10%, approximately, regardless of temperature and type of operation. When both digesters were run at the same SRT, the thermophilic process increased the LAS elimination, regardless of type of operation.

The biodegradation mechanism of LAS was described by Balson and Felix (1995) and it involves three steps: the degradation of the straight alkyl chain, the sulphonate group and finally the benzene ring. There is recent evidence that anaerobic desulphonation can take place (Denger and Cook, 1999). Desulphonation with assimilation of the sulphur moiety by strictly anaerobic bacteria (Chien *et al.,* 1995; Denger and Cook, 1999) is followed by the reduction of the sulphonate as a source of electrons and carbon under anaerobic nitrate-respiring conditions (Laue *et al.,* 1997; Denger *et al.,* 1997).

8.4. Conclusions

The debate on sludge recycling and disposal has recently been the target of growing interest. This is due to the fact that some concern was expressed about the potential risks of the agricultural use of sludge for health and the environment. Parallel to this, the government policy and regulations regarding the application of sludge in agriculture have changed considerably (Spinoza, 2001).

Therefore, due to the urgency to develop more sustainable scenarios for sludge treatment, an increasing growth is observed in research into innovative sludge treatment processes. The general objectives are the recovery and reuse of valuable products from the sludge and the improvement of the sludge quality in terms of pathogens, dewatering properties, heavy metals and organic pollutants.

In this work, the four parameters mentioned before have been chosen in order to characterize the digested sludge obtained by different treatment technologies with the anaerobic digestion as a basis. It was observed that the results were different depending on four parameters: type of operation (conventional or

advanced), type of pretreatment (alkaline, thermal or ozone), SRT (high or low) and temperature (mesophilic or thermophilic).

In general, the digested sludge possesses a high agronomic value due to its organic matter and nutrients content, 20-50% and 2-10%, respectively. The concentrations of heavy metals were below the current legal requirements (EU Directive 86/278/EEC; RD 1310/1990) and also below the more stringent limit values proposed in the Working Document on Sludge (EU 2000). The hygienic properties were different for the mesophilic and thermophilic digested sludge, having the latter the best sanitizing conditions which allow it to be classified as a Class A product according to the US EPA restriction. The LAS content, chosen as a model organic pollutant, in the digested sludge (90-550 $mg \cdot kg^{-1}$) was much lower than the proposed limit value (2,600 $mg \cdot kg^{-1}$). Finally, the dewatering properties of the sludge were improved after all treatments, except for the ozonation process.

Consequently, the digested sludge obtained at any conditions tested in this work is suitable for land disposal regarding the legal requirements. However, sludge management has to be considered as a dynamic activity always looking for the most efficient sustainable solution at the location of concern and valid within the present and future boundary conditions.

8.5. References

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Conclusions

In recent years, an increasing concern about the presence of Pharmaceutical and Personal Care Products (PPCPs) in the environment and the unknown long-term effects on aquatic organisms and human health has arisen. In the present work, the occurrence and fate of PPCPs in a municipal Sewage Treatment Plant (STP) has been studied, paying special attention to the sludge treatment by anaerobic digestion. Next, the results obtained and the main conclusions are presented:

Sewage Treatment Plant

i) Eight out of the thirteen substances selected in this work have been detected up to $\mu g \cdot L^{-1}$-level in both influent and effluent of the STP: Galaxolide, Tonalide, Ibuprofen, Naproxen, Iopromide, Sulfamethoxazole, Estrone and 17β-estradiol. The other compounds (Carbamazepine, Diazepam, Diclofenac, Roxithromycin and 17α-ethinylestradiol) were not detected or below the limit of quantification. An increase in the concentrations in the inlet of the primary sedimentation compared with the raw influent of the STP was observed for some compounds, which suggests either the contribution of recycling streams from sludge treatment processes or the cleavage of glucuronide forms.

ii) Most substances detected were not eliminated completely in the STP, thus being discharged as contaminants into the receiving waters.

iii) A higher elimination of the substances was achieved in the secondary treatment compared with the primary treatment, although the degree of reduction depends on each substance.

iv) Musks (Galaxolide and Tonalide) were removed during both primary and secondary treatment mainly due to sorption processes, leading to overall efficiencies up to 90%.

v) Anti-inflammatories (Ibuprofen and Naproxen) were highly reduced during biological treatment with efficiencies ranging between 40 and 65%. Due to their hydrophilic nature, this elimination is mainly biological degradation.

vi) Sulfamethoxazole was also noticeable removed (around 60%) during biological treatment, being its reduction carried out biologically.

vii) High persistence of Iopromide was observed in this work since no elimination was obtained for this compound along the STP.

viii) For the natural estrogens, a significant removal was achieved for E2 (around 65%), whereas the concentrations of E1 increased along the treatment probably due to the cleavage of glucuronide forms and the oxidation of E2.

ix) The elimination pathways of PPCPs in STPs depend on their physico-chemical properties. Due to the low Henry coefficient values, volatilisation is negligible (up to 5% for musks), thus being sorption and degradation the main mechanisms involved in PPCPs removal in STPs.

x) Mass balances through the different units of the STP including the sludge phase are needed to differentiate between sorption and degradation. The selection of the method to calculate the amount sorbed onto sludge is crucial for sorptive compounds because an inaccurate estimation of this parameter can lead to misunderstandings in the establishment of the mechanism responsible for their elimination.

xi) Method I, using the concentrations measured in the sludge, seems to be more suitable for mass balance calculations of sorptive substances, such as musks. However, the inherent errors of the analytical methodology should be considered.

xii) For hydrophilic compounds, such as most pharmaceuticals, Method II, using the solid-water distribution coefficients (K_d) to calculate the concentrations in the sludge, is appropriate. This would avoid the analysis in the sludge phase.

xiii) Musks are mainly eliminated via sorption (although biodegradation also occurs), while pharmaceuticals, which are more polar, are mainly degraded in the plant.

Physico-chemical processes

xiv) Coagulation-flocculation processes can be successfully applied for the removal of Galaxolide, Tonalide and Diclofenac, around 70%, and in less extent, Naproxen and Diazepam (around 20%). Carbamazepine and Ibuprofen were not affected.

xv) Nor influence of temperature neither of coagulant dose was observed for any compound tested, being $FeCl_3$ selected as the most suitable additive.

xvi) In the flotation assays, all PPCPs tested were removed in some extent (20-60%), being the higher efficiencies achieved for musks. High fat content in the wastewaters increases the elimination of these substances.

