Chapter I

THE BIODIGESTION PROCESS

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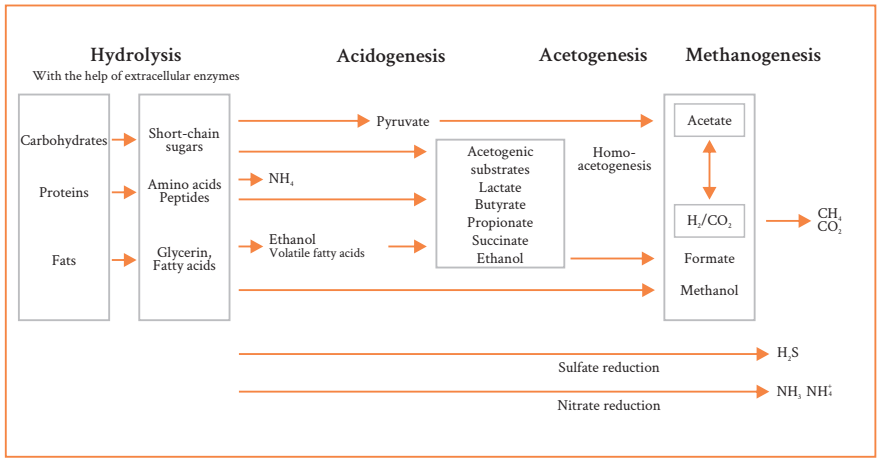
Introduction

Anaerobic digestion is a complex metabolic process that requires anaerobic conditions (redox potential ≤ 200 mV) and depends on the joint activity of an association of microorganisms to transform organic material into carbon dioxide and methane. The process can be divided into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each phase is performed by different groups of microorganisms, in syntrophy, and may require different environmental conditions (Figure 1).



Source: Adapted from Deublein and Steinhauser (2011).

Figure 1. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis processes.

Hydrolysis

The hydrolysis phase degrades high molecular weight compounds, such as lipids, polysaccharides, and proteins into simpler (monomers) and soluble organic substances. This process occurs through the action of extracellular enzymes excreted by hydrolytic bacteria. The importance of the hydrolysis phase in the degradation rate depends on the characteristic of the involved substrate. Hydrolysis is of great importance in the global degradation rate when the organic matter present is complex and difficult to degrade, being considered a limiting step in the anaerobic digestion rate. The duration of the hydrolysis phase varies according to the substrate characteristics, that is, a few hours for carbohydrates and a few days for proteins and lipids. Lignocellulose and lignin are hydrolyzed more slowly, often incompletely.

Acidogenesis

Monomers formed in the hydrolytic phase are used as substrates by different anaerobic and facultative bacteria, being degraded in the acidogenic phase into short-chain organic acids, molecules with 1 to 5 carbons (e.g., butyric, propionic, and acetic acids), alcohols, nitrogen

oxides, hydrogen sulfide, hydrogen, and carbon dioxide. The hydrogen partial pressure during the process directly affects the oxidation state of the products. If it is too high, it will result in products with a higher amount of carbon.

Carbohydrates such as glucose are broken down to pyruvate in acidogenesis. This product is converted into lactic acid by *Lactobacillales* and ethanol by the action of yeasts. Fatty acids are degraded, for example, by *Acetobacter* by β-oxidation. Therefore, fatty acids must be linked to coenzyme A and oxidation occurs step by step through the sequential release of two carbon units in the form of acetate. Amino acids are degraded in pairs by *Clostridium botulinum* through the Stickland reaction (Figure 2), in which one amino acid serves as an electron donor and another as an acceptor. This reaction results in the formation of acetate, ammonia, carbon dioxide, and hydrogen sulfide.

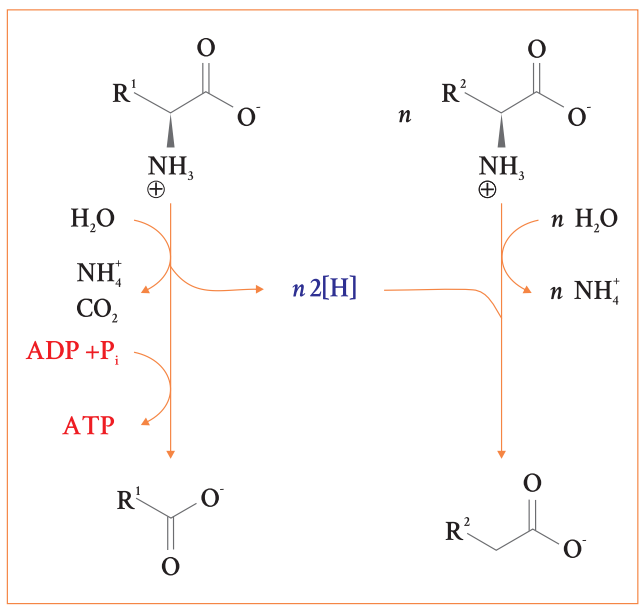


Figure 2. Example of a Stickland reaction, in which a pair of amino acids is degraded, forming acetate, ammonia, and carbon dioxide.

Acetogenesis

The third phase of anaerobic digestion is considered critical to the process, being conducted by a group of bacteria called acetogenic. The acetogenic reactions are endothermic (Table 1). For instance, the propionic acid degradation into acetate and carbon dioxide has $\Delta G = +74$ kJ.mol^{-1}.

Table 1. Acetogenic reactions. Decomposition to low molecular weight elements.

Name	Conjugate acid	Chemical reaction/chemical compound	ΔG (k J.mol^{-1})
Carbon dioxide/ hydrogen		$2CO_2 + 4H_2 \rightleftharpoons CH_3COOH + 2H_2O$	-104.6
Formate	Formic acid	HCOOH	
Acetate	Acetic acid	CH_3COOH	
Propionate	Propionic acid	$CH_3(CH_2)COOH + 2H_2O \rightleftharpoons CH_3COOH + CO_2 + 3H_2$	+76.1
Butyrate	Butyric acid	$CH_3(CH_2)_2COOH + 2H_2O \rightleftharpoons 2CH_3COOH + 2H_2$	+48.1
Lactate	Lactic acid	$CH_3CHOHCOOH + 2H_2O \rightleftharpoons CH_3COOH + HCO_3 + H^+ + 2H_2$	-4.2
	Ethanol	$CH_3(CH_2)OH + H_2O \rightleftharpoons CH_3COOH + 2H_2$	+9.6

Source: Adapted from Deublein and Steinhauser (2011); Chernicharro (2007).

Acetogenic bacteria establish a syntrophic relationship with methanogenic archaea and homoacetogenic bacteria. In this phase, long-chain acids are transformed into acids with only one or two carbon atoms (formic and acetic), with the concomitant hydrogen and carbon dioxide production. Homoacetogenic bacteria govern the balance of the direction of hydrogen and carbon dioxide consumption reaction for acetate production (Equation 1). A thermodynamically favorable formation of short-chain acids must occur associated with the consumption of gaseous hydrogen by methanogenic archaea. Syntrophy between organisms from different microbial groups allows both to grow, ensuring the feasibility of producing acetate from organic acids.

$$\boldsymbol{2CO_2 + 4H_2 \rightleftharpoons CH_3COOOH + 2H_2O} \qquad \textit{Equation 1}$$

Methanogenesis

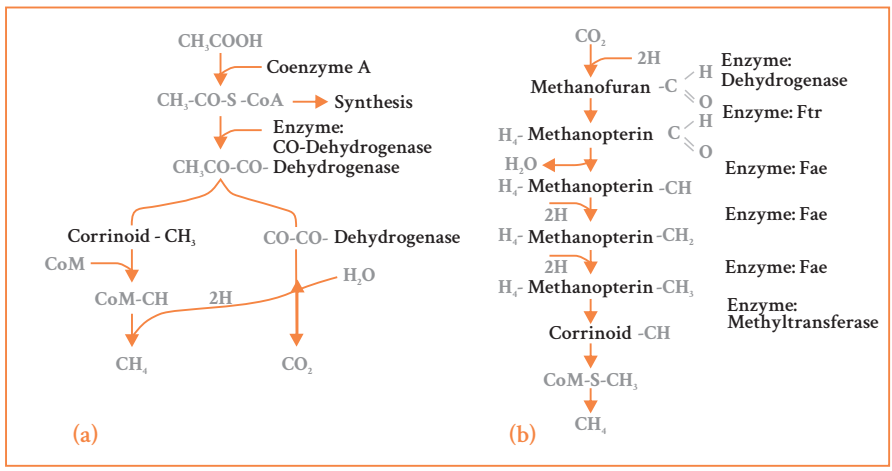
The final phase, methanogenesis, takes place under strictly anaerobic conditions. Thus, the carbon contained in the biomass is converted into carbon dioxide and methane through the action of methanogenic archaea. The archaeal domain is polymorphic, allowing it to be distinguished from other domains only by the 16S rRNA sequence. The reactions that occur in methanogenesis are exothermic (Table 2).

Table 2. Methanogenic reactions. Decomposition to low molecular weight elements.

Chemical reaction	ΔG (k J.mol^{-1})	Name
$4H_2 + HCO_3^- + H^+ \rightleftharpoons CH_4 + 3H_2O$	-135.4	Several species
$CO_2 + 4H_2 \rightleftharpoons CH_4 + 2H_2O$	-131.0	
$4HCOO^- + H_2O + H^+ \rightleftharpoons CH_4 + 3HCO_3^-$	-130.4	Several species
$CH3COO^- + H_2O \rightleftharpoons CH_4 + HCO_3^-$	-30.9	Some species
$4CH_3OH \rightleftharpoons 3CH_4 + HCO_3^- + H^+ + H_2O$	-314.3	
$CH_3OH + H_2 \rightleftharpoons CH_4 + H_2O$	-113.0	*Methanobacterium* *Methanospirillum*
$2CH_3CH_2OH + CO_2 \rightleftharpoons CH_4 + 2CH_3COOH$	-116.3	*Methanosarcina*

Source: Adapted from Deublein and Steinhauser (2011).

Methanogenic archaea are divided according to their metabolic pathways into acetoclastic and hydrogenotrophic. Acetoclastic methanogenic archaea (e.g., *Methanosarcina*) convert acetate into methane, while hydrogenotrophic methanogenic archaea (e.g., *Methanobacterium* and *Methanospirillum*) convert hydrogen and carbon dioxide into methane. Both reactions are exothermic. The pathways for methane formation via acetate or carbon dioxide are shown in Figure 3



Source: Adapted from Deublein and Steinhauser (2011).

Figure 3. (a) Formation of methane by acetate; (b) methane formation by carbon dioxide. CoA = coenzyme A; CoM = coenzyme M.

Many authors report a 70/30% collaboration in methane production between acetoclastic/hydrogenotrophic methanogenic archaea. However, recent studies have shown the dynamism of this relationship (Silva et al., 2014). Acetoclastic methanogenic archaea are more sensitive to changes in pH and high ammonia concentrations, which is a characteristic of substrates from agriculture (other than sanitary sewage). It may imply the predominance of methanogenic hydrogenotrophic archaea. The acetate produced during biodigestion in the absence of acetoclastic methanogenic archaea is oxidized by homoacetogenic bacteria, producing CO_2 and H_2 (Figure 1), which are used as a substrate by hydrogenotrophic methanogenic archaea, producing methane.

Process parameters

The metabolism of anaerobic mesophilic microorganisms depends on several factors (Table 3). Therefore, multiple parameters must be considered and controlled for an optimal fermentation process.

Table 3. Environmental requirements of anaerobic mesophilic microorganisms.

Parameter	Hydrolysis/Acidogenesis	Methanogenesis
Temperature	25 - 35	32 - 42
pH	5.2 - 6.3	6.7 - 7.5
C:N ratio	10-45	20-30
Dry matter concentration (%)	<40	<30
Redox potential (mV)	+ 400 to - 300	<-200
Required C:N:P:S ratio	500 : 15 : 5 : 3	600 : 15 : 5 : 3
Trace elements	-	Essential: Ni, Co, Mo, Se

Source: Adapted from Wellinger et al., (2013).

Hydrogen partial pressure

Hydrogen partial pressure plays a key role in methanogenesis. Therefore, a narrow symbiosis between H_2-producing and H_2-consuming microorganisms is necessary. Overall, a biochemical reaction needs to be exothermic for it to occur spontaneously, that is, the Gibbs free energy must be negative ($\Delta G < 0$).

The hydrogen concentration must be balanced, as methanogenic microorganisms need hydrogen to produce methane (hydrogenotrophic methanogenic archaea). On the other hand, the hydrogen partial pressure must be low enough (10^{-4} to 10^{-6} bar) so that acetogenic bacteria are not inhibited by excess hydrogen, paralyzing the production of short-chain acids.

The maximum hydrogen partial pressure depends on the involved microorganism species and also the substrate characteristics. The energy window is especially small for anaerobic conversion of propionate via acetic acid and carbon dioxide/hydrogen into methane. Low partial pressures can only be maintained if the formed hydrogen is quickly and effectively removed by hydrogen-consuming microorganisms.

Temperature

The temperature has important effects on the physicochemical properties of the components found in anaerobic substrates. It also influences the growth rate and metabolism of microorganisms and, therefore, the population dynamics in a biodigester. Microorganisms can be classified into three large groups, according to the temperature (Table 4).

Table 4. Classification of microorganisms according to the temperature.

	Optimal growth temperature (°C)
Thermophilic	60
Mesophilic	37
Psychrophilic	15

Acetoclastic methanogenic archaea are the group most sensitive to temperature increase. The temperature affects the hydrogen partial pressure in a biodigester, influencing the syntrophic metabolism kinetics. Thermodynamically, endothermic reactions under standard conditions, such as the breakdown of propionate into acetate, carbon dioxide, and hydrogen, become energetically more favorable at high temperatures, but exothermic reactions (e.g., hydrogenotrophic methanogenic) are less favored at high temperatures.

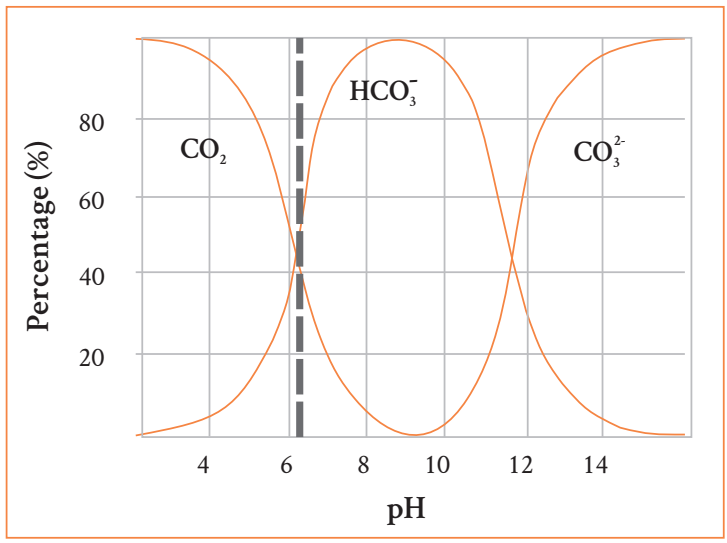
Increasing temperature has numerous benefits, including an increase in the solubility of organic compounds, improving the biochemical reaction rates. There is also an increase in the pathogen elimination rate. However, temperature influences parameters such as ammonia dissociation, which may have an inhibitory effect (Kunz; Saqib, 2016). The chemical balance is shifted from NH_4^+ to NH_3 (aqueous) as the temperature increases, which may lead to failure in the process. Free ammonia is toxic to methanogenic archaea, as it easily diffuses through the cell membrane of microorganisms, causing ionic imbalance and/or potassium (K^+) deficiency.

Biogas production in regions with a large thermal amplitude can be compromised due to high-temperature variations. The reactor temperature should not vary sharply more than 2 °C to avoid possible problems. Biomass temperature control is of paramount importance to ensure uniformity of biogas generation.

pH, alkalinity and volatily fatty acids

Each microorganism group has a different optimum pH value. Methanogenic archaea are extremely pH sensitive, with an optimum value between 6.7 and 7.5. Fermentative microorganisms are less sensitive and can adapt to greater pH variations between 4.0 and 8.5. The main products at low pH values are acetic and butyric acids, while the main products at pH close to 8.0 are acetic and propionic acids. Volatile acids produced during biodigestion tend to reduce the pH of the reaction medium. This reduction is usually countered by the activity of methanogenic archaea, which also produce alkalinity in the form of carbon dioxide, ammonia, and bicarbonate.

The system pH is controlled by the concentration of carbon dioxide in the gas phase and HCO_3-alkaline in the liquid phase (Figure 4). CO_2 is continuously released as a gas during biodigestion. A higher CO_2 amount will remain dissolved in the reaction medium if the system pH decreases excessively. On the other hand, dissolved CO_2 will form carbonic acid if the system pH increases, thus releasing hydrogen ions (Deublein; Steinhauser, 2011).



Source: Adapted from Deublein and Steinhauser (2011).

Figure 4. Chemical balance between carbon dioxide – bicarbonate ion – carbonate ion.

Almost all CO_2 will be in free molecule form at pH 4 and dissolved as carbonate in the substrate at pH 13. The chemical equilibrium point between gaseous and soluble forms in the system will occur at pH 6.52 (Figure 4). Therefore, the increase in pH will result in a lower CO_2 concentration in the gas phase. Bicarbonate has a strong buffering effect at concentrations of 2.5 $g.L^{-1}$ – 5 $g.L^{-1}$.

Most problems in anaerobic digestion can be attributed to the accumulation of volatile acids and, consequently, the decrease in pH. The main adverse effects of volatile fatty acis (VFAs) in the anaerobic digestion process are related to the fact that they are intermediate species. The decrease in pH below 6.6 implies the growth inhibition of methanogenic archaea. However, acidogenic bacteria continue their functions up to pH 4.5. The result is a rapid VFA accumulation.

A strategy for controlling the buffering system and indirectly monitoring the acids produced during the anaerobic digestion is the intermediate alkalinity/partial alkalinity (VFA/TA) ratio, the former providing values equivalent to the alkalinity by bicarbonate and the latter to

alkalinity from volatile acids. Table 5 shows the importance of monitoring the VFA/TA ratio and the reactor relationships and characteristics according to empirical experience.

Table 5. Evolution of the VFA/TA ratio and reactor characteristics.

VFA/TA ratio	Reactor characteristics
>0.4	Reactor under overload
0.3 – 0.4	Optimal range
< 0.3	Reactor under underload

Source: Adapted from Mézes et al., (2011).

The optimal value may vary depending on the reactor and substrate characteristics. It is recommended to monitor the VFA/TA ratio constantly, as observing sudden variations and taking corrective measures when necessary are the most important.

Nutrients

Cells of anaerobic microorganisms contain nitrogen, phosphorus, and sulfur at approximate dry matter proportions of 12%, 2% and 1%, respectively. The anaerobic process requires biological oxygen, N, and P demand ratios of 700:5:1. Sulfur, potassium, calcium, magnesium, chlorine, and sulfate ions are necessary for the proper functioning of anaerobic digestion. Trace elements such as iron, copper, zinc, magnesium, molybdenum, and vanadium are important for cell growth.

