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Microaeration improves the removal/biotransformation of organic micropollutants in anaerobic wastewater treatment systems

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ABSTRACT

This work assessed the effect of increasing microaeration flow rates (1–6 mL min^{-1} at 28 °C and 1 atm, equivalent to 0.025–0.152 L O_2 L^{-1} feed) on the removal/biotransformation of seven organic micropollutants (OMPs) (three hormones, one xenoestrogen, and three pharmaceuticals), at 200 μg L^{-1} each, in a lab-scale upflow anaerobic sludge blanket reactor operated at a hydraulic retention time (HRT) of 7.4 h. Additionally, the operational stability of the system and the evolution of its microbial community under microaerobic conditions were evaluated. Microaeration was demonstrated to be an effective strategy to improve the limited removal/biotransformation of the evaluated OMPs in short-HRT anaerobic wastewater treatment systems. The rise in the airflow rate considerably increased the removal efficiencies of all OMPs. However, there seems to be a saturation limit for the biochemical reactions. Then, the best results were obtained with 4 mL air min^{-1} (0.101 L O_2 L^{-1} feed) (~90%) because, above this flow rate, the efficiency increase was negligible. The long-term exposure to microaerobic conditions (249 days) led the microbiota to a gradual evolution. Consequently, there was some enrichment with species potentially associated with the biotransformation of OMPs, which may explain the better performance at the end of the microaerobic term even with the lowest airflow rate tested.

1. Introduction

In the last decades, organic micropollutants (OMPs), such as pharmaceuticals, pesticides, hormones, personal care products, and others, have drawn much attention due to their potential negative impacts on ecosystems and public health (e.g., development of antibiotic-resistant pathogens and reproductive disorder in animals and humans by endocrine disruptors) (Gogoi et al., 2018; Harb et al., 2019). The main sources of water pollution with such compounds are raw or treated domestic, agricultural, and some industrial wastewaters along with urban and rural runoff, in which OMPs may be found at concentrations ranging from few ng to several μg per liter (Gogoi et al., 2018; Luo et al., 2014), indicating that conventional wastewater treatment technologies are usually ineffective in removing OMPs (Wang and Wang, 2016).

The use of biological processes for the removal of OMPs from wastewater is extensively reported in the literature, but the efficiencies vary widely, depending on the technology and redox conditions used (Grandclément et al., 2017; Luo et al., 2014). Usually, anaerobic systems are less efficient than the aerobic ones for most OMPs (Alvarino et al., 2014, 2018, 2018; Harb et al., 2019), particularly when operated at short hydraulic retention times (HRTs) (<10 h) (Brandt et al., 2013; Buarque et al., 2019; Vassalle et al., 2020).

The upflow anaerobic sludge blanket (UASB) reactor is a consolidated wastewater treatment technology in developing countries, especially those with tropical climate, such as Brazil, Colombia, and India (Chernicharo et al., 2015). Thus, the use of approaches to enhance the removal of OMPs in such an anaerobic system treating domestic wastewater, specifically designed to be operated at short HRTs (usually between 6 and 8 h), is needed.

According to Harb et al. (2019), the compound-biomass contact time is an important factor for the anaerobic biotransformation of OMPs, i.e., longer contact times favors the process. The easiest way to increase the contact time is to use a longer HRT. However, there is a practical limit for low-strength wastewaters, such as domestic wastewater. Thus, other strategies have been recommended to increase the retention and, consequently, biotransformation of OMPs inside the reactors, such as the

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use of adsorbents (e.g., activated carbon) as supporting material (attached growth reactors) or highly selective membranes (anaerobic membrane bioreactors) (Harb et al., 2019). However, depending on the socioeconomics scenario of some regions of the aforementioned developing countries, these modifications may be technically and economically unfeasible.

In this context, an alternative that may be relatively more cost-effective and easier to operate is to microaerate the anaerobic reactors, which consists of injecting small amounts of oxygen, i.e., up to 1 L O_2 L^{-1} feed (Krayzelova et al., 2015), into these systems to facilitate the initial degradation of recalcitrant compounds (e.g., aromatic hydrocarbons) by probable monooxygenase-producing microorganisms (Firmino et al., 2018; Fuchs, 2008; Siqueira et al., 2018). In fact, this technique was already demonstrated to improve considerably the removal of OMPs (from less than 10% to more than 50%) in an anaerobic reactor without compromising its overall performance and stability (Buarque et al., 2019). However, this previous work investigated only one airflow rate (1 mL min^{-1} at 27 °C and 1 atm, equivalent to 0.021 L O_2 L^{-1} feed). Consequently, as the equivalent dose of oxygen used by Buarque et al. (2019) was far below the aforementioned limit of 1 L O_2 L^{-1} feed, it opens up the possibility of using higher microaeration flow rates to increase even more the removal efficiencies of OMPs in anaerobic reactors. On the other hand, the higher availability of oxygen in the system may inhibit the methanogenic activity, which is reported to be directly related to the anaerobic biotransformation of some OMPs (Gonzalez-Gil et al., 2018). Thus, evaluating different airflow rates is critical to know the limits of this engineering approach regarding both system efficiency and stability and then consolidate microaeration as a viable technology to be applied to full-scale anaerobic systems to improve their removal of OMPs. However, to the best of the authors' knