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Can microaeration boost the biotransformation of parabens in high-rate anaerobic systems?

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ABSTRACT

The main objective of the present study was to demonstrate microaeration as an effective strategy to boost the biotransformation of four parabens (methylparaben, ethylparaben, propylparaben, and butylparaben) in an upflow anaerobic sludge blanket reactor operated at a short hydraulic retention time (8 h). Moreover, the effect of different airflow rates (1–4 mL min^{-1}) was also assessed from an engineering and microbiological perspective. Low mean removal efficiencies (REs) (14–20 %) were achieved under anaerobic conditions. However, the addition of only 1 mL air min^{-1} (0.027 L O_2 L^{-1} feed) remarkably boosted the biotransformation of parabens, ensuring mean REs above 85 % for all compounds. In contrast, the increase in the airflow rate had a minor impact on the process, and an apparent saturation in the removal capacity was observed, noticeably from 2 to 4 mL air min^{-1}. The reactor presented high stability throughout the experiment, and microaeration did not impair the organic matter removal and methanogenesis. However, high airflow rates can dilute biogas, compromising its use as a fuel in combined heat and power units. The microaerobic conditions increased both richness and diversity of the reactor's microbiota, likely favoring the growth of oxygenase-producing microorganisms, which may have played a role in the biotransformation of parabens. Finally, the high REs of parabens reached in the microaerated reactor, a more cost-effective technology, are comparable to those found in high-cost wastewater treatment systems, such as activated sludge and its variants.

1. Introduction

The increasing presence of organic micropollutants (OMPs) in the environment is a result of the excessive use of synthetic organic compounds, namely pesticides, personal care/cosmetic products (PCPs), industrial chemicals, food additives and detergents, and some naturally occurring substances, such as estrogens (Goswami et al., 2018). Among these compounds, parabens, i.e., esters of p-hydroxybenzoic acid, are widely used in food, pharmaceuticals, and PCPs because they have excellent preservative properties, preventing the growth of Gram-positive bacteria, yeast, and mold (Haman et al., 2015; Ma et al., 2018). The parabens usually found on product labels are methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), butylparaben (BuP), isobutylparaben (iBuP), and benzylparaben (BeP) (Gałwa-Widera, 2019).

As an emerging group of endocrine-disrupting chemicals (EDCs), parabens have attracted growing attention due to their potential long-term effects on human health and aquatic organisms, such as increase in estrogenic activity, alteration in spermatogenesis and steroidogenesis, lipid accumulation, adipogenesis, breast cancer, and aquatic toxicity (Giulivo et al., 2016; Li et al., 2015). For instance, these compounds, even at very low concentrations (e.g., 1.52 μg L^{-1} of MeP, 18.0 μg L^{-1} of PrP, and 19.4 μg L^{-1} of BuP), were reported to be associated with the incidence of breast cancer (Błędzka et al., 2014; Lillo et al., 2017). Thus, the occurrence of parabens in water (0.0005–1062 ng L^{-1} of MeP, 0.17–147 ng L^{-1} of EtP, 0.1–2142 ng L^{-1} of PrP, and 0.03–181 ng L^{-1} of BuP) and wastewater (0.001–79.6 μg L^{-1} of MeP, 0.001–41 μg L^{-1} of EtP, 0.001–23.6 μg L^{-1} of PrP, and 0.001–17 μg L^{-1} of BuP) is an emerging concern, and it is necessary to remove them

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from wastewater before their release into water bodies (Haman et al., 2015).

These compounds can be efficiently removed (> 80 %) in high-cost wastewater treatment systems, such as activated sludge and its variants (Ashfaq et al., 2017; Karthikraj et al., 2017; Ma et al., 2018; Wang and Kannan, 2016). However, there are very few studies on the removal of parabens in continuous-flow anaerobic systems (Hernández Leal et al., 2010; Londoño and Peñuela, 2015), such as upflow anaerobic sludge blanket (UASB) reactors, a more cost-effective option for domestic wastewater treatment, widely used in warm-climate developing countries (e.g., Brazil, Colombia, and India) (Chernicharo et al., 2015). Additionally, although parabens can be anaerobically biotransformed, the reaction is very slow (Wu et al., 2017). Consequently, their removal may be limited in UASB reactors designed for domestic wastewater treatment, which usually are operated at short hydraulic retention times (HRTs) (6–8 h) (Chernicharo et al., 2015).

Therefore, some relatively simple and inexpensive strategies should be implemented to overcome this limitation, such as the injection of small amounts of oxygen into these systems (microaeration). In fact, despite being initially proposed for sulfide removal (Krayzelova et al., 2015), this technique has been recently reported to enhance considerably the biotransformation of recalcitrant compounds in high-rate anaerobic reactors, such as monoaromatic hydrocarbons (benzene, toluene, ethylbenzene and xylenes, i.e., BTEX) (Firmino et al., 2018; Siqueira et al., 2018) and even OMPs (hormones, pharmaceuticals, and bisphenol A) (Buarque et al., 2019). However, to the best of the authors' knowledge, there is no investigation into the removal of parabens under microaerobic conditions.

Hence, the main objective of the present study was to demonstrate microaeration as an effective strategy to boost the biotransformation of four parabens (MeP, EtP, PrP, and BuP) in a short-HRT (8 h) UASB reactor. Moreover, the effect of different airflow rates (1–4 mL min^{-1}) was also assessed from an engineering and microbiological perspective.

2. Material and methods

2.1. Synthetic wastewater

The synthetic wastewater consisted of an aqueous solution containing MeP, EtP, PrP, and BuP (~200 μg L^{-1} each) (> 99 %, Sigma-Aldrich, USA), ethanol (1 g COD L^{-1}), basal medium (macro and micronutrients), prepared according to Firmino et al. (2010), and sodium bicarbonate (1 g L^{-1}), to maintain the pH near 7.0.

2.2. Experimental set-up

The experiment was performed in a UASB reactor (working volume of 3.5 L) inoculated with anaerobic sludge (~50 g VSS L^{-1}) from a mesophilic UASB reactor of a domestic wastewater treatment plant (WWTP) (Fortaleza, Ceará, Brazil) and operated at an HRT of 8 h and room temperature of approximately 28 °C. The reactor was fed with the synthetic paraben-containing wastewater by a peristaltic pump (Minipuls 3, Gilson, USA) and, in some experimental periods, was microaerated with synthetic air (80 % N_2:20 % O_2, White Martins, Brazil) at its feeding line by a mass flow controller (GFC17, Aalborg, USA). The biogas produced was measured by a Mariotte flask containing a 3% sodium chloride solution at pH 2 (Fig. 1).

2.3. Experimental procedure

In period I, the removal of parabens was assessed under anaerobic conditions. Subsequently, from period II to IV, to investigate their removal under microaerobic conditions, the reactor was microaerated at increasing flow rates (1, 2, and 4 mL air min^{-1} at 28 °C and 1 atm, equivalent to 0.027, 0.055, and 0.110 L O_2 L^{-1} feed, respectively). Afterwards, in period V, to evaluate a likely adaptation of microbiota to microaerobic conditions, the microaeration flow rate was reduced to 1 mL min^{-1} (28 °C and 1 atm). Finally, in period VI, to reinforce the oxygen effect and eliminate the hypothesis of microbiota adaptation to the parabens throughout the experiment, the reactor was again operated under anaerobic conditions. The operational parameters of the reactor in each period are shown in Table 1.

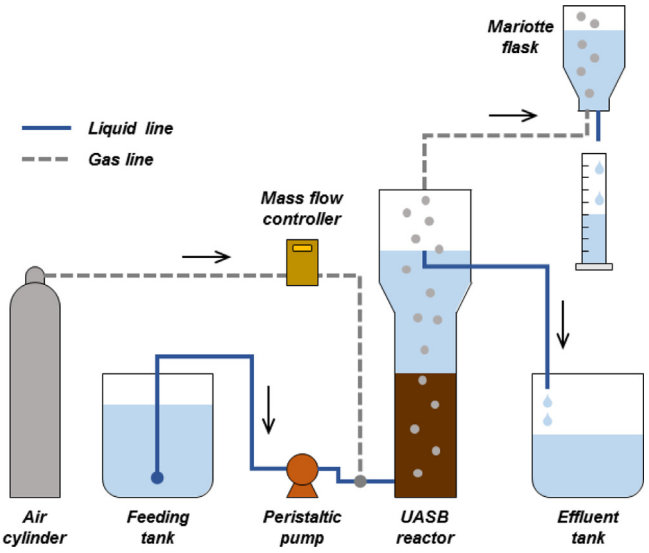

Fig. 1. Schematic of the experimental set-up.

2.4. Chemical analysis

The parabens were determined by an LC-20A Prominence high-performance liquid chromatograph (HPLC) equipped with a Shim-pack CLC-ODS(M)® C18 column (4.6 × 150 mm, 5 μm) and a UV–vis SPD-20A detector (215 nm) (Shimadzu Corporation, Japan). Firstly, 500 mL of pre-filtered samples (0.45 μm) were percolated through Oasis HLB cartridges (3 cc, 60 mg, 30 μm) (Waters Corporation, USA) for solid-phase extraction of parabens, which were then eluted by 4 mL of HPLC/UV grade methanol (99.8 %, Neon, Brazil). Afterwards, 10 μL of this methanolic solution was injected in the HPLC and eluted by a mobile phase composed of ultrapure water and HPLC/UV grade acetonitrile (99.9 %, Sigma-Aldrich, Germany) at a constant flow of 1.2 mL min^{-1}, using the following gradient: increase from 10 % to 60 % of acetonitrile in 8 min, followed by 10 % reduction in 4 min. The temperature of the oven was maintained at 35 °C throughout the run.

COD, alkalinity, and pH were determined according to APHA (2012). The volatile fatty acids (VFA) were determined by the Kapp titrimetric method (Buchauer, 1998). The levels of CH_4 and CO_2 in the biogas were determined by gas chromatography with barrier-discharge ionization detection (GC-BID) (GC-2010 Plus, Shimadzu Corporation, Japan). The biogas sample (1.0 mL) was injected in split mode (split ratio of 30), and the chromatographic separation was performed on a GS-GasPro column (60 m, 0.32 mm I.D.) (Agilent Technologies, USA). The temperatures of the injector and the detector were 100 and 250 °C, respectively. The temperature of the oven started at 50 °C, was raised to 75 °C at 5 °C min^{-1}, then to 105 °C at 8 °C min^{-1}, and was finally maintained at 105 °C for 0.25 min (total run time of 9 min). Helium (White Martins, Brazil) was used as the carrier gas at a flow rate of 2.0 mL min^{-1}. The levels of O_2 and N_2 in

Table 1
Operational parameters of the reactor throughout the experiment.

Period	I	II	III	IV	V	VI
End of period (day)	46	96	148	179	228	263
Microaeration (mL min^{-1})	–	1	2	4	1	–
Dose of oxygen (L O_2 L^{-1} feed)	–	0.027	0.055	0.110	0.027	–
HRT (h)	8.0	8.0	8.0	8.0	8.0	8.0
COD (g L^{-1})	1.0	1.1	1.0	1.1	1.1	1.0
MeP (μg L^{-1})	210	202	202	205	224	199
EtP (μg L^{-1})	222	210	204	210	222	195
PrP (μg L^{-1})	202	206	199	197	219	197
BuP (μg L^{-1})	203	186	206	205	197	204

BuP, buthylparaben; COD, chemical oxygen demand; EtP, ethylparaben; HRT, hydraulic retention time; MeP, methylparaben; PrP, propylparaben.

the biogas were determined by gas chromatography with thermal conductivity detection (GC-TCD) (GC-17A, Shimadzu Corporation, Japan). The biogas sample (1.0 mL) was injected in splitless mode, and the chromatographic separation was performed on a Mol Sieve 5A PLOT column (30 m, 0.32 mm I.D.) (Restek Corporation, USA). The temperatures of the injector, oven, and detector were 40, 35, and 230 °C, respectively. Helium (White Martins, Brazil) was used as the carrier gas at a flow of 7 mL min^{-1}, and the run time was 5 min.

2.5. Microbiological analysis

To evaluate the dynamics of the microbial community under microaerobic conditions, the DNAs of the inoculum and sludge samples collected at the end of the periods I (anaerobic), II (1 mL air min^{-1}), IV (4 mL air min^{-1}), and V (1 mL air min^{-1}) were extracted and then sequenced by an Illumina MiSeq Desktop Sequencer as detailed elsewhere (Rollemberg et al., 2019). Some ecological indices, namely Chao1 (richness), inverse Simpson (diversity), and Bray-Curtis dissimilarity, were calculated by Mothur software. Based on the latter index, UPGMA (unweighted pair group method with arithmetic mean) cluster analysis at genus level was also performed.

2.6. Statistical analysis

The Mann-Whitney and Kruskal-Wallis non-parametric tests, which do not require a specific data distribution, were used to compare the performance of the reactor during the different experimental periods at a 95 % confidence level.

3. Results and discussion

3.1. Removal of parabens under anaerobic conditions

In period I, under anaerobic conditions, the removal efficiencies (REs) of all parabens fluctuated significantly (Fig. 2), and the mean values were quite low (≤ 20 %), particularly for EtP (14 %) (Table 2). Nevertheless, there was no statistical difference among the REs of these compounds ($p = 0.158$).

According to Wu et al. (2017), although parabens can be anaerobically degraded, the reaction is very slow even in the presence of alternative terminal electron acceptors (SO_4^{2-}, Fe^{3+}, and NO_3^-), with REs of 43.2–70.1 % for MeP (1–10 mg L^{-1}) and 94.1–97.8 % for PrP (1–10 mg L^{-1}) being achieved only after 72 h and 48 h, respectively, in batch assays with activated sludge. Probably, the recalcitrance of parabens under such conditions is related to their ester functional group (—COOR), as electron-withdrawing groups tends to hamper anaerobic biotransformation (Wijekoon et al., 2015). Therefore, in short-HRT anaerobic systems (< 10 h), such as the UASB reactors designed for domestic wastewater treatment, whose usual HRT ranges from 6 to 8 h (Chernicharo et al., 2015), the removal of parabens may be limited.

In fact, in anaerobic reactors operated at longer HRTs (> 10 h), higher REs of parabens were reached. For instance, Londoño and Peñuela (2015) found REs of MeP above 80 % in an anaerobic expanded granular sludge bed (EGSB) reactor fed with synthetic wastewater containing different concentrations of this compound (300–1000 μg L^{-1}) and glucose (~1 g COD L^{-1}) when operated at a long HRT (26–27 h). Additionally, Hernández Leal et al. (2010) evaluated a UASB reactor operated at an HRT of 12 h for the treatment of gray water (830 mg COD L^{-1}) containing some xenobiotics, including PrP (2.9 μg L^{-1}) and BuP (0.9 μg L^{-1}), and found mean REs of approximately 75 % and 67 %, respectively.

3.2. Removal of parabens under microaerobic conditions

In period II, with the airflow rate of 1 mL min^{-1} (0.027 L O_2 L^{-1} feed), the mean REs of all evaluated compounds were above 85 % (Table 2). Therefore, it was evident that the addition of small amounts of oxygen to the reactor significantly favored the removal of parabens ($p < 0.001$). Interestingly, even at a very low airflow rate, there was no apparent limitation on the gas-liquid mass transfer, since a low oxygen content was found in the biogas (< 15 % of the added amount) (data not shown), i.e., oxygen seemed to be effectively solubilized in the liquid and promptly used by microaerophilic or facultative microorganisms.

Although there are no reports on the effect of microaeration on the removal of parabens in anaerobic reactors, previous studies have shown that this technique can significantly improve the biotransformation of recalcitrant compounds, such as BTEX (Firmino et al., 2018; Siqueira et al., 2018) and OMPs (Buarque et al., 2019). For example, Siqueira et al. (2018) found that microaeration at a flow rate of 1 mL air min^{-1} (0.14 L O_2 L^{-1} feed at 27 °C and 1 atm) increased the REs of BTEX, mainly for benzene (from 55 % to 84 %), in a UASB reactor (HRT of 24 h) fed with water contaminated with ethanol (1 g COD L^{-1}) and these monoaromatics (4–5 mg L^{-1} each). Similarly, Buarque et al. (2019) also observed an increase in the REs of seven OMPs (~230 μg L^{-1} each) from less than 10 % to more than 50 % in a UASB reactor (HRT of 7 h) treating synthetic wastewater (~1 g COD L^{-1}) after being microaerated at 1 mL air min^{-1} (0.021 L O_2 L^{-1} feed at 27 °C and 1 atm).

Under both aerobic and anaerobic conditions, the initial degradation of esters can take place through the hydrolysis of the ester bond by esterases or lipases, producing a carboxylic acid and an alcohol (Ghattas et al., 2017; Valkova et al., 2001). Specifically for parabens, the generated carboxylic acid is p-hydroxybenzoic acid (Wang et al., 2018), which can be further degraded through different pathways depending on the redox condition. Accordingly, under aerobic conditions, p-hydroxybenzoate (the conjugate base of the aforementioned acid) is converted into protocatechuate and then cleaved, both reactions catalyzed by oxygenases (β-ketoadipate pathway), whereas, under anaerobic conditions,

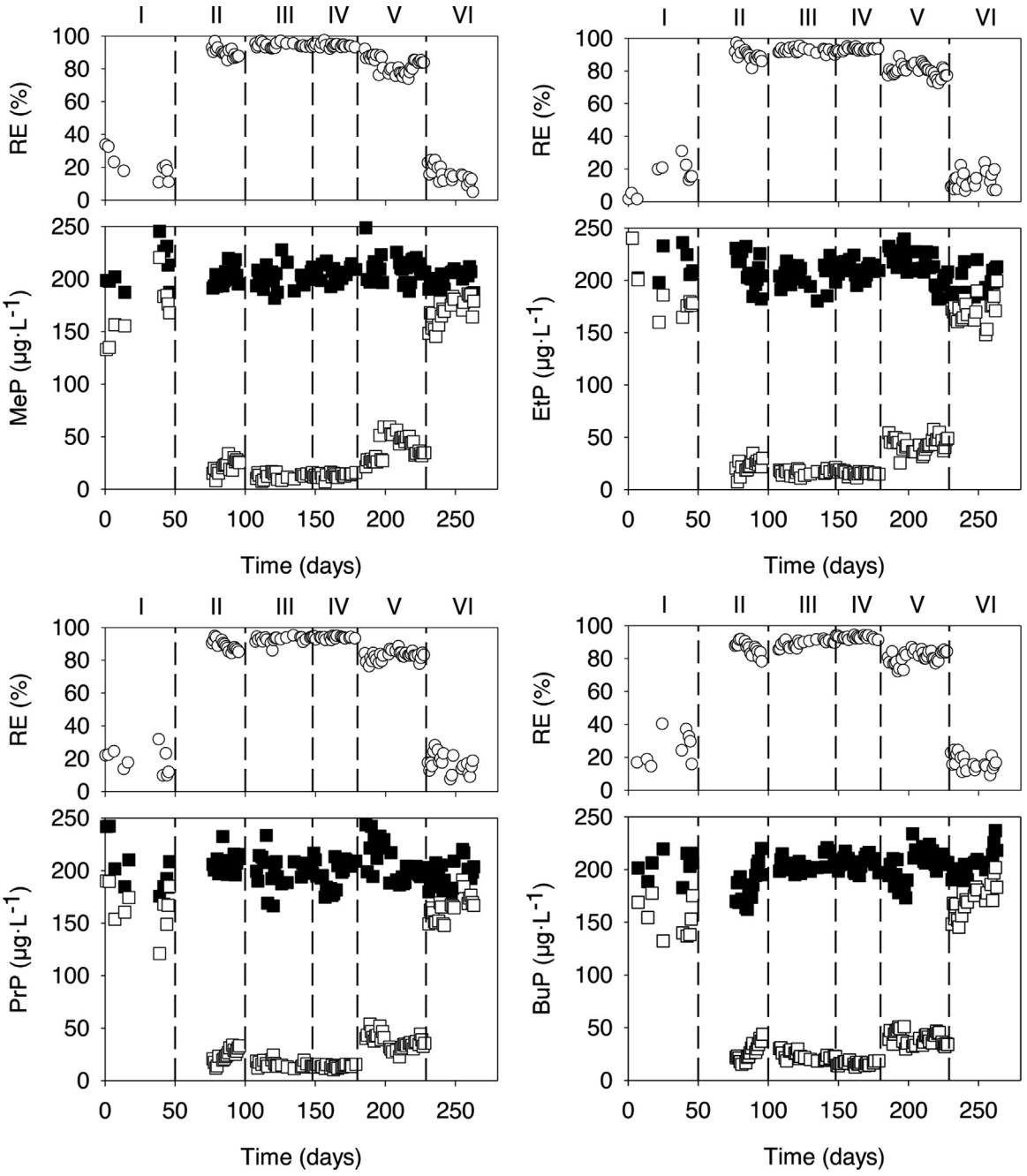

Fig. 2. Influent (■) and effluent (□) concentrations and removal efficiencies (REs) (○) of methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), and butylparaben (BuP) throughout the experiment.

Table 2
Mean removal efficiencies of the parabens throughout the experiment.

Period	I	II	III	IV	V	VI
Microaeration ($mL\,min^{-1}$)	–	1	2	4	1	–
MeP (%)	20.2 (8.1)	89.2 (2.8)	93.6 (1.4)	93.7 (1.1)	81.7 (4.8)	15.5 (4.6)
EtP (%)	14.0 (9.6)	89.0 (3.6)	91.8 (1.4)	92.5 (0.9)	79.6 (3.6)	12.4 (5.1)
PrP (%)	18.0 (7.3)	88.3 (3.1)	92.0 (1.9)	93.1 (0.7)	82.1 (2.7)	16.5 (5.5)
BuP (%)	20.0 (13.9)	85.5 (3.8)	88.5 (2.0)	92.2 (0.9)	80.3 (3.8)	16.1 (4.3)

BuP, buthylparaben; EtP, ethylparaben; MeP, methylparaben; PrP, propylparaben.
The standard deviation is shown in parentheses.

Table 3
Parameters of operational stability of the reactor throughout the experiment.

Period	I	II	III	IV	V	VI
Microaeration (mL min^{-1})	–	1	2	4	1	–
COD removal (%)	87.7 (1.6)	89.9 (0.8)	87.7 (1.6)	90.1 (1.0)	89.4 (0.9)	86.6 (1.9)
Biogas production (L d^{-1})	2.1 (0.1)	3.4 (0.3)	6.7 (0.2)	10.5 (0.2)	3.6 (0.1)	2.2 (0.2)
CH_4 in the biogas (%)	81 (2)	65 (2)	46 (6)	21 (5)	79 (2)	85 (2)
pH	7.2 (0.4)	7.1 (0.4)	7.1 (0.3)	7.0 (0.4)	7.2 (0.2)	7.4 (0.3)
VFA (mg L^{-1})	260 (27)	186 (54)	233 (42)	214 (40)	247 (25)	237 (85)
VFA/TA	0.4 (0.1)	0.3 (0.1)	0.4 (0.1)	0.3 (0.1)	0.4 (0.1)	0.4 (0.1)

COD, chemical oxygen demand; TA, total alkalinity; VFA, volatile fatty acids.
The standard deviation is shown in parentheses.

Table 4
Ecological indices of richness (Chao1) and diversity (inverse Simpson) for the inoculum and samples collected at the end of periods I (anaerobic), II (1 mL air min^{-1}), IV (4 mL air min^{-1}), and V (1 mL air min^{-1}).

Sample	OTUs[a]	Chao1	Inverse Simpson
Inoculum	1196	2042	7.68
I	1622	2695	8.67
II	1869	3235	9.85
IV	2188	4225	30.43
V	1419	2466	22.09

[a] Number of operational taxonomic units.

p-hydroxybenzoate is converted into benzoyl-CoA, the central intermediate of anaerobic degradation of aromatic compounds (Fuchs et al., 2011).

According to the literature, the aerobic degradation of aromatic compounds is faster than the anaerobic one because oxygen is a much more favorable terminal electron acceptor (Ghattas et al., 2017; Weelink et al., 2010). However, under microaerobic conditions, oxygen is not used as the terminal electron acceptor. Actually, oxygenase-producing microorganisms only use oxygen for the hydroxylation of the aromatic ring, facilitating its cleavage and further anaerobic degradation, i.e., a hybrid pathway (Fuchs, 2008). Therefore, in the current investigation, the addition of small amounts of oxygen to the UASB reactor may have stimulated the activity of oxygenase enzymes, favoring the biotransformation of parabens, probably though hydroxylation.

In period III, the microaeration flow rate was increased to 2 mL air min^{-1} (0.055 L O_2 L^{-1} feed), and the mean REs of parabens were higher than 90 %, except for BuP (Table 2). Although the difference between the mean REs obtained in periods II and III was not so high (2.7–4.5 % depending on the compound) (Table 2), it was still statistically significant for all parabens ($p < 0.050$). Moreover, the efficiency values in period III were more stable than in period II (Fig. 2). Thus, the increase in the airflow rate from 1 to 2 mL min^{-1} had indeed a positive effect on the biotransformation of parabens. In period IV, when the microaeration flow rate was 4 mL air min^{-1} (0.110 L O_2 L^{-1} feed), the mean REs of all parabens were higher than 90 % and slightly above the values observed in period III (Table 2). However, this increase was significant only for PrP ($p = 0.013$) and BuP ($p < 0.001$). Therefore, a saturation in the biotransformation capacity of parabens in the microaerobic system may have occurred. Since the mass transfer remained effective (residual oxygen in the biogas < 27 % of the added amount), this saturation is likely related to biochemical limitations rather than a lack of oxygen in the medium. These results agree with those by Siqueira et al. (2018), who also observed a positive correlation between the microaeration flow rate and the REs of BTEX and a saturation in the removal capacity of their UASB reactor when the airflow rate was raised from 1 to 2 mL min^{-1} (from 0.14 to 0.27 L O_2 L^{-1} feed).

In period V, when the airflow rate was reduced back to 1 mL min^{-1} (0.027 L O_2 L^{-1} feed), as expected, the mean REs of all parabens decreased (11–13 %). Surprisingly, the values were even lower than those obtained in period II ($p < 0.001$), when the same airflow rate was used (Table 2). Nevertheless, they remained close to 80 %. Finally, in period VI, microaeration was interrupted to reestablish the anaerobic conditions. Consequently, the REs of parabens were similar to those obtained at the beginning of the experiment, when the reactor was also operated under anaerobic conditions (period I). Therefore, these results reinforce the importance of oxygen availability to the biotransformation of parabens and exclude the hypothesis of microbiota adaptation to these compounds throughout the experiment.