Anaerobic treatment of sludge

xvii) In sludge treatment, the limit of relevance below which the PPCPs sorption can be neglected is around $K_d<1$ $L \cdot kgTSS^{-1}$, much lower than that accepted for wastewater treatment ($K_d<500$ $L \cdot kgTSS^{-1}$).

xviii) The removal of solids and organic matter ranged from 50 to 70% in both digesters. The sludge stabilisation increased when operating at higher SRT, regardless of temperature of operation. Thermophilic conditions led to slightly better results than the mesophilic ones.

xix) The behaviour of the different PPCPs during conventional anaerobic digestion of sludge depends on the nature and characteristics of each single substance: i) very high removal (>80%) of Naproxen, Sulfamethoxazole and Roxithromycin; ii) high removal (60-80%) of Galaxolide, Tonalide and natural estrogens; iii) medium removal (30-60%) of Ibuprofen; iv) low elimination (<40%) of Iopromide, and v) no removal of Carbamazepine (<20%). The elimination of Diazepam, Diclofenac and 17α-ethinylestradiol occurred after sludge adaptation. In general, no influence of SRT and temperature was observed.

xx) The use of pre-treatments (alkaline, thermal and ozonation) leads to COD solubilization percentages between 55 and 80%, the highest values being obtained when applying the alkaline process. Therefore, the biogas productions and soluble organic matter removal efficiencies during anaerobic digestion of pretreated sludge are higher. However, the

elimination of solids and total COD stays in the same range, with small differences depending on the SRT or type of pre-treatment.

xxi) Concerning PPCPs, the elimination of Naproxen, Iopromide and Sulfamethoxazole was not affected by the sludge pre-treatments. In contrast, Carbamazepine was only removed when the ozone process was applied. For the other substances, small influences were observed: i) Alkaline process affected negatively the removal of Roxithromycin in mesophilic range; ii) Thermal treatment affected positively Ibuprofen elimination under mesophilic conditions; and, iii) Ozonation process affected positively the removal of musks in the mesophilic digester and negatively the elimination of Tonalide (in thermophilic range) and Ibuprofen (in both digesters). Once again, the removal of Diazepam, Diclofenac and estrogens was more related with sludge adaptation than with the operational conditions in the digesters.

xxii) The digested sludge is suitable for agronomic purposes (20-50% of organic matter and 2-10% of nutrients). The concentrations of heavy metals were below the current legal requirements and also below the more stringent limit values proposed in the Working Document on Sludge. The hygienic properties were better for thermophilic (Class A) than for mesophilic (Class B) conditions. The LAS content (90-550 $mg \cdot kg^{-1}$) was much lower than the proposed limit value (2,600 $mg \cdot kg^{-1}$) and the dewatering properties were improved after all treatments, except for the ozonation process.

To sum up, municipal STPs represent a key point between the anthropogenic activities and the environment. Mass balance calculations indicate where efforts must be made in order to reduce the amounts of PPCPs being discharged into the environment. For sorptive substances, they should be focused in the sludge treatment. This work shows a significant removal of some PPCPs during anaerobic digestion of sewage sludge. On the contrary, for non-sorptive substances, a tertiary treatment of the final effluent must be considered.

Finally, a hazard-assessment is needed to, on one hand, determine the toxicological relevance of exposure to trace quantities of PPCPs, and on the other hand, to know the safe concentration levels (no effect). This fact would let know the removal efficiencies to be achieved in the STPs in order to reach those safe concentration levels.

Conclusiones

Recientemente ha aparecido una preocupación creciente sobre la presencia de productos farmacéuticos y de cuidado personal (Pharmaceutical and Personal Care Products, PPCPs) en el medioambiente y sobre los efectos desconocidos a largo plazo sobre los organismos acuáticos y la salud humana. En el presente trabajo se ha estudiado la presencia y el comportamiento de los PPCPs en una Estación depuradora de aguas residuales urbanas (EDAR), prestando especial atención al tratamiento de lodos mediante digestión anaerobia. A continuación, se presentan los resultados obtenidos y las principales conclusiones:

Estación depuradora de aguas residuales urbanas

i) Se han detectado 8 de los 13 compuestos seleccionados en este trabajo a niveles de $\mu g \cdot L^{-1}$ tanto en el influente como en el efluente de la EDAR: Galaxolide, Tonalide, Ibuprofen, Naproxen, Iopromide, Sulfamethoxazol, Estrona y 17β-estradiol. Las otras sustancias (Carbamazepina, Diazepam, Diclofenac, Roxithromicina y 17α-ethinylestradiol), bien no se detectaron o por debajo del límite de cuantificación. Se observó un aumento de las concentraciones a la entrada del sedimentador primario en comparación con el influente de la planta para algunos compuestos, lo que indica la contribución de las corrientes de reciclaje procedentes de los procesos de tratamiento de lodos o la ruptura de formas glucurónicas.

ii) La mayoría de las sustancias detectadas no son eliminadas completamente en la EDAR, siendo por lo tanto emitidas a las aguas receptoras.

iii) Se observó una mayor eliminación en el tratamiento biológico que en el tratamiento primario, aunque el grado de reducción depende de cada sustancia por separado.

iv) Las fragancias (Galaxolide y Tonalide) se eliminan tanto durante el tratamiento primario como biológico con eficacias del 90%, fundamentalmente debido a procesos de absorción.

v) Los antiinflamatorios (Ibuprofen y Naproxen) se eliminan significativamente durante el tratamiento biológico con eficacias entre 40 y 65%. Debido a su naturaleza hidrofílica, su eliminación es principalmente debida a la degradación microbiana.

vi) Sulfamethoxazol se elimina notablemente (alrededor del 60%) durante el tratamiento biológico, siendo su eliminación principalmente debida a la degradación microbiana.

vii) En este trabajo se observó una alta persistencia de Iopromide, ya que no se elimina a lo largo del tratamiento en la planta.

viii) En cuanto a los estrógenos naturales, se obtuvo una eliminación significativa de E2 (alrededor del 65%), mientras que las concentraciones de E1 aumentan a lo largo del tratamiento, debido probablemente a la ruptura de formas glucurónicas y a la oxidación parcial de E2.

ix) Los mecanismos de eliminación de PPCPs en EDAR dependen de sus propiedades físico-químicas. Volatilización es despreciable debido a los bajos valores de los coeficientes de Henry (hasta un 5% para las fragancias). Por lo tanto, los principales mecanismos responsables de la eliminación de PPCPs en EDAR son la adsorción/absorción y la degradación.

x) Para diferenciar entre absorción/adsorción y degradación, es necesario realizar balances de materia a lo largo de las diferentes unidades de tratamiento de las EDAR, incluyendo la fase sólida. La selección del método de cálculo para determinar la fracción asociada al lodo es crucial para compuestos con gran afinidad por los sólidos, ya que una estimación imprecisa de este parámetro puede llevar a confusiones en el establecimiento del mecanismo responsable de su eliminación.

xi) El método I, que usa concentraciones medidas en la fase sólida, parece adecuado para la realización de balances de materia de compuestos con alta afinidad por los sólidos, tales como las fragancias. Sin embargo, deben ser considerados los errores inherentes de la metodología analítica utilizada.

xii) Para sustancias hidrofílicas, tales como los compuestos farmacéuticos, es adecuado el método II, que usa los coeficientes de distribución sólido-líquido (K_d) para calcular las concentraciones en el lodo. De este modo, se evitarían los análisis de la fase sólida.

xiii) Las fragancias se eliminan principalmente por absorción (aunque también se degradan) y los compuestos farmacéuticos, que son más polares, son fundamentalmente degradados en la planta.

Procesos físico-químicos

xiv) Procesos de coagulación-floculación pueden aplicarse con éxito para la eliminación de Galaxolide, Tonalide y Diclofenac (70%), y en menor medida para la eliminación de Naproxen y Diazepam (20%). Carbamazepina e Ibuprofen no se ven afectados.

xv) No se observó influencia ni de la temperatura ni de la dosis de coagulante sobre la eliminación de ningún compuesto, resultando el $FeCl_3$ el aditivo más eficaz.

xvi) En los ensayos de flotación, todos los PPCPs seleccionados se eliminan en cierta medida (20-60%), lográndose las eficacias más altas para las fragancias. Esta eliminación es mayor en aguas residuales con un alto contenido en grasa.

Tratamiento anaerobio de lodos

xvii) En el tratamiento de lodos, el valor límite por debajo del cual la adsorción/absorción de PPCPs es despreciable es $K_d<1 L \cdot kgTSS^{-1}$ aproximadamente, mucho menor que el establecido en el tratamiento de aguas residuales ($K_d<500\ L \cdot kgTSS^{-1}$).

xviii) La eliminación de sólidos y materia orgánica varía entre 50 y 70% en ambos digestores. El grado de estabilización del lodo aumenta operando a SRT altos, independientemente de la temperatura de operación. Condiciones termófilas mejoran ligeramente el grado de estabilización del lodo en comparación con las condiciones mesófilas.

xix) El comportamiento de los diferentes PPCPs durante la digestión anaerobia convencional de lodos depende de la naturaleza y características de cada sustancia por separado: i) eliminación muy alta (>80%) de Naproxen, Sulfamethoxazol y Roxithromicina; ii) eliminación alta (60-80%) de Galaxolide, Tonalide y los estrógenos naturales; iii) eliminación media (30-60%) de Ibuprofen; iv) eliminación baja (<40%) de Iopromide; y, v) no eliminación de Carbamazepina (<20%). La eliminación de Diazepam, Diclofenac y 17α-ethinylestradiol tiene lugar tras un proceso de adaptación

del lodo. En general, no se observó influencia ni de la temperatura ni del SRT.

xx) El uso de pretratamientos (alcalino, térmico y ozonización) conduce a porcentajes de solubilización de materia orgánica entre 55 y 80%, alcanzándose los valores más altos con el tratamiento alcalino. Por lo tanto, la producción de biogás y las eficacias de eliminación de materia orgánica soluble durante el proceso de digestión anaerobia son más altas. Sin embargo, las eliminaciones de sólidos y materia orgánica particulada permanecen en el mismo rango, con pequeñas diferencias dependiendo del SRT o del tipo de pretratamiento.

xxi) En lo que se refiere a PPCPs, la eliminación de Naproxen, Iopromide y Sulfamethoxazol no se ve afectada por los pretratamientos. Por el contrario, Carbamazepina solo se elimina cuando se aplica el tratamiento con ozono. Para las otras sustancias, se observan pequeñas diferencias: i) El tratamiento alcalino influye negativamente en la eliminación de Roxithromicina en rango mesófilo; ii) El tratamiento térmico influye positivamente en la eliminación de Ibuprofen en rango mesófilo; y, iii) Ozonización influye positivamente en la eliminación de fragancias en rango mesófilo y negativamente en la eliminación de Tonalide (en rango termófilo) y de Ibuprofen (en ambos digestores). De nuevo, la eliminación de Diazepam, Diclofenac y estrógenos está más relacionada con la adaptación del lodo que con las condiciones de operación en los digestores.

xxii) El lodo digerido es adecuado para fines agronómicos (20-50% de materia orgánica y 2-10% de nutrientes). Las concentraciones de metales pesados están por debajo de los límites legales actuales y también por debajo de los límites más estrictos propuestos en el Documento de Trabajo sobre lodos. Se obtienen mejores propiedades higiénicas en rango termófilo (Clase A) que en mesófilo (Clase B). El contenido en LAS (90-550 $mg \cdot kg^{-1}$) es muy inferior al valor límite propuesto (2,600 $mg \cdot kg^{-1}$) y las propiedades de deshidratación mejoran después de todos los tratamientos, excepto con el proceso de ozonización.

Resumiendo, las EDAR urbanas representan un punto crucial entre la contaminación de origen antropogénico y el medioambiente. La realización de balances de materia indica dónde deben centrarse los esfuerzos para reducir las cantidades de PPCPs emitidas al medioambiente. Para compuestos con gran

afinidad por los sólidos, estos esfuerzos deben estar enfocados en el tratamiento de lodos. Este trabajo indica eliminaciones importantes para algunas sustancias durante la digestión anaerobia de lodos. Por el contrario, para sustancias con tendencia a permanecer en la fase líquida, el tratamiento terciario del efluente final es la opción a considerar.

Por último, es necesaria una valoración de riesgo para, por un lado, determinar la relevancia toxicológica de la exposición a concentraciones traza de PPCPs, y por otro, para conocer los niveles de concentración seguros (sin riesgo). Esto permitiría conocer las eficacias de eliminación necesarias en EDAR para alcanzar dichos niveles de concentración sin riesgo.

Conclusións

Recentemente apareceu unha preocupación crecente sobre a presencia de productos farmacéuticos e de coidado persoal (Pharmaceutical and Personal Care Products, PPCPs) no medioambiente e sobre os efectos descoñecidos a longo prazo sobre os organismos acuáticos e a saúde humana. No presente traballo estudiouse a presencia e o comportamento dos PPCPs nunha Estación depuradora de augas residuais urbanas (EDAR), prestando especial atención ó tratamento dos lodos mediante dixestión anaerobia. A continuación, preséntanse os resultados obtidos e as principais conclusións:

Estación depuradora de augas residuais urbanas

i) Detectáronse 8 dos 13 compostos seleccionados neste traballo a niveis de $\mu g \cdot L^{-1}$ tanto no influente como no efluente da EDAR: Galaxolide, Tonalide, Ibuprofen, Naproxen, Iopromide, Sulfamethoxazol, Estrona e 17β-estradiol. As outras sustancias (Carbamazepina, Diazepam, Diclofenac, Roxithromicina e 17α-ethinylestradiol) foron detectadas por debaixo do límite de cuantificación. Observouse un aumento das concentracións á entrada do sedimentador primario en comparación co influente da planta para algúns compostos, o que indica unha contribución das correntes de reciclaxe procedentes dos procesos de tratamento de lodos ou a ruptura das formas glucurónicas.

ii) A maioría das sustancias detectadas non son eliminadas completamente na EDAR, e polo tanto son emitidas ás augas receptoras.

iii) Observouse unha maior eliminación no tratamento biolóxico que no tratamento primario, aínda que o grado de reducción depende de cada sustancia por separado.

iv) As fragancias (Galaxolide e Tonalide) elimínanse tanto durante o tratamento primario como biolóxico con eficacias do 90%, fundamentalmente debido a procesos de absorción.

v) Os antiinflamatorios (Ibuprofen e Naproxen) elimínanse significativamente durante o tratamento biolóxico con eficacias entre 40 e 65%. Debido á súa natureza hidrofílica, a súa eliminación é principalmente debida á degradación microbiana.

vi) Sulfamethoxazol elíminase notablemente (ó redor do 60%) durante o tratamento biolóxico, sendo a súa eliminación principalmente debida á degradación microbiana.

vii) Neste traballo observouse unha alta persistencia de Iopromide, xa que non se elimina ó longo do tratamento na planta.

viii) En canto ós estróxenos naturais, obtívose unha eliminación significativa de E2 (ó redor do 65%), mentres que as concentracións de E1 aumentan ó longo do tratamento, debido probablemente á ruptura de formas glucurónicas e á oxidación parcial de E2.

ix) Os mecanismos de eliminación de PPCPs en EDAR dependen das súas propiedades físico-químicas. Volatilización é desprezable debido ós baixos valores dos coeficientes de Henry (ata un 5% para as fragrancias). Polo tanto, os principais mecanismos responsables da eliminación de PPCPs en EDAR son a adsorción/absorción e a degradación.

x) Para diferenciar entre absorción/adsorción e degradación, é necesario realizar balances de materia ó longo das diferentes unidades de tratamento das EDAR, incluíndo a fase sólida. A selección do método de cálculo para determinar a fracción asociada ó lodo é crucial para compostos con gran afinidade polos sólidos, xa que unha estimación imprecisa deste parámetro pode levar a confusións no establecemento do mecanismo responsable da súa eliminación.

xi) O método I, que usa concentracións medidas na fase sólida, parece adecuado para a realización de balances de masa de compostos con alta afinidade polos sólidos, como son as fragrancias. Sen embargo, deben terse en contan os erros inherentes da metodoloxía analítica utilizada.

xii) Para sustancias hidrofílicas, como son os compostos farmacéuticos, é adecuado o método II, que usa os coeficientes de distribución sólido-líquido (K_d) para calcular as concentracións no lodo. Deste xeito, evitaríanse as análises da fase sólida.

xiii) As fragrancias elimínanse principalmente por absorción (aínda que tamén se degradan) e os compostos farmacéuticos, que son máis polares, son fundamentalmente degradados na planta.

Procesos físico-químicos

xiv) Procesos de coagulación-floculación poden aplicarse con éxito para a eliminación de Galaxolide, Tonalide e Diclofenac (70%), e en menor medida para a eliminación de Naproxen e Diazepam (20%). Carbamazepina e Ibuprofen non se ven afectados.

xv) Non se observou influencia nin da temperatura nin da dose de coagulante sobre a eliminación de ningún composto, resultando o $FeCl_3$ o aditivo máis eficaz.

xvi) Nos ensaios de flotación, tódolos PPCPs seleccionados se eliminan en certa medida (20-60%), acadándose as eficacias máis altas para as fragrancias. Esta eliminación é maior en augas residuais con un alto contido en graxa.

Tratamento anaerobio de lodos

xvii) No tratamento de lodos, o valor límite por debaixo do cal a adsorción/absorción de PPCPs é desprezable é K_d<1L·kgTSS^{-1} aproximadamente, moito máis baixo que o establecido no tratamento de augas residuais (K_d<500 L·kgTSS^{-1}).

xviii) A eliminación de sólidos e materia orgánica varía entre 50 e 70% nos dous dixestores. O grado de estabilización do lodo aumenta cando se traballa a SRT altos, independentemente da temperatura de operación. Condicións termófilas melloran lixeiramente o grado de estabilización do lodo en comparación coas condicións mesófilas.

xix) O comportamento dos diferentes PPCPs durante a dixestión anaerobia convencional de lodos depende da natureza e características de cada sustancia por separado: i) eliminación moi alta (>80%) de Naproxen, Sulfamethoxazol e Roxithromicina; ii) eliminación alta (60-80%) de Galaxolide, Tonalide e dos estróxenos naturais; iii) eliminación media (30-60%) de Ibuprofen; iv) eliminación baixa (<40%) de Iopromide; e, v) non eliminación de Carbamazepina (<20%). A eliminación de Diazepam, Diclofenac e 17α-ethinylestradiol ten lugar tras un proceso de adaptación do lodo. En xeral, non se observou influencia nin da temperatura nin do tempo de retención.

xx) O uso de pretratamentos (alcalino, térmico e ozonización) conduce a porcentaxes de solubilización de materia orgánica entre 55 e 80%, acadándose os valores máis altos co tratamento alcalino. Polo tanto, a producción de biogás e as eficacias de eliminación de materia orgánica soluble durante o proceso de dixestión anaerobia son máis altas. Sen embargo, as eliminacións de sólidos e materia orgánica particulada permanecen no mesmo rango, con pequenas diferencias dependendo do tempo de retención ou do tipo de pretratamento.

xxi) No que se refire ós PPCPs, a eliminación de Naproxen, Iopromide e Sulfamethoxazol non se ve afectada polos pretratamentos. Polo contrario, Carbamazepina só se elimina cando se aplica o tratamento con ozono. Para as outras sustancias, obsérvanse pequenas diferencias: i) O tratamento alcalino inflúe negativamente na eliminación de Roxithromicina en rango mesófilo; ii) O tratamento térmico inflúe positivamente na eliminación de Ibuprofen en rango mesófilo; e, iii) Ozonización inflúe positivamente na eliminación das fragancias en rango mesófilo e negativamente na eliminación de Tonalide (en rango termófilo) e de Ibuprofen (nos dous dixestores). De novo, a eliminación de Diazepam, Diclofenac e estróxenos está máis relacionada coa adaptación do lodo que coas condicións de operación nos dixestores.

xxii) O lodo dixerido é adecuado para fins agronómicos (20-50% de materia orgánica e 2-10% de nutrientes). As concentracións de metais pesados están por debaixo dos límites legais actuais e tamén por debaixo dos límites más estrictos propostos no Documento de Traballo sobre lodos. Obtéñense mellores propiedades hixiénicas no rango termófilo (Clase A) que no mesófilo (Clase B). O contido en LAS (90-550 $mg \cdot kg^{-1}$) é moi inferior ó valor límite proposto (2,600 $mg \cdot kg^{-1}$) e as propiedades de deshidratación melloran despois de tódolos tratamentos, excepto co proceso de ozonización.

Resumindo, as EDAR urbanas representan un punto crucial entre a contaminación de orixe antropoxénico e o medioambiente. A realización de balances de materia indica ónde deben poñerse os esforzos para reducir as cantidades de PPCPs emitidas ó medioambiente. Para compostos con gran afinidade polos sólidos, estes esforzos deben estar enfocados no tratamento de lodos. Este traballo indica eliminacións importantes para algunhas sustancias durante a dixestión anaerobia de lodos. Polo contrario, para sustancias con

tendencia a permanecer na fase líquida, o tratamento terciario do efluente final é a opción a ter en conta.

Para rematar, é necesaria unha valoración de risco para, por un lado, determinar a relevancia toxicolóxica da exposición a concentracións traza de PPCPs, e por outro, para coñecer os niveis de concentración seguros (sen risco). Isto permitiría coñecer as eficacias de eliminación necesarias nas EDAR para acadar eses niveis de concentración sen risco.

Annex I

Sensitivity analysis of PPCPs mass balance

Summary

In this Annex, the influence of the most crucial parameter (the concentration of PPCPs in the feeding) in the mass balance calculations of PPCPs in the anaerobic digesters is analysed.

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1. Inlet concentration

The PPCPs concentration in the feeding (C_{in}) depends on the background content in the sludge (C_{raw}) and the spike (C_{spike}), as indicated in equation I.1.

$$C_{in} = C_{raw} + C_{spike} \qquad \text{I.1}$$

Since the spike has been performed manually and the pilot plant has been working for more than two years, it can be considered that a steady state was achieved, thus remaining this variable constant. Therefore, the background content is the parameter analysed in this Annex and, consequently, only for those substances detected in the STP studied: Galaxolide, Tonalide, Carbamazepine, Ibuprofen, Naproxen, Iopromide, Sulfamethoxazole, Estrone, 17β-estradiol and 17α-ethinylestradiol.

2. Background concentration

To calculate the background content, it must be taken into account that the feeding is a mixture of primary and secondary sludge, thus both contributions should be considered.

The total content of PPCPs in each type of sludge is the sum of the concentrations in the liquid and sludge phase, respectively (Eq. I.2). The PPCPs concentrations in the liquid phase have been measured, but the PPCPs concentrations in the sludge phase (X) have been calculated from those in the liquid phase (S) using the K_d values and the TSS content.

$$C_{raw} = \frac{V_P \cdot S_P \cdot (1 + K_{d,p} \cdot TSS_P) + V_B \cdot S_B \cdot (1 + K_{d,B} \cdot TSS_B)}{V_P + V_B} \qquad \text{I.2}$$

where C_{raw} is the total PPCPs concentration present in the sludge ($\mu g \cdot L^{-1}$),

V_P is the volume of primary sludge (L),

S_P is the dissolved PPCPs concentration in the primary sludge ($\mu g \cdot L^{-1}$),

$K_{d,P}$ is the solid-water distribution coefficient for primary sludge ($L \cdot kg^{-1}$),

TSS_P is the solids concentration in the primary sludge ($kg \cdot L^{-1}$),

V_B is the volume of biological sludge (L),

S_B is the dissolved PPCPs concentration in the biological sludge ($\mu g \cdot L^{-1}$),

$K_{d,B}$ is the solid-water distribution coefficient for biological sludge ($L \cdot kg^{-1}$), and

TSS_B is the solids concentration in the biological sludge ($kg \cdot L^{-1}$).

The influence of the three main parameters involved in the background concentration calculation (S, TSS and K_d) is analysed in the following sections.

2.1. Effect of TSS content

Keeping constant the K_d values and the PPCPs concentrations in the liquid phase, the influence of TSS content in the primary and secondary sludge has been analysed. The results are shown in Table I.1. It can be observed that the minimum and maximum background concentrations correspond to the minimum and maximum TSS content in the primary sludge (70% of feeding mixture).

2.2. Effect of K_d value

Keeping constant the PPCPs concentrations in the liquid phase and considering the minimum, average and maximum TSS content in primary and secondary sludge (Table I.1), the influence of the K_d values has been analysed. Three K_d values (minimum, average and maximum) have been selected among the different information available (Table I.2).

The results are shown in Table I.3. It can be observed that the minimum and maximum background concentrations correspond to the combinations $TSS_{minimum}$-$K_{d,minimum}$ and $TSS_{maximum}$-$K_{d,maximum}$, respectively.

2.3. Effect of PPCP concentration

Keeping constant the TSS content and the K_d values, the influence of PPCPs concentrations in the liquid phase has been analysed. For that purpose, a value 50% lower and higher than that measured was chosen as minimum and maximum, respectively (Table I.4). Concerning the TSS content and the K_d values, the minimum, average and maximum values were selected.

The results are shown in Table I.5. As expected, the maximum value obtained is three times higher than the minimum for the same K_d and TSS content.

A summary of the minimum and maximum background concentrations obtained in the analysis of each parameter (TSS, K_d values and S) is presented in Table I.6.

Table I-1. Influence of TSS content in primary and secondary sludge on PPCPs background concentration (µg·L^{-1}).

	TSS content (g·L^{-1})		Background concentration (µg·L^{-1})									
	Primary	**Biological**	**HHCB**	**AHTN**	**CBZ**	**IBP**	**NPX**	**IPM**	**SMX**	**E1**	**E2**	**EE2**
May 2002	57.1	16.9	1,079	371	0.45	10	9	10	7	0.10	1.01	0.19
June 2002	61.0	17.8	1,151	396	0.47	11	9	10	8	0.11	1.08	0.21
July 2002	73.8	21.0	1,391	477	0.51	13	11	10	9	0.13	1.30	0.25
August 2002	83.2	21.1	1,558	530	0.54	14	12	10	10	0.14	1.46	0.27
September 2002	53.7	20.1	1,031	362	0.44	10	9	10	7	0.10	0.96	0.19
October 2002	60.4	21.1	1,153	402	0.46	11	10	10	8	0.11	1.08	0.21
November 2002	60.9	35.9	1,218	449	0.47	12	11	10	8	0.12	1.13	0.24
December 2002	79.5	35.9	1,548	553	0.53	14	13	11	10	0.14	1.45	0.29
January 2003	79.8	34.3	1,547	550	0.53	14	13	11	10	0.14	1.45	0.29
February 2003	50.5	23.7	987	354	0.43	10	9	10	7	0.10	0.92	0.19
March 2003	117.9	23.7	2,184	732	0.66	18	15	11	14	0.19	2.05	0.37
April 2003	94.0	15.9	1,730	575	0.58	15	12	10	11	0.15	1.63	0.29
May 2003	72.3	19.6	1,359	464	0.50	12	11	10	9	0.12	1.28	0.24
June 2003	59.4	32.8	1,180	432	0.46	11	11	10	8	0.11	1.10	0.23
July 2003	**122.8**	**33.2**	**2,307**	**788**	**0.68**	**19**	**16**	**12**	**15**	**0.21**	**2.17**	**0.41**
August 2003	63.0	24.2	1,211	426	0.47	11	10	10	8	0.11	1.13	0.22
September 2003	63.0	24.2	1,211	426	0.47	11	10	10	8	0.11	1.13	0.22
October 2003	81.0	20.7	1,517	516	0.54	13	12	10	10	0.14	1.42	0.27
November 2003	64.4	21.6	1,226	426	0.48	11	10	10	8	0.11	1.15	0.22
June 2004	55.7	20.9	1,069	375	0.45	10	9	10	7	0.10	1.00	0.20
July 2004	**51.0**	**20.9**	**978**	**343**	**0.43**	**10**	**9**	**9**	**7**	**0.09**	**0.92**	**0.18**
Average	72	24	1,364	474	0.50	12	11	10	9	0.12	1.28	0.25
St. deviation	20	6	361	118	0.07	3	2	1	2	0.03	0.34	0.06
Minimum	51	16	978	343	0.43	10	9	9	7	0.09	0.92	0.18
Maximum	123	36	2,307	788	0.68	19	16	12	15	0.21	2.17	0.41

Table I-2. Minimum, average and maximum log K_d values ($L \cdot kg^{-1}$) for PPCPs in primary and secondary sludge.

PPCPs	Primary sludge			Secondary sludge		
	Minimum	Average	Maximum	Minimum	Average	Maximum
HHCB	4.0	4.2	4.4	4.0	4.1	4.2
AHTN	3.6	4.0	4.4	4.2	4.3	4.4
CBZ	0.1	1.3	1.8	0.1	1.3	1.8
IBP	1.4	1.6	1.8	2.3	2.4	2.5
NPX	1.4	1.6	1.8	2.3	2.4	2.5
IPM	0.5	0.7	1.5	0.5	1.0	1.5
SMX	1.0	2.4	2.6	1.0	2.4	2.6
E1	2.7	2.8	2.9	2.8	2.9	3.0
E2	3.9	4.0	4.1	3.5	4.0	4.5
EE2	3.3	3.6	3.9	3.1	3.8	4.5

Table I-4. Minimum, average and maximum PPCPs concentration in the aqueous phase ($\mu g \cdot L^{-1}$) of primary and secondary sludge.

PPCPs	Primary sludge			Secondary sludge		
	Minimum	Average	Maximum	Minimum	Average	Maximum
HHCB	0.80	1.60	2.40	0.50	1.00	1.50
AHTN	0.40	0.80	1.20	0.25	0.50	0.75
CBZ	0.13	0.25	0.38	0.13	0.25	0.38
IBP	2.15	4.30	6.45	0.20	0.40	0.60
NPX	1.60	3.20	4.80	0.55	1.10	1.65
IPM	3.70	7.20	11.90	4.40	8.80	13.20
SMX	0.32	0.64	0.96	0.13	0.25	0.38
E1	0.002	0.003	0.005	0.001	0.003	0.004
E2	0.001	0.002	0.004	0.001	0.001	0.002
EE2	0.001	0.001	0.002	0.001	0.001	0.002

Table I-6. Minimum and maximum PPCPs background concentrations ($\mu g \cdot L^{-1}$) obtained in the sensitivity analysis.

PPCPs	TSS influence		K_d influence		S influence	
	Min.	Max.	Min.	Max.	Min.	Max.
HHCB	978	2,307	621	3,633	310	5,449
AHTN	343	788	152	1,867	76	2,800
CBZ	0.43	0.68	0.26	1,78	0.14	2.70
IBP	10	19	7	28	4	42
NPX	9	16	6	24	3	36
IPM	9	12	9	30	4	46
SMX	7	15	1	24	0.38	35
E1	0.09	0.21	0.07	0.26	0.04	0.40
E2	0.92	2.17	0.70	2.95	0.35	4.42
EE2	0.18	0.41	0.08	1.03	0.04	1.54

Table I-3. Influence of K_d values for primary and secondary sludge on PPCPs background concentration ($\mu g \cdot L^{-1}$).

K_d[1]	TSS[2]	Background concentration ($\mu g \cdot L^{-1}$)									
		HHCB	**AHTN**	**CBZ**	**IBP**	**NPX**	**IPM**	**SMX**	**E1**	**E2**	**EE2**
Minimum	Minimum	**621**	**152**	**0.26**	**7**	**6**	**9**	**1**	**0.07**	**0.70**	**0.08**
	Average	869	216	0.27	9	8	9	1	0.10	0.97	0.11
	Maximum	1,487	361	0.28	13	12	10	1	0.17	1.68	0.19
Average	Minimum	967	334	0.43	10	8	9	7	0.09	0.91	0.17
	Average	1,352	470	0.50	12	11	10	9	0.12	1.27	0.24
	Maximum	2,321	797	0.68	19	17	12	15	0.21	2.18	0.41
Maximum	Minimum	1,512	778	0.89	13	1	17	10	0.11	1.23	0.44
	Average	2,113	1,090	1.15	18	15	21	14	0.16	1.73	0.62
	Maximum	**3,633**	**1,867**	**1.78**	**28**	**24**	**30**	**24**	**0.26**	**2.95**	**1.03**
Average		1,653	674	0.69	14	13	14	9	0.14	1.51	0.37
St. deviation		926	543	0.51	6	5	7	8	0.06	0.71	0.31
Minimum		621	152	0.26	7	6	9	1	0.07	0.70	0.08
Maximum		3,633	1,867	1.78	28	24	30	24	0.26	2.95	1.03

[1]See Table I.2 for K_d values; [2]See Table I.1 for TSS concentrations.

Table I-5. Influence of PPCPs dissolved concentrations (S) on PPCPs background concentration (µg·L^{-1}).

K_d[1] TSS[2]	S[3] (µg·L^{-1})	Background concentration (µg·L^{-1})									
		HHCB	**AHTN**	**CBZ**	**IBP**	**NPX**	**IPM**	**SMX**	**E1**	**E2**	**EE2**
Minimum	Minimum	**310**	**76**	**0.14**	**4**	**3**	**4**	**0.38**	**0.04**	**0.35**	**0.04**
	Average	621	152	0.26	7	6	9	0.76	0.07	0.70	0.08
	Maximum	931	229	0.40	11	10	13	1.15	0.11	1.05	0.12
Average	Minimum	676	235	0.26	7	5	5	4.49	0.06	0.63	0.12
	Average	1,352	470	0.50	12	11	10	8.96	0.12	1.27	0.24
	Maximum	2,029	705	0.76	18	16	15	13.46	0.19	1.90	0.37
Maximum	Minimum	1,816	933	0.96	15	12	15	11.79	0.13	1.47	0.51
	Average	3,633	1,867	1.78	29	24	30	23.54	0.26	2.95	1.03
	Maximum	**5,449**	**2,800**	**2.70**	**42**	**36**	**46**	**35.33**	**0.40**	**4.42**	**1.54**
Average		1,869	830	0.86	16	14	16	11	0.15	1.64	0.45
St. deviation		1,678	926	0.86	12	10	13	12	0.11	1.30	0.51
Minimum		310	76	0.14	4	3	4	0.38	0.04	0.35	0.04
Maximum		5,449	2,800	2.70	42	36	46	35	0.40	4.42	1.51

[1]See Table I.2 for K_d values; [2]See Table I.1 for TSS concentrations; [3]See Table I.4 for PPCPs concentrations.

3. Effect of background concentration on PPCPs mass balance

In this section, the influence of the background concentration in the sludge on PPCPs mass balance results (i.e. removal during anaerobic digestion) was analysed. For that purpose, the mass balances were carried out with the minimum and maximum background concentrations calculated in the previous section (Table I.6) and considering average values for the outlet parameters (Table I.7).

Table I-7. Average values of the outlet parameters (TSS, S and X) used in the mass balance calculations.

	Mesophilic		**Thermophilic**	
TSS ($g \cdot L^{-1}$)	36		40	
	S ($\mu g \cdot L^{-1}$)	**X ($\mu g \cdot g^{-1}$)**	**S ($\mu g \cdot L^{-1}$)**	**X ($\mu g \cdot g^{-1}$)**
HHCB	1.3	16.7	1.9	14.6
AHTN	0.6	8.1	1.1	6.9
CBZ	8.3	0.4	9.8	0.3
IBP	7.2	0.2	7.1	0.2
NPX	1.7	0.1	1.2	0.1
IPM	28.9	0.3	24.8	0.3
SMX	0.2	0.01	0.4	0.01
E1+E2	0.5	0.20	0.3	0.14
EE2	0.1	0.02	0.1	0.02

Table I.8 shows the results obtained as well as the average values showed in Chapter 6 and 7.

Table I-8. PPCPs removal efficiencies (%) during sludge anaerobic digestion calculated with the minimum and maximum background concentrations and average values obtained in Chapter 6 and 7.

PPCPs	**Background**		**Chapter 6 and 7**
	Min.	**Max.**	
HHCB	15	90	65 - 85
AHTN	0	90	30 - 80
CBZ	0	0	0
IBP	0	70	20 - 50
NPX	72	92	85 - 90
IPM	20	50	10 - 30
SMX	>98	>98	>98
E1+E2	40	60	>50
EE2	>75	>85	>60

From Table I.8, it can be concluded that only three compounds are affected by the background concentration: Galaxolide, Tonalide and Ibuprofen. This fact can be explained taking into account the ratio between the background concentration and the spike. For Galaxolide and Tonalide, this ratio is 4 and 2, respectively, and for Ibuprofen, it is 1.

The minimum and maximum background concentrations lead to no or very high removal of these substances, respectively. However, it should be taken into account that these conditions are really extreme with punctual occurrence in the STP. Therefore, an average value between both extremes (minimum and maximum) should be considered as the removal efficiency achieved for these substances, which fits with the results showed in Chapter 6 and 7.

Annex II

Soluble and sorbed concentrations of PPCPs in the digested sludge

Summary

In this Annex, a summary of PPCPs concentrations in the liquid and solid phase of digested sludge is presented. The values highlighted did not fit the statistical selection described in section 6.2.5.

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1. Galaxolide	**II-3**
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1. Galaxolide

Table II-1. Concentrations of Galaxolide in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		***S* (μg·L^{-1})**	***X* (μg·g^{-1})**	***S* (μg·L^{-1})**	***X* (μg·g^{-1})**
Conventional	30 (M); 20 (T)	1.6	32	1.4	14
		1.6	27	2.1	21
		3.5	22	2.7	19
		-	-	0.9	25
	20 (M); 10 (T)	0.7	29	2.6	22
		1.2	21	3.9	11
		1.2	14	1.7	9
	10 (M); 6 (T)	1.8	14	2.6	9
		2.1	-	2.3	-
Alkaline	20 (M); 10 (T)	0.9	15	1.6	11
		0.9	17	1.7	17
	10 (M); 6 (T)	0.9	13	0.8	12
Thermal	20 (M); 10 (T)	1.0	13	1.8	10
		2.0	18	1.9	13
	10 (M); 6 (T)	1.3	19	1.4	20
Ozonation	20 (M); 10 (T)	0.6	-	2.0	-
		0.7	-	2.3	-

*M: Mesophilic digester; T: Thermophilic digester.

2. Tonalide

Table II-2. Concentrations of Tonalide in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		***S* (µg·L^{-1})**	***X* (µg·g^{-1})**	***S* (µg·L^{-1})**	***X* (µg·g^{-1})**
Conventional	30 (M); 20 (T)	0.6	14	0.5	7
		0.8	12	1.1	12
		1.9	12	1.2	11
		-	-	0.3	10
	20 (M); 10 (T)	0.3	8	1.5	5
		0.4	7	3.0	4
		0.4	4	0.5	3
	10 (M); 6 (T)	0.9	7	2.0	4
		1.1	-	2.7	-
Alkaline	20 (M); 10 (T)	0.4	8	0.8	5
		0.4	9	0.8	9
	10 (M); 6 (T)	0.5	7	0.4	6
Thermal	20 (M); 10 (T)	0.5	7	0.9	5
		1.0	9	0.8	6
	10 (M); 6 (T)	0.6	11	0.7	10
Ozonation	20 (M); 10 (T)	0.2	-	1.4	-
		0.3	-	1.5	-

*M: Mesophilic digester; T: Thermophilic digester.

3. Carbamazepine

Table II-3. Concentrations of Carbamazepine in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		***S* ($\mu g \cdot L^{-1}$)**	***X* ($\mu g \cdot g^{-1}$)**	***S* ($\mu g \cdot L^{-1}$)**	***X* ($\mu g \cdot g^{-1}$)**
Conventional	30 (M); 20 (T)	15	-	13	-
		16	-	12	-
		15	-	15	-
		-	-	12	-
	20 (M); 10 (T)	19	-	13	-
		11	-	15	-
		15	-	14	-
	10 (M); 6 (T)	8	0.28	9	0.18
		23	-	29	-
Alkaline	20 (M); 10 (T)	5	-	10	-
		7	0.43	7	0.27
	10 (M); 6 (T)	6	-	12	-
Thermal	20 (M); 10 (T)	7	-	9	-
		8	0.33	9	0.33
	10 (M); 6 (T)	3	-	8	-
Ozonation	20 (M); 10 (T)	8	0.38	5	-
		6	0.41	4	0.15

*M: Mesophilic digester; T: Thermophilic digester.

4. Diazepam

Table II-4. Concentrations of Diazepam in the aqueous phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		S (µg·L^{-1})	*X (µg·g^{-1})*	*S (µg·L^{-1})*	*X (µg·g^{-1})*
Conventional	30 (M); 20 (T)	11	-	8	-
		9	-	8	-
		14	-	12	-
		-	-	13	-
	20 (M); 10 (T)	7	-	12	-
		9	-	13	-
		7	-	7	-
	10 (M); 6 (T)	4	-	4	-
		5	-	9	-
Alkaline	20 (M); 10 (T)	2.6	-	4.7	-
		3.3	-	5.8	-
	10 (M); 6 (T)	2.6	-	4.9	-
Thermal	20 (M); 10 (T)	2.6	-	5.8	-
		5.4	-	5.5	-
	10 (M); 6 (T)	2.3	-	4.7	-
Ozonation	20 (M); 10 (T)	5.3	-	4.8	-
		7.3	-	5.5	-

*M: Mesophilic digester; T: Thermophilic digester.

5. Ibuprofen

Table II-5. Concentrations of Ibuprofen in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		***S* (μg·L^{-1})**	***X* (μg·g^{-1})**	***S* (μg·L^{-1})**	***X* (μg·g^{-1})**
Conventional	30 (M); 20 (T)	7	0.42	5	< 0.05**
		6	0.30	5	0.15
		11	0.26	7	0.20
		-	-	7	0.29
	20 (M); 10 (T)	7	0.26	9	0.15
		6	0.22	7	0.15
		5	0.17	7	0.07
	10 (M); 6 (T)	5	0.10	8	0.08
		5	-	7	-
Alkaline	20 (M); 10 (T)	10	0.21	12	0.19
		10	0.28	13	0.34
	10 (M); 6 (T)	7	0.08	7	0.13
Thermal	20 (M); 10 (T)	4	0.13	5	0.18
		8	0.13	4	0.20
	10 (M); 6 (T)	6	0.12	6	0.18
Ozonation	20 (M); 10 (T)	9	0.21	8	0.29
		9	0.35	5	0.26

*M: Mesophilic digester; T: Thermophilic digester. **Limit of Quantification (LOQ).

6. Naproxen

Table II-6. Concentrations of Naproxen in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		S (µg·L^{-1})	*X (µg·g^{-1})*	*S (µg·L^{-1})*	*X (µg·g^{-1})*
Conventional	30 (M); 20 (T)	3.3	-	0.5	-
		2.0	-	0.8	-
		2.2	-	0.6	-
		-	-	1.1	-
	20 (M); 10 (T)	2.4	-	1.1	-
		1.2	-	0.9	-
		1.3	-	0.6	-
	10 (M); 6 (T)	1.9	0.02	2.4	0.03
		1.9	-	2.0	-
Alkaline	20 (M); 10 (T)	1.8	0.03	1.3	0.03
		1.8	0.03	1.5	0.03
	10 (M); 6 (T)	2.0	0.03	1.1	0.03
Thermal	20 (M); 10 (T)	1.1	0.03	0.3	0.03
		1.3	0.03	0.6	0.03
	10 (M); 6 (T)	1.3	0.03	1.4	0.03
Ozonation	20 (M); 10 (T)	8.8	-	12.5	-
		8.4	-	9.1	-

*M: Mesophilic digester; T: Thermophilic digester.

7. Diclofenac

Table II-7. Concentrations of Diclofenac in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		S (μg·L^{-1})	*X (μg·g^{-1})*	*S (μg·L^{-1})*	*X (μg·g^{-1})*
Conventional	30 (M); 20 (T)	4.6	0.30	0.1	0.28
		2.5	0.24	1.4	0.17
		4.2	0.21	3.7	0.09
		-	-	1.4	0.21
	20 (M); 10 (T)	3.4	0.26	4.2	0.11
		3.3	0.28	4.1	0.21
		3.9	0.24	8.3	0.19
	10 (M); 6 (T)	1.2	0.03	0.8	0.03
		1.1	-	1.3	-
Alkaline	20 (M); 10 (T)	3.3	0.17	4.5	0.14
		3.6	0.20	4.2	0.19
	10 (M); 6 (T)	1.7	0.03	1.1	0.04
Thermal	20 (M); 10 (T)	2.7	0.13	1.6	0.11
		4.9	0.12	2.0	0.18
	10 (M); 6 (T)	2.1	0.08	1.1	0.06
Ozonation	20 (M); 10 (T)	0.5	0.10	0.5	0.10
		0.5	0.10	0.5	0.10

*M: Mesophilic digester; T: Thermophilic digester.

8. Iopromide

Table II-8. Concentrations of Iopromide in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		***S* (µg·L^{-1})**	***X* (µg·g^{-1})**	***S* (µg·L^{-1})**	***X* (µg·g^{-1})**
Conventional	30 (M); 20 (T)	24	-	4	-
		25	-	11	-
		28	-	-	-
		-	-	-	-
	20 (M); 10 (T)	33	-	29	-
		63	-	31	-
		34	-	23	-
	10 (M); 6 (T)	33	0.23	17	0.05
		63	-	33	-
Alkaline	20 (M); 10 (T)	24	-	27	-
		32	0.51	36	0.61
	10 (M); 6 (T)	30	-	21	-
Thermal	20 (M); 10 (T)	25	-	12	-
		29	0.15	24	0.23
	10 (M); 6 (T)	26	-	19	-
Ozonation	20 (M); 10 (T)	33	0.36	11	-
		25	0.58	4	0.13

*M: Mesophilic digester; T: Thermophilic digester.

9. Sulfamethoxazol

Table II-9. Concentrations of Sulfamethoxazol in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		***S* (μg·L^{-1})**	***X* (μg·g^{-1})**	***S* (μg·L^{-1})**	***X* (μg·g^{-1})**
Conventional	30 (M); 20 (T)	0.39	-	5.40	-
		0.05**	-	4.10	-
		0.05**	-	1.20	-
		-	-	-	-
	20 (M); 10 (T)	0.05	-	0.09	-
		0.04	-	0.08	-
		0.08	-	0.09	-
	10 (M); 6 (T)	0.35	0.008**	0.53	0.008**
		0.20	-	0.20	-
Alkaline	20 (M); 10 (T)	0.18	-	0.55	-
		0.13	0.008	0.15	0.008
	10 (M); 6 (T)	0.22	-	0.15	-
Thermal	20 (M); 10 (T)	0.28	-	0.12	-
		0.83	0.008	1.37	0.008
	10 (M); 6 (T)	0.28	-	0.52	-
Ozonation	20 (M); 10 (T)	0.10	0.016	0.10	0.016
		0.10	0.016	0.10	0.016

*M: Mesophilic digester; T: Thermophilic digester. **Limit of Quantification (LOQ).

10. Roxithromycin

Table II-10. Concentrations of Roxithromycin in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		***S* (µg·L^{-1})**	***X* (µg·g^{-1})**	***S* (µg·L^{-1})**	***X* (µg·g^{-1})**
Conventional	30 (M); 20 (T)	0.05**	-	0.05**	-
		0.05**	-	0.05**	-
		0.05**	-	0.05**	-
		-	-	-	-
	20 (M); 10 (T)	1.00	-	0.10	-
		0.54	-	0.75	-
		1.01	-	0.49	-
	10 (M); 6 (T)	11.89	0.99	18.20	0.25
		8.60	-	8.63	-
Alkaline	20 (M); 10 (T)	0.13	-	2.39	-
		0.16	0.96	20.26	1.22
	10 (M); 6 (T)	2.46	-	12.87	-
Thermal	20 (M); 10 (T)	0.04	-	2.47	-
		2.19	0.18	22.91	0.68
	10 (M); 6 (T)	0.07	-	0.62	-
Ozonation	20 (M); 10 (T)	-	-	-	-
		-	-	-	-

*M: Mesophilic digester; T: Thermophilic digester. **Limit of Quantification (LOQ).

11. Estrone + 17β-estradiol

Table II-11. Concentrations of natural estrogens in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		***S* (μg·L^{-1})**	***X* (μg·g^{-1})**	***S* (μg·L^{-1})**	***X* (μg·g^{-1})**
Conventional	30 (M); 20 (T)	0.436	0.048	0.690	0.073
		0.436	0.192	0.692	0.065
		0.624	0.102	1.130	0.004
		-	-	-	-
	20 (M); 10 (T)	1.158	-	2.720	-
		1.660	-	1.762	-
		0.062	-	1.994	-
	10 (M); 6 (T)	0.111	0.017	0.333	0.030
		-	-	-	-
Alkaline	20 (M); 10 (T)	0.753	0.240	1.105	0.250
		1.369	0.210	1.526	0.280
	10 (M); 6 (T)	0.106	0.016	0.181	0.022
Thermal	20 (M); 10 (T)	0.464	0.164	0.257	0.106
		0.653	0.218	0.331	0.128
	10 (M); 6 (T)	0.022	0.010	0.143	0.032
Ozonation	20 (M); 10 (T)	0.263	0.085	0.182	0.045
		0.220	0.091	0.270	0.048

*M: Mesophilic digester; T: Thermophilic digester.

12. 17α-ethinylestradiol

Table II-12. Concentrations of 17α-ethinylestradiol in the aqueous and sludge phase of mesophilic and thermophilic digested sludge.

	SRT* (d)	Mesophilic digester		Thermophilic digester	
		S (µg·L^{-1})	***X (µg·g^{-1})***	***S (µg·L^{-1})***	***X (µg·g^{-1})***
Conventional	30 (M); 20 (T)	0.350	0.332	0.734	0.088
		0.330	0.356	0.516	0.063
		0.310	0.082	0.606	0.002**
		-	-	-	-
	20 (M); 10 (T)	0.640	-	1.860	-
		0.740	-	1.920	-
		0.003	-	1.900	-
	10 (M); 6 (T)	0.015	0.009	0.049	0.008**
		-	-	-	-
Alkaline	20 (M); 10 (T)	0.266	0.279	0.435	0.197
		0.638	0.194	0.585	0.362
	10 (M); 6 (T)	0.034	0.016	0.044	0.008
Thermal	20 (M); 10 (T)	0.206	0.236	0.107	0.158
		0.314	0.316	0.180	0.300
	10 (M); 6 (T)	0.098	0.070	0.030	0.036
Ozonation	20 (M); 10 (T)	0.100	0.020	0.100	0.020
		0.100	0.020	0.100	0.020

*M: Mesophilic digester; T: Thermophilic digester. **Limit of Quantification (LOQ).

Table II-13. Improvement of EE2 results from K_d values.

		SRT	***S (µg·L^{-1})***	***X (µg·g^{-1})***	***Removal (%)***
Mesophilic	**Alkaline**	20 d	0.266	*0.067*	No
	Thermal	20 d	0.206	*0.052*	No
			0.314	*0.079*	21.8
Thermophilic	**Alkaline**	10 d	0.435	*0.109*	No
			0.585	*0.147*	No
	Thermal	10 d	0.107	*0.027*	62.7
			0.180	*0.045*	38.5
		6 d	*0.143*	0.036	60.1

Calculated values in italics.