Sulfur compounds can cause problems for the anaerobic process, as they lead to the precipitation of essential nutrients at trace levels, such as iron, nickel, copper, and molybdenum, which are insoluble at low redox potentials (precipitation as a sulfide). Heavy metal ions such as Cu^{++} and Zn^{++}, alkali and alkaline earth metal ions, and NH_4^+ can also cause inhibitory effects. Toxicity is reversible in many cases and a high acclimatization potential is observed when sufficient time is given to anaerobic microorganisms.

Ammoniacal nitrogen and free ammonia

Ammonia is an essential nutrient for the growth of anaerobic microorganisms, but it can also be toxic at high concentrations. Fermentation of urea and protein-rich materials releases ammonia. A high generation of free ammonia may be reached as a function of the pH and temperature of the reaction medium (De Prá et al., 2013). The chemical equilibrium of the system for free ammonia (FA) formation can be calculated using Equation 2.

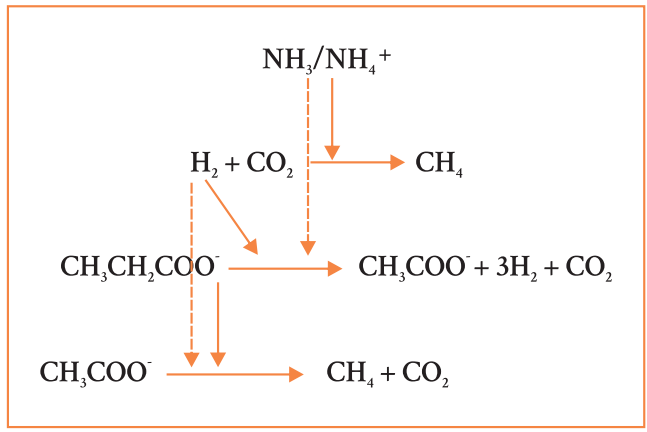
$$FA\ (NH_3, mg.L^{-1}) = \frac{17}{14}\ X\ \frac{[total\ ammonia\ as\ N] \cdot 10^{pH}}{e^{[6344/(273+T(°C))]} + 10^{pH}} \qquad \textit{Equation 2}$$

Table 6 shows the relationship between pH and temperature with free ammonia concentration in an effluent with a high ammoniacal nitrogen concentration, using Equation 2.

Table 6. Effect of pH and temperature on free ammonia (FA) concentration in an effluent with high ammoniacal nitrogen concentration.

NH_3-N ($mg.L^{-1}$)	Reactor pH	Temperature (°C)	FA ($mg.L^{-1}$)
3,000	5	20	0.14
3,000	7	20	14.34
3,000	9	20	1,031.68
3,000	5	37	0.47
3,000	7	37	46.58
3,000	9	37	2,055.77
3,000	5	55	0.15
3,000	7	55	139.51
3,000	9	55	2,911.66

Free ammonia is toxic to methanogenic archaea as it easily diffuses through the cell membrane of microorganisms. Figure 5 shows a scheme to exemplify the inhibitory action of free ammonia. Continuous arrows indicate reaction inhibition and dashed arrows indicate possible inhibitory actions.



Source: Wiegant and Zeeman (1986).

Figure 5. Scheme proposed to explain the inhibitory action of free ammonia. Horizontal arrows: inhibited reactions; vertical arrows: inhibitory action. Dotted arrows indicate possible inhibiting actions.

The literature shows anaerobic digestion inhibition at different free ammonia concentrations. Garcia and Angenent (2009) studied the digestion of swine manure and reported inhibition of methane production at concentrations from 200 $mg.L^{-1}$ at 35 °C with pH 7.6. Rodríguez et al., (2011) reported that levels of up to 375 $mg.L^{-1}$ of free ammonia did not affect the efficiency of the digestion process.

The acclimatization of microorganisms in the presence of free ammonia is a key factor for the process efficiency. It can occur due to the adaptation of methanogenic archaea species present in the reactor or through population selection, standing out species more adapted to the reactor conditions (Silva et al., 2014).

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Further reading

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