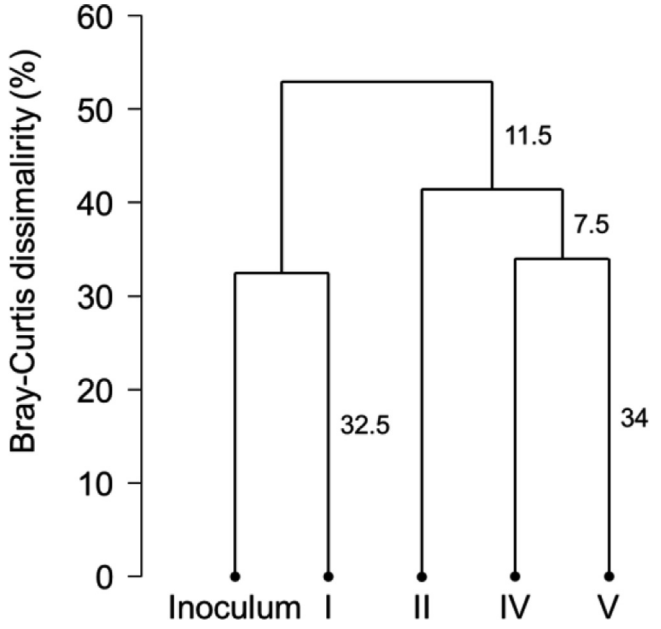

Fig. 3. UPGMA cluster analysis at genus level based on Bray-Curtis dissimilarity index for the inoculum and samples collected at the end of periods I (anaerobic), II (1 mL air min^{-1}), IV (4 mL air min^{-1}), and V (1 mL air min^{-1}).

Lastly, it is important to highlight that the high REs of parabens (> 90 %) reached in the present work, with a more cost-effective system (microaerated UASB reactor), are comparable to those found in high-cost wastewater treatment systems, such as activated sludge and its variants (Ashfaq et al., 2017; Karthikraj et al., 2017; Ma et al., 2018; Wang and Kannan, 2016).

3.3. Operational stability of the system

During the entire experiment, the reactor remained remarkably stable, with high mean COD REs (85–90 %), no accumulation of VFA, and pH values close to the neutral range (Table 3). Therefore, neither the parabens nor microaeration impaired organic matter removal. Regarding the methane content in the biogas, the higher

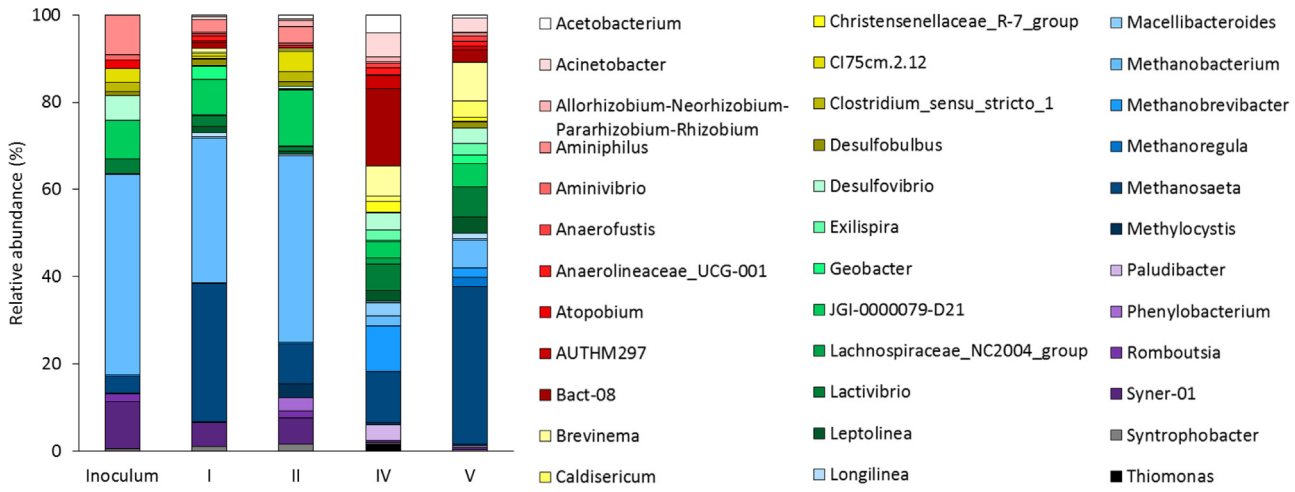

Fig. 4. Microbial diversity at genus level of the inoculum and samples collected at the end of periods I (anaerobic), II (1 mL air min^{-1}), IV (4 mL air min^{-1}), and V (1 mL air min^{-1}).

the airflow rate, the lower this content (Table 3). However, this reduction in the methane content is only a consequence of biogas dilution with nitrogen from the synthetic air (80 %) injected into the system, i.e., microaeration did not harm methanogenesis. However, a very low methane content, as observed in period IV (Table 3), may be a problem if the biogas is intended to be used in combined heat and power plants, which require a minimum limit of 40 % (Haubrichs and Widmann, 2006). Therefore, for situations in which a greater availability of oxygen is necessary to ensure high REs of the target pollutants, the use of pure oxygen as the microaeration source is a way of overcoming the biogas dilution problem.

3.4. Dynamics of the microbial community of the system

Apparently, in period I, the exposure to a different substrate (ethanol + parabens) increased the richness and diversity of the microbiota when compared to the inoculum (Table 4), anaerobic sludge from a full-scale UASB reactor that treats domestic wastewater. From period II to IV, when the reactor was continuously microaerated at increasing flow rates (from 1 to 4 mL air min^{-1}), both ecological attributes continued to increase, reaching their maximum values at the end of the period IV (Table 4), when the highest airflow rate was applied to the system. Therefore, microaeration seemed to exert a significant selection pressure on the microbial community, favoring the growth of different species, probably those microaerophilic or facultative able to synthesize oxygenase enzymes, which may have played a role in the biotransformation of parabens. In agreement with the present results, Buarque et al. (2019) and Firmino et al. (2018) also observed an increase in the richness and diversity of the microbiota of their UASB reactors when operated under microaerobic conditions.

In period V, when the airflow rate was set back to 1 mL min^{-1}, the number of observed species (or OTUs, operational taxonomic units) decreased remarkably, reaching a value even lower than that observed in period I (anaerobic) (Table 4). Probably, the lower availability of oxygen may have impaired the survival of some microaerophilic microorganisms that grew throughout the previous microaerobic periods. Nevertheless, the inverse Simpson index remained high (Table 4), i.e., the microbial community maintained a high diversity/evenness (no dominance of specific groups). In fact, the sample of this period is more similar to that of period IV (4 mL air min^{-1}) than that of period II (Fig. 3), when the reactor was also microaerated at 1 mL air min^{-1}.

Concerning the archaeal community, its relative abundance remained above 45 % except in period IV, when it decreased to approximately 25 %. Although the granular structure of the anaerobic sludge tends to protect these strictly anaerobic microorganisms from oxygen in the granule core (Baloch et al., 2008; Picioreanu et al., 2005), an airflow rate as high as 4 mL min^{-1} may have allowed a deeper diffusion of this gas into the sludge granule, inhibiting some less oxygen-resistant archaeal species. Nevertheless, even in this period, the methanogenic activity was not compromised (Section 3.3). This is an important observation because, in anaerobic consortia, the degradation of aromatic compounds is hardly performed by a single species, but most likely through syntrophic relationships between fermentative bacteria and methanogenic archaea (Gieg et al., 2014).

With respect to the bacterial community, the relative abundance of some genera seemed to increase under microaerobic conditions, namely *Acetobacterium*, *Acinetobacter*, *Brevinema*, *Caldisericum*, *Desulfovibrio*, *Exilispira*, *Lactivibrio*, *Leptolinea*, and *Longilinea* (Fig. 4). However, just a few have been reported to be directly related to the degradation of aromatic compounds, such as *Acinetobacter* (Jung and Park, 2015) and *Longilinea* (Zhu et al., 2018), and nitrogen-based heterocyclic compounds, such as *Caldisericum* (Shi et al., 2019).

4. Conclusions

Low mean REs (14–20 %) were achieved under anaerobic conditions, but the addition of only of 1 mL air min^{-1} (0.027 L O_2 L^{-1} feed) remarkably boosted the biotransformation of parabens, ensuring mean REs above 85 % for all compounds. In contrast, the increase in the airflow rate had a minor impact on the process, and an apparent saturation in the removal capacity was observed, noticeably from 2 to 4 mL air min^{-1}.

The reactor presented high stability throughout the experiment, and microaeration did not impair the organic matter removal and methanogenesis. However, high airflow rates can dilute biogas, compromising its use as a fuel in combined heat and power units.

The microaerobic conditions increased both richness and diversity of the microbiota of the reactor, likely favoring the growth of oxygenase-producing microorganisms, which may have played a role in the biotransformation of parabens.

Finally, the high REs of parabens reached in the microaerated reactor, a more cost-effective technology, are comparable to those found in high-cost wastewater treatment systems, such as activated sludge and its variants.

Declaration of Competing Interest

The authors report no declarations of interest.

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