Biodegradation of two organic UV-Filters by single bacteria strains

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Research Article

Keywords: UV-filters, Gram-negative bacteria, Gram-positive bacteria, biodegradation products, LC-MS/MS, kinetics

Posted Date: March 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-302964/v1

Abstract

Organic UV-filters, including 4-hydroxybenzophenone (4-HBP) and 2,4-dihydroxybenzophenone (BP-1), are persistent emerging contaminants whose presence in the environment poses a threat to aquatic organisms due to their endocrine disruptor's properties. For this reason, finding suitable technological processes for their safety and efficient removal from the environment represent a priority for the scientific community. To the author's knowledge, until now, there are no studies reporting the biodegradation of 4-HBP and BP-1 by a single bacteria strain. In this paper, there were tested the 4-HBP and BP-1 biodegradation potential of two Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and two Gram-negative (*Salmonella typhimurium* and *Serratia rubidae*).

The 4-HPB biodegradation process was observed only in the presence of Gram-negative bacterial strains. Thus, the biodegradation rates of 4-HBP reached up to 12.7% after 24h of incubation in presence of *Salmonella thyphimurium* and up to 24.0% after 24h of incubation with *Serratia rubidae*. *Staphylococcus aureus* was able to biodegrade 26.7% of BP-1, while *Salmonella thiphymurium* was able to biodegrade 14.7% of BP-1 after 24h of incubation. Their biodegradation products generated during the 4-HBP biodegradation process by *Serratia rubidae* were analyzed through LC-MS/MS analysis. The (bio)degradation products were benzophenone and a multi-hydroxylated derivative of 4-HBP and the degradation pathways were proposed. The data obtained in this study gave important information regarding the 4-HBP and BP-1 potential biodegradation by single bacterial strains.

1. Introduction

Benzophenone-type UV filters are chemical substances used extensively in sunscreens and other personal care products such as body lotions, shampoos, lipstick and skin creams to protect lips, hair and skin against the solar irradiation (Mao et al., 2019). Due to their extensively applications, organic UV filters and their metabolites may be released into environment through human activities or through wastewater treatment plant effluent discharges, which are not always capable of completely remove these micro-pollutants. In recent years, many studies reported the presence of organic UV filters, including 2-hydroxy-4-methoxy-benzophenone (BP-3), 4-hydroxibenzophenone (4-HBP) and 2,4-dixydroxybenzophenone (BP-1), in concentration levels ranging from nanograms per liter to microgram per liter in surface waters and sediments (Chiriac et al., 2021; O'Malley et al., 2021; Fagervold et al., 2019; Apel et al., 2018; Tsui et al., 2019; Mitchelmore et al., 2019), wastewaters and sewage sludge (Chiriac et al., 2021; Wu et al., 2018; Mao et al., 2019; O'Malley et al., 2019). The major concern regarding these chemical compounds is related to the easiness with which they can bioaccumulate in living organisms, especially due to the negative properties they can have: alter sex hormonal balance, affect endocrine signaling and impede reproductive capacity. In the last decade, organic UV-filters were discovered more often bioaccumulated in aquatic organisms, such as: coral (Tsui et al., 2017; Fel et al., 2019; Mitchelmore et al., 2019; He et al., 2019; Stien et al., 2020), dolphin (Alonso et al., 2015; Gago-Ferrero et al., 2013a) fishes (Horricks et al., 2019; Araújo et al., 2018; Lu et al., 2018; Gago-Ferrerro et al., 2015), algae (Teoh et al., 2020; Zhong et al.,

2019; Mao et al., 2017; Mao et al., 2018; Seoane et al., 2017), benthic mollusks (Vidal-Linan et al., 2018; Giraldo 2017).

The frequent and increasing determination of organic UV filters leads to the need of fast and efficient methods capable to eliminate them before they reach the environment. Many degradation studies are focusing on BP-3 and only very few on BP-1 or 4-HBP. The most common methods for removing these emerging organic micropollutants from environment are, photodegradation (Luo et al., 2019; Vione et al., 2013; Gong et al., 2015), ozonation (Gago-Ferrero et al., 2013b; Guo et al., 2016), electro-Fenton degradation (Ye et al., 2019), persulfate-based oxidation (Pan et al., 2017) and chlorination (Lee et al., 2020; Manasfi et al., 2017). Compared to chemical methods, biological treatment is a more economical, environmentally friendly alternative and has gained very high public acceptance (Nita-Lazar et al., 2016a, b). Biological treatment is one of the most important method of removing organic pollutants in a wastewater treatment plant (Ponce-Robles et al., 2018). The biodegradation process involves the total or partial removal of organic chemicals by transforming them into substances less dangerous for the environment. These processes are based on microorganisms existing in natural ecosystems that can degrade and eliminate organic pollutants using them as growth substrates. Until now, activated sludge and anaerobic digested sludge were used as inoculum for BP-3 biodegradation (Liu et al., 2012b). White rot fungus, *Trametes versicolor*, proved to be very efficient for BP-3 biodegradation (Badia-Fabregat et al., 2012; Rodríguez-Rodríguez et al., 2014). Moreover, fungal treatment managed to degrade more than 99% of both BP-3 and BP-1 in less than 24 h (Gago-Ferrero et al., 2012).

However, according to the authors' knowledge, there are no reports in the literature regarding the degradation of 4-HBP or BP-1 by a single bacterial strain. Unveiling bacterial strains capable of efficient degradation of emerging organic pollutants such as 4-HBP and BP-1, could be useful for increasing the wastewater treatment plants removal capacity, by bioaugmentation or other bioremediation process.

The aim of this study was to find single bacterial strains with the ability to degrade two of the most common UV filters which are often found in wastewater treatment plants: 4-HBP and BP-1, without additional carbon sources and to evaluate, for the first time, the tolerance to 4-HBP and BP-1 stress of gram-negative and gram-positive bacteria strains. Moreover, removal efficiency and biodegradation kinetic parameters were determined and a possible (bio)degradation pathway was discussed.

2. Material And Methods

2.1. Chemicals

4-HBP and BP-1 (analytical grade > 99%) were purchased from Sigma-Aldrich (Germany). The acetonitrile HPLC-gradient grade and Formic acid were acquired from Sigma-Aldrich (Germany). Gram-negative bacterial strain *Salmonella thyphimurium*- ATCC 14028 and gram-positive bacterial strain *Enterococcus faecalis* - ATCC 29212 were purchased from ATCC (American Type Culture Collection), while gram-negative bacterial strain *Serratia rubidae* and gram-positive bacterial strain *Staphylococcus aureus* were

obtained from our bacterial data bank collection. Solid growth medium - soybean agar tryptone (Oxoid, UK) and liquid growth medium - lauryl sulfate broth, used for bacterial growth, were acquired from Himedia Laboratories Pvt. Ltd.

2.2. Biodegradation assays

For biodegradation studies, the bacterial strains were initially seeded on a solid nutrient medium (soy agar tryptone) and incubated at 37°C for 24 hours. After incubation, one colony of each bacterial strain was transferred to specific liquid culture medium - lauryl sulfate broth (tryptose 20g/L, lactose 5g/L, sodium chloride 5g/L, dipotassium phosphate 2.75g/L, monopotassium phosphate 2.75g/L, sodium lauryl sulfate 0.1g/L) and incubated at 37°C. Incubation in liquid medium was performed for 24h in an incubator under gentle rotation 130 rpm) (New Brunswick Scientific, Innova 44). Bacterial growth was spectrophotometrically measured by optical density (OD) detection at 600 nm wavelength using the UV-VIS spectrometer (VWR International, USA). A 10ml of 0.2 OD 600nm bacterial inoculum were incubated in presence of lauryl sulfate broth medium containing 10 mg/L BP-1 or 4-HBP, then incubated in sterile condition up to 24h at 37°C and 130 rpm. Growth inhibition rate was calculated based on bacterial density, measured as optical density at 600 nm.

2.3. Chemical analysis

The concentration of 4-HBP and BP-1 was quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS). Experiments were carried out in triplicates and control experiments without bacterial inoculation were performed under the same conditions.

Standard solutions (without the bacterial strain incubation), control samples (bacterial strain incubated in liquid culture medium and 4-HBP or BP-1 in liquid culture medium) and test samples (bacterial strains incubated in presence of 4-HBP or BP-1) were collected and compared at 0h, 6h and 24h in liquid culture medium - lauryl sulfate broth. LC-MS/MS analysis was performed using an Agilent 1260 series LC system (Agilent, Waldbronn, Germany) coupled with an Agilent 6410B triple-quadrupole mass spectrometer with electrospray ionization source (ESI). The chromatographic separation was done on a hydrophobic chromatographic column (Luna(C18), 150 x 2.0 mm, 3.0 µm, Phenomenex) maintained at 30°C, with suitable guard column. The mobile phase consisted of 0.15% formic acid in ultrapure water (A) and Acetonitrile (B) 40/60 v/v, in isocratic mode. The flow rate of the mobile phase was 0.2 mL/min and the injection volume 1 µL. Determination of chemical concentration and metabolite formation were done in SCAN operation mode. Full-scan chromatograms were recorded, using the electrospray ionization source in both negative and positive modes, respectively. The scan ranged between 80 and 400 Da. Parameters of the ionization source were: capillary voltage (5000V), drying gas temperature (300°C), drying gas flow (8 L/min) and nebulizer pressure (40 psi). For quantification of 4-HBP, BP-1 and unknown metabolites, the retention times of samples collected after specific hours (0h, 6h and 24h) were matched and compared for areas. 4-HBP and BP-1 peaks were observed at 7.6 and respectively 8.3 min retention times in standard solutions (Fig. 1) control samples and test samples (data not shown).

The preparation method for standard solutions, control samples and test samples focused on proteins precipitation with Acetonitrile to reduce the matrix effect that may interfere with mass spectrometric detection. Thus, 200 µL aliquots of the all samples were deproteinized with 400 µL Acetonitrile. The mixtures were vortexed for 1 min and then centrifuged (5 min at 14000 rpm). After centrifugation, the supernatant was transferred to the LC-MS injection vial.

4-HBP and BP-1 removal efficiencies (%) were calculated with the following equation:

$$Removalefficiency(\%) = 100 \times \frac{(C_{MB} - C_T)}{C_0}$$

1

Where C_{MB} represented the concentration of 4-HBP or BP-1 in liquid culture medium at different sampling times, C_T represented the 4-HBP or BP-1 in treatment medium at each sampling time and C_0 was the initial concentration of 4-HBP or BP-1.

3. Results And Discussion

3.1. 4-HBP and BP-1 effect on Gram-positive and negative bacterial strains

Four bacterial strains, two Gram-negative and two Gram-positive strains were incubated in presence of organic UV filters, 4-HBP or BP-1. The impact of organic UV filters on the bacterial strains was different based on the timing and strains, where the growth curves were more robust in case of gram-negative bacteria incubated in presence of both 4-HBP (Fig. 2) and BP-1 (Fig. 3), compared with gram-positive bacteria.

Salmonella thyphimurium had a robust growth in the first 2h of incubation in the presence of 4-HBP followed by a less increase growth phase after 4h to 6h of incubation (Fig. 2a), compared with *Serratia rubidae* which had an exponential growth throughout the entire test period (Fig. 2b). *Enterococcus faecalis* had a slightly decrease after 6h contact with 4-HBP (Fig. 2c) compared with *Staphylococcus aureus* (Fig. 2d), however both gram-positive bacteria recorded a lower growth rate compared to gram-negative ones.

According with Turner et al. (2000), within the stationary phase of growth, the cell phenotypes of bacterial species could oscillate due to the increase of cell heterogeneity. Moreover, numerous bacterial strains, including *Salmonella thyphimurium* has been reported as being able to cope environmental pressures by expression of specific genes (Child et al., 2002).

Gram-negative bacteria incubated in the presence of BP-1 had a continuous growth compared to Gram-positive bacteria which had a short exponential phase (Fig. 3a and 3b). Overall growth profile of all for bacterial

strains in presence of organic UV filters had the same growth pattern as the control samples, indicating that organic UV filters did not significantly modify the growth pattern of the analyzed bacterial strains. The overall growth inhibition was up to 16% for *Salmonella typhimurium* incubated in presence of 4-HBP, which pointed to the hypothesis that bacterial strains could metabolize the organic UV filters.

3.2. Bacterial biodegradation potential of organic UV filters, *4-HBP and BP-1*

The assay of bacterial incubation in presence of the organic UV filters continued with the quantitative monitoring of 4-HBP and BP-1 from the bacterial broth in which incubation took place. The control monitoring of organic UV filters was performed in absence of bacterial strains (data not shown). The abiotic degradation of 4-HBP was around 6%, while the BP-1 abiotic degradation was up to 2.6% during 24h of incubation. Overall the abiotic degradation was not significant, but there were taken into consideration when biodegradation results were analyzed.

Experiments performed in the presence of gram-positive bacteria, *Staphyloccocus aureus* and *Enterococcus faecalis*, showed the biodegradation degree of 4-HBP was less than 4%, in addition to the abiotic degradation percentage. A longer incubation time did not enhance the biodegradation (Fig. 4a).

In contrast, both gram-negative bacterial strains, *Salmonella typhymurium* and *Serratia rubidae*, were efficient in 4-HBP removal after 6h of incubation. The highest degradation degree was recorded in the presence of *Serratia rubidae*, up to 24%, while *Salmonella typhimurium* managed to biodegrade only 12.7% of 4-HBP (Fig. 4a). In both experiments, the biodegradation degree wasn't significantly increased after 6 hours of incubation. The results were analyzed after subtracting the abiotic degradation values.

Experiments performed in the presence of the gram-positive bacteria *Staphyloccocus aureus* resulted in a steady decrease of BP-1 concentration throughout the entire test period. About 27% of BP-1 was degraded by strain *Staphyloccocus aureus* in 24h (Fig. 4b). BP-1 removal efficiencies of only 3% were achieved by incubation with *Enterococcus faecalis*, after the correction with abiotic degradation was made. *Serratia rubidae* achieved to remove only 6% of BP-1 after 12h of incubation, while for *Salmonella thyphimurium*, the removal efficiency was up to 15% of the initial amount of BP-1 after 24h of incubation (Fig. 4b). All values reported were corrected with abiotic degradation results.

It was observed that after 6h incubation time both *Salmonella thyphimurium* and *Serratia rubidae* were able to metabolize 4-HBP, in contrast to BP-1. We assume that the lower removal efficiency of BP-1 after 12h incubation was due to expression of efflux pumps described as the key mechanism of resistance in bacteria. Therefore, the efflux pumps allowed the microorganisms to regulate their internal environment by removing toxic substances, metabolites and quorum sensing signal molecules out of the cell (Pearson et al., 1999).

There is only one study in the literature in which the authors reported the degradation of an organic UV filter, BP-3 by a single bacterial strain, namely *Methylophilus sp.* strain FP-6. In that study *Methylophilus*

sp efficiently removed about 65% of BP-3 after 8 days, using methanol as additional carbon sources (Jin et al., 2019).

3.3. Kinetics biodegradation of 4-HBP and BP-1

Both UV-Filters biodegradation kinetics was simulated and all experiments found to be fitted with second-order model, in which the time (t) dependence of the concentration (C) variation is given by the Eq. 2:

$$\frac{1}{[C]} = \frac{1}{[C]_0} + \mathrm{kt}$$

2

Where C_0 is the initial concentration and k is the biodegradation rate constants.

The half-life of biodegradation ($t_{1/2}$) for both UV-Filters by gram-positive and gram-negative bacterial strains was estimated from k, using the following equation:

$$t_{\frac{1}{2}} = \frac{1}{k[C]_0}$$

3

Two-gram positive bacterial strain, meaning *Staphylococcus aureus* and *Enterococcus faecalis* and two gram-negative bacterial strain, *Serratia rubidae* and *Salmonella thyphimurium*, were tested for their ability to degrade 4-HBP and BP-1. The kinetic parameters are summarized in Table 1. 4-HBP was resistant to biodegradation by both *Staphylococcus aureus* and *Enterococcus faecalis*, the higher removal rate being only up to 3%. The observed rate constants (k) of 4-HBP degradation were determined to be 4.64×10^{-4} h^{-1} for *Staphyloccocus aureus* and 2.37×10^{-5} h^{-1} for *Enteroccocus faecalis*, respectively. The half-life ($t_{1/2}$) values were calculated as 11.1 days for the interaction of 4-HBP with *Staphylococcus aureus* and 17.4 days for the interaction with *Enterococcus faecalis*.

Table 1. Second-order rate constant (k) and half-life ($t_{1/2}$) for biodegradation of 4-HBP and BP-1 by bacterial strains

Compound	Bacterial strain	Removal efficiency (%)	Kinetics order	R^2	k	$t_{1/2}$ (days)
4-HBP	*Staphyloccocus aureus*	3.75	2^{nd} order	0.9685	$4.64x10^4$	11.1
	Enterococcus faecalis	0.96	2^{nd} order	0.9117	$2.37x10^4$	17.4
	Salmonella typhimurium	12.7	2^{nd} order	0.9034	$8.61x10^4$	5.5
	Serratia rubidae	24.03	2^{nd} order	0.9363	$1.58x10^3$	2.6
BP-1	*Staphyloccocus aureus*	26.7	2^{nd} order	0.9894	$1.56x10^3$	2.5
	Enterococcus faecalis	2.5	2^{nd} order	0.9487	$2.14x10^4$	19.7
	Salmonella typhimurium	14.7	2^{nd} order	0.9308	$6.91x10^4$	6.03
	Serratia rubidae	3.40	2^{nd} order	0.9088	$2.82x10^4$	17.1

The biodegradation of 4-HBP by the gram-negative bacterial strains exhibit the highest rate constant values: 1.58×10^{-3} h^{-1} for *Serratia rubidae* and 8.61×10^{-4} h^{-1} for *Salmonella thyphimurium*. The half-life ($t_{1/2}$) values of 4-HBP biodegradation by *Serratia rubidae* and *Salmonella thyphimurium* were estimated at 2.6 days and 5.5 days respectively. The correlation coefficient (R^2) values ranged from 0.90 to 0.97, which indicate a good relationship between the biodegradation data and the second-order kinetic model.

Unlike 4-HBP, for which biodegradation has been favored by gram-negative bacterial strains, the degradation of the BP-1 no longer depended on the type of bacteria used. The higher degradation rates were obtained for *Staphyloccocus aureus* and *Salmonella typhimurium*, with the second-order degradation rate constant of $1.56x10^3$ and $6.91x10^4$, respectively. Based on the half-life time calculated values, it takes approximatively 2.5 days for *Staphyloccocus aureus* to biodegrade half of BP-1 amount and 6.03 days for *Salmonella typhimurium* to biodegrade half of the same amount of BP-1. For the other two tested bacterial strain, *Enterococcus faecalis* and *Serratia rubidae*, the BP-1 biodegradation was very small (up to 3.4%). The biodegradation rate constant of *Enterococcus faecalis* was $2.14x10^4$, while the k value of *Serratia rubidae* incubated with BP-1 was $2.82x10^4$. The half-life of BP-1 biodegradation by *Enterococcus faecalis* was estimated as 19.7 days while by *Serratia rubidae* was determined as 17.1 according to the BP-1 degradation curves. High correlation coefficient values were also obtained for the second-order kinetic degradation model of BP-1 by all bacterial strains, with values ranged between 0.90 and 0.99.

The half-life values are similar with those reported for BP-3 degradation by *Methylophilus* sp. strain FP-6, which was estimated as 2.95 days under optimum culture conditions (Jin et al., 2019).

3.4 (Bio)degradation products and proposed degradation pathway

The highest biodegradation of BP-1 was obtained in presence of *Salmonella typhimurium* and *Staphyloccocus aureus* and the next step was to analyzed the mass spectra of BP-1, in order to identify its possible by products. Although the BP-1 concentration decreased during the biodegradation assays, no biodegradation product could be observed on the spectra (data not shown). Possible explanations of no biodegradation products detection could reside either on the low sensitivity of the SCAN method or on obtaining biodegradation compounds with molecular masses smaller than the tested SCAN range, between 80 and 400 Da.

The analysis of the 4-HBP mass spectra after *Salmonella typhimurium* and *Serratia rubidae* incubation was more successful in biodegradation products identification compared to BP-1 biodegradation. As shown in the chromatograms of Figure 5, only 4-HBP (t_R=7.6 min) was observed before starting the biodegradation experiments. The peak area of 4-HBP was observed to decrease after 6h of both bacterial treatment and in their absence (only in the liquid medium), but much more slowly. After 6 hours of incubation the chromatographic peak width corresponding to 4-HBP increases which suggesting the formation of an unknown degradation product (**DP1**) at the same retention time with 4-HBP for both gram-negative bacterial strains (Figure 5B and Figure 5C). The same thing was also observed for the experiment which does not contain the biotic factor (Figure 5A), which means that this unknown compound results from the abiotic degradation. However, as it can be seen in Figure 5C, LC-MS/MS analysis showed one unknown chromatographic peak (**DP2**) after 24 hours of incubation, indicating the formation of a degradation product of 4-HBP by *Serratia rubidae* (t_R=8.6 min). The intermediate derived from 4-HBP were further determined through MS spectrum obtained from LC (ESI)-MS/MS (Figure 5).

Based on the molecular ion masses from MS fragmentation, the molecular ion peaks $[M+H]^+$ of compounds **DP1** and **DP2** were 184.0 Da (molecular weight 183.0) and 310.1 Da (molecular weight 309.1), respectively (Fig. 6). It was proposed that 4-HBP could be transformed into benzophenone (DP1) through dihydroxylation under abiotic condition. Multi-hydroxylated intermediate (DP2) was formed in the presence of the *Serratia rubidae* bacterial strain by successive hydroxylation of 4-HBP. In a similar study, performed with BP-3, it was reported that the reaction between hydroxyl radical and benzene ring is performed in a gradual way, leading to the formation of mono-, di- and tri-hydroxylated BP-3 analogs (Guo et al., 2016). Based on the identified products, (bio)transformation pathways of 4-HBP under biotic and abiotic condition have been proposed in Figure 6.

Conclusion

In this study, we investigated the effect of 4-HBP and BP-1 on the growth of four single bacterial strains, gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and two gram-negative (*Salmonella typhimurium* and *Serratia rubidae*), and evaluated their ability of biodegrade 4-HBP and BP-1 in culture. 4-HBP reached about 12.7% after 24 hours of incubation with strain *Salmonella thiphymurium* and about 24.0% after 24 hours of incubation with *Serratia rubidae*. The half-life ($t_{1/2}$) of 4-HBP biodegradation was estimated as 5.5 days for *Salmonella thyphimurium* and 2.6 days for *Serratia rubidae*. Degradation of 4-HBP by strain *Serratia rubidae* resulted in the formation of one biodegradation products and a biodegradation pathway was proposed, while for *Salmonella thyphimurium*, no biodegradation product was observed.

The degradation achieved for BP-1 was 26.7% for *Staphylococcus aureus* and 14.7% for *Salmonella thyphimurium*, while for the other two bacterial strains, the degradation was less than 5%. The biodegradation half-life of BP-1 was determined to be 2.5 days for *Staphylococcus aureus* and 6.03 days for *Salmonella thyphimurium*. No biodegradation products were observed during the biodegradation processes. The obtained data provides the first proof that single bacterial strains can degrade 4-HBP and BP-1 and can be successfully involved in bioaugmentation processes.

Future researches will be focused on longer incubation time of bacteria in presence of UV filters to highlight the expression of genes encoding proteins.

Declarations

Funding

This study was supported by The National Research Program “Nucleu”, contract no. 20N/2019, Project code PN 19 04 01 01

Author contribution

The authors contribution to the paper was as follows:

Florentina Laura Chiriac: methodology, design, data interpretation, writing - original draft; Catalina Stoica: methodology, design, data interpretation, writing - original draft; Florinela Pirvu: resources, material preparation and performed analysis; Iuliana Paun: resources, material preparation and performed analysis; Toma Galaon: writing - review & editing; Mihai Nita-Lazar: conceptualization, supervision, writing - review & editing. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Not applicable.

Consent to participate

The authors were informed and agreed to the study.

Consent for publish

The authors agreed to publication in the journal.

Competing interest

The authors declared that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

All data generated or analyzed during this study are included in this published article

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Figures

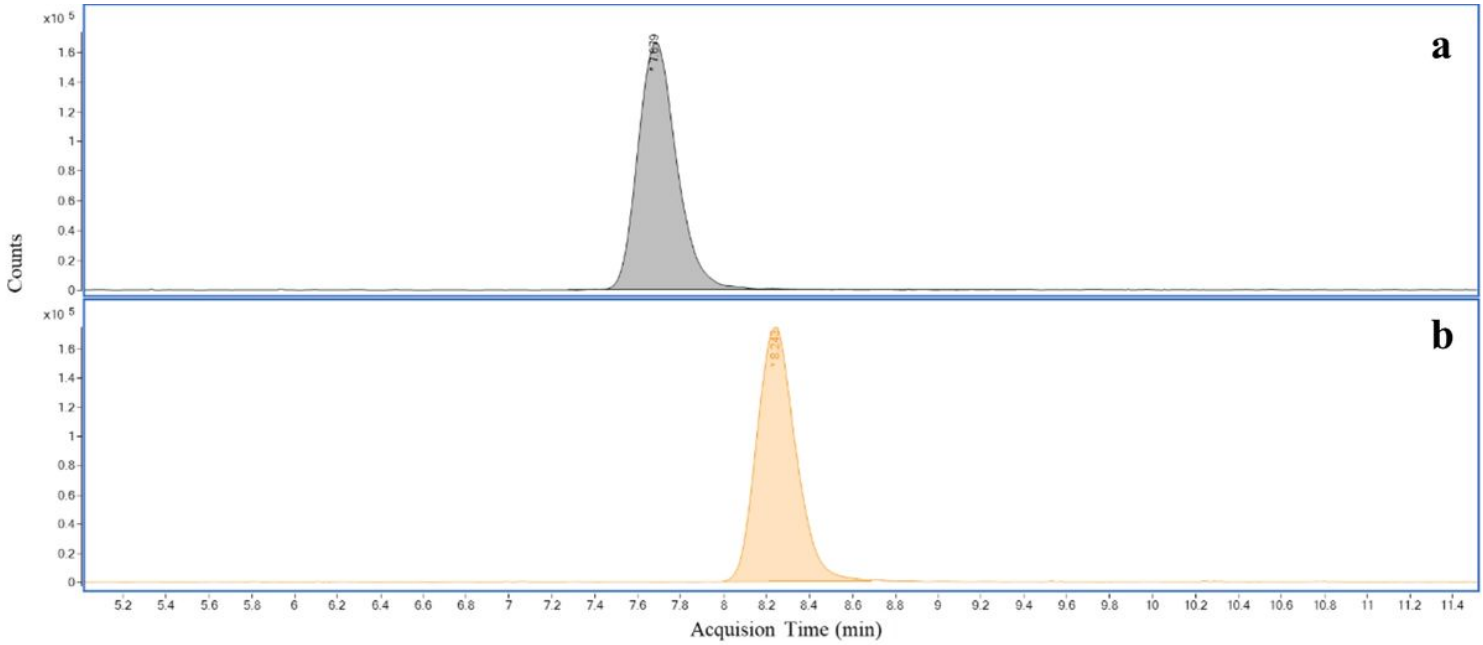

Figure 1

TIC Scan chromatograms registered in positive mode for a. 4HBP and b. BP1 in standard solution.

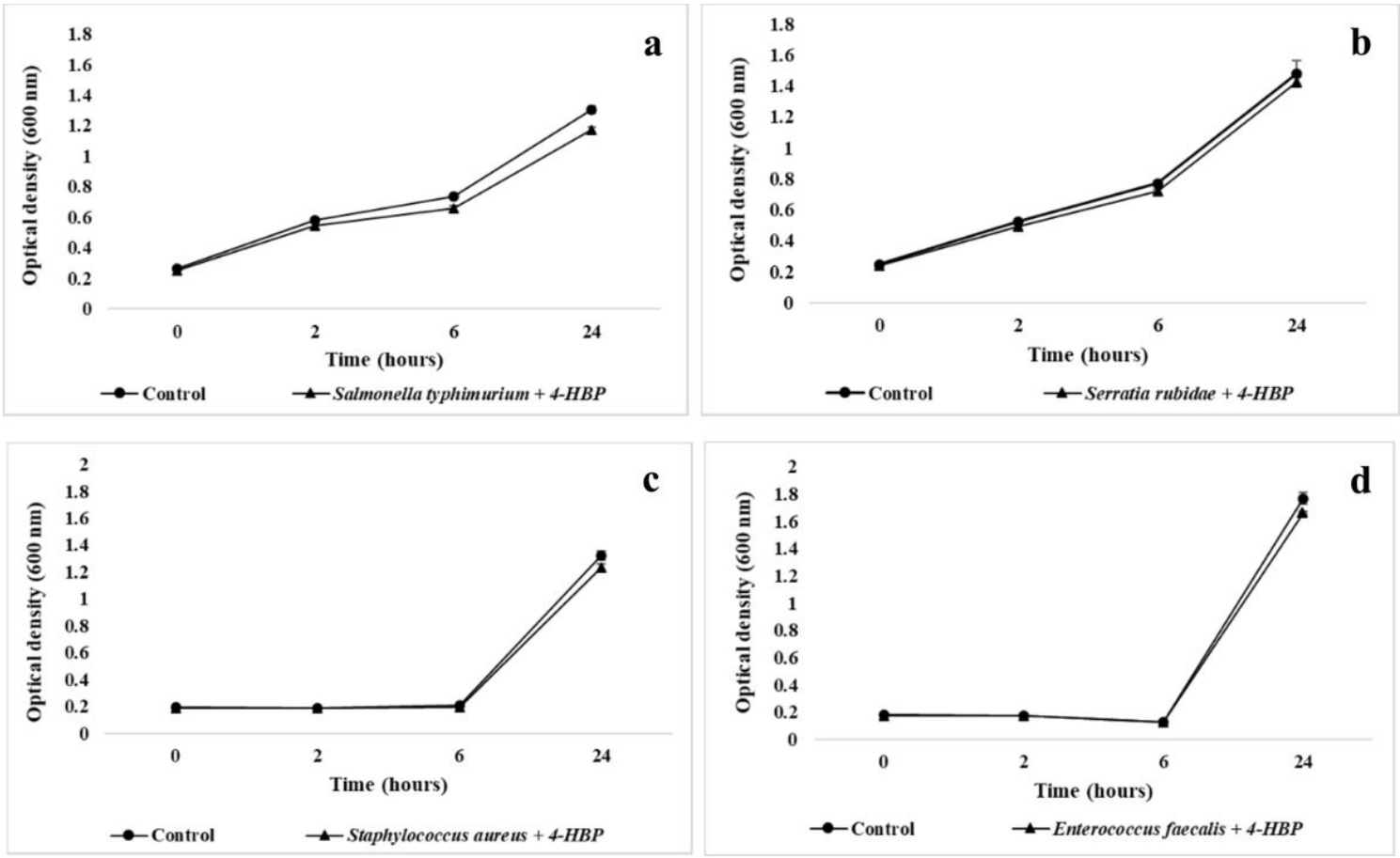

Figure 2

4-HBP impact on bacterial growth. Gram-negative bacteria a, Salmonella typhimurium and b, Serratia rubidae and Gram-positive bacteria c, Staphylococcus aureus and d, Enterococcus faecalis. Control, bacteria incubated without 4-HBP. All studies represent one of three independent experiments.

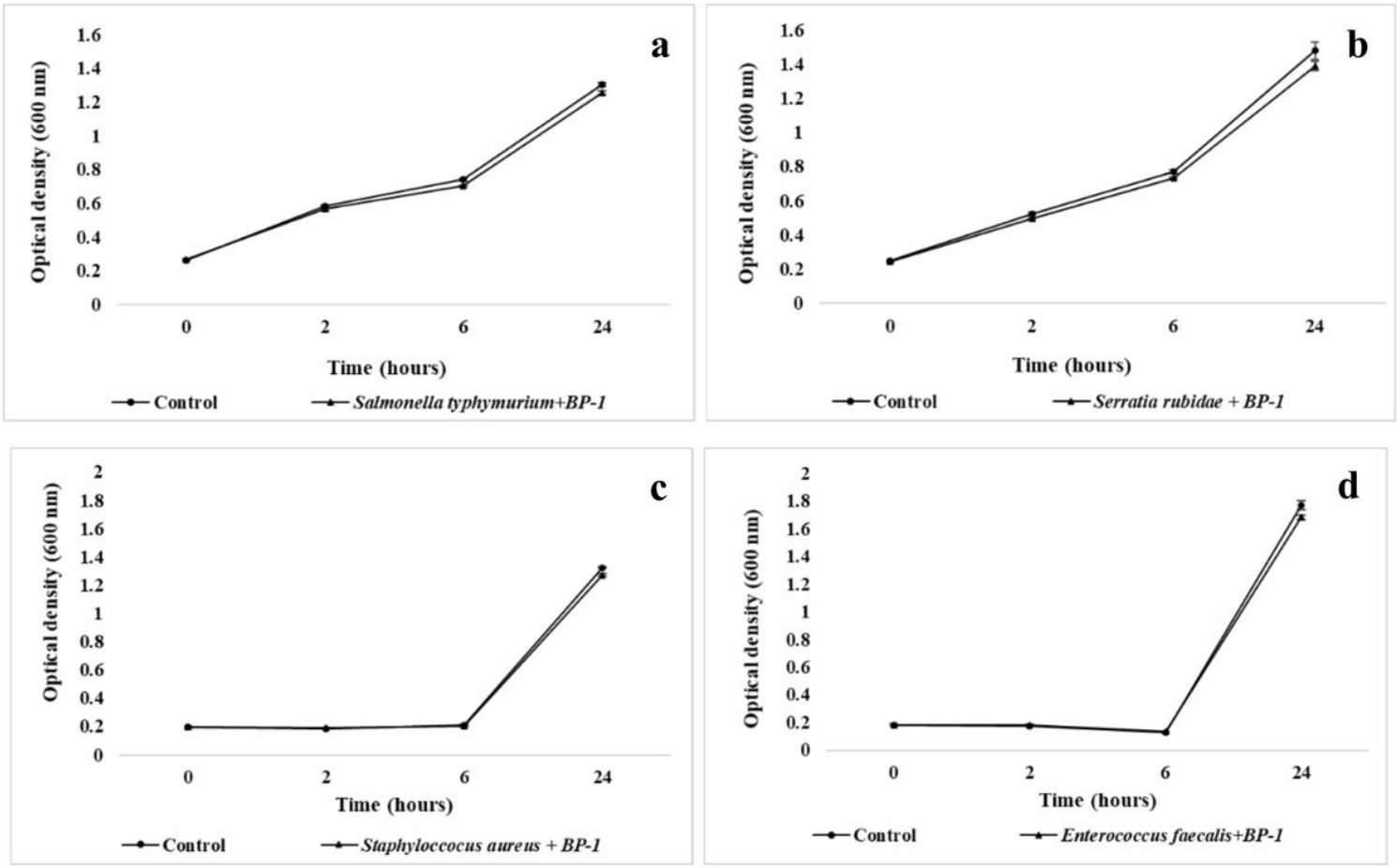

Figure 3

BP-1 impact on bacterial growth. Gram-negative bacteria a, Salmonella typhimurium and b, Serratia rubidae and Gram-positive bacteria c, Staphylococcus aureus and d, Enterococcus faecalis. Control, bacteria incubated without BP-1. All studies represent one of three independent experiments.

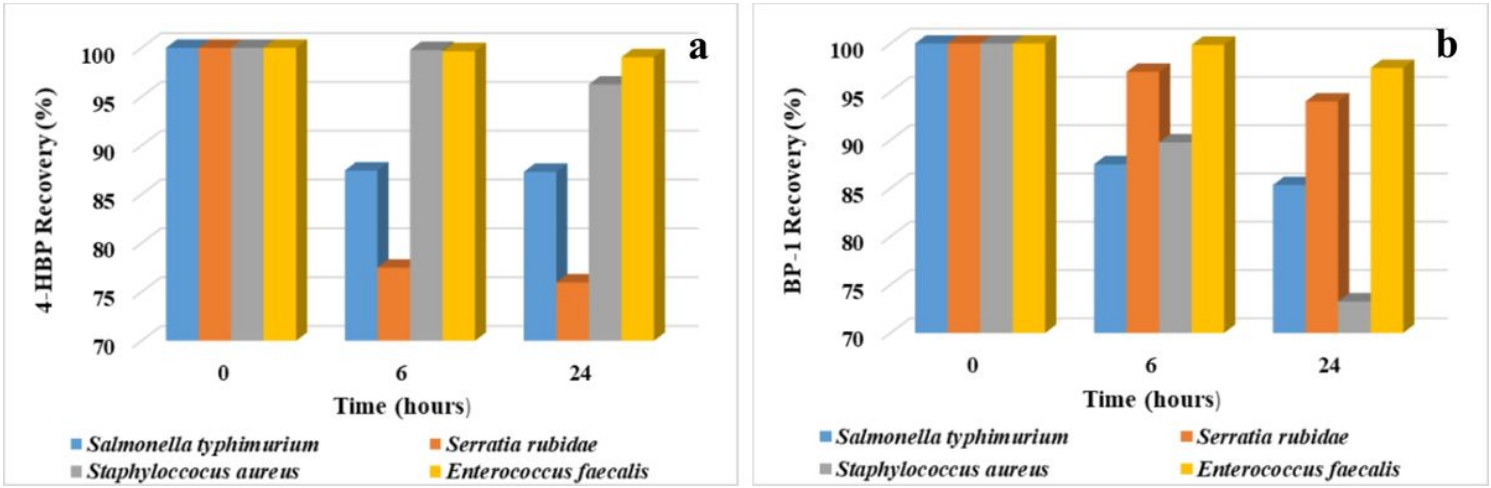

Figure 4

Biodegradation of a, 4-HBP and b, BP-1 by gram-positive and gram-negative bacterial strains. All studies represent one of three independent experiments.

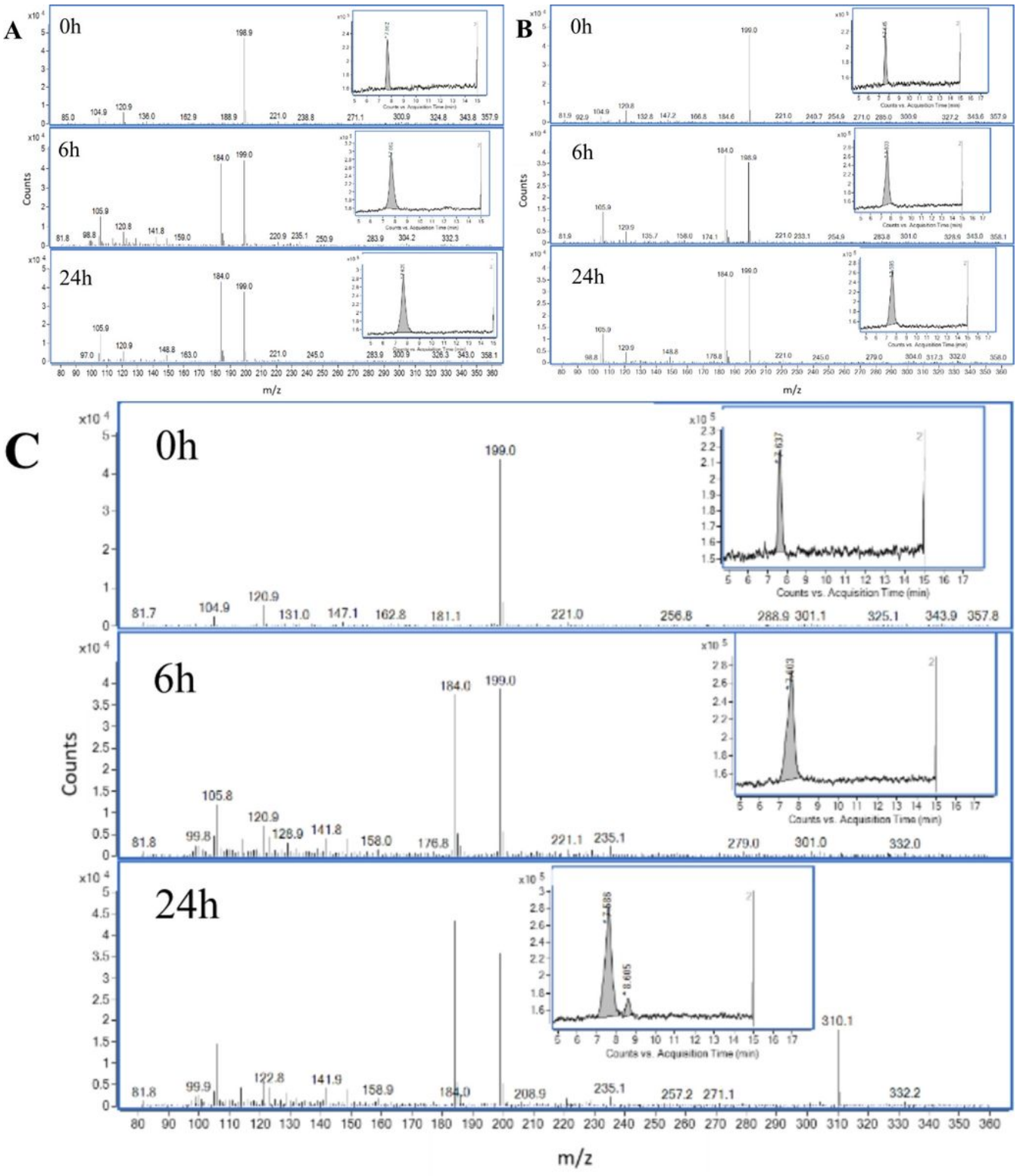

Figure 5

A: Mass spectra of 4-HBP and abiotic degradation product in liquid medium at 0h, 6h and 24h; B: Mass spectra of 4-HBP and degradation product by Salmonella typhimurium at 0h, 6h and 24h; and C: Mass spectra of 4-HBP and degradation product by Serratia rubidae at 0h, 6h and 24h.

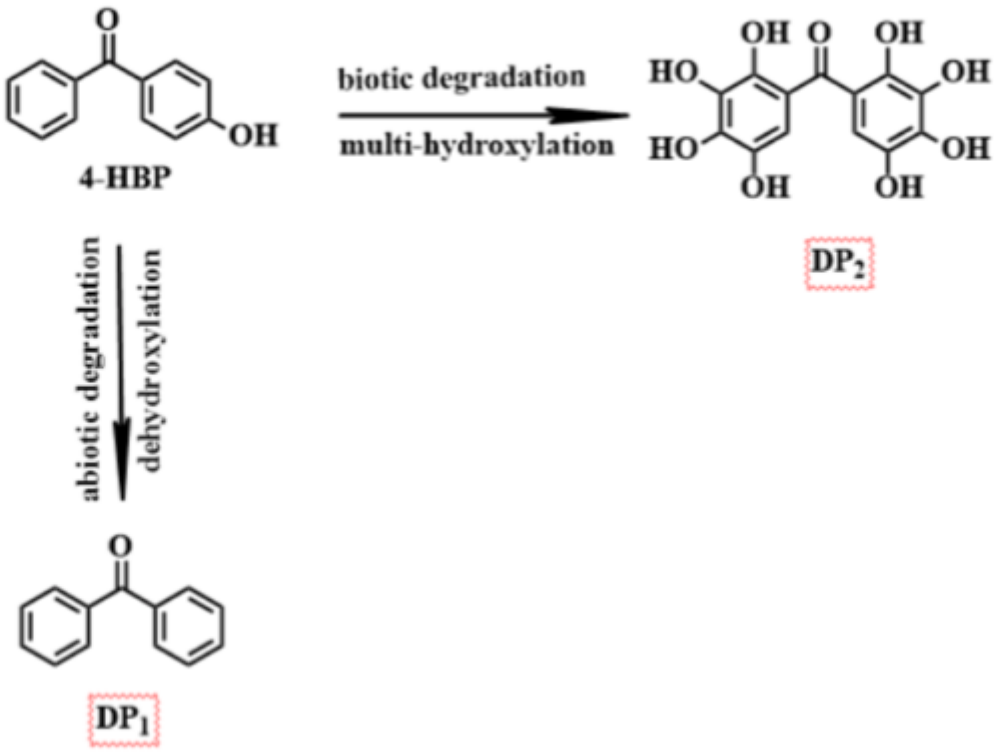

Figure 6

Proposed pathway for the biotic degradation of 4-HBP by Serratia rubidae strain and abiotic degradation in liquid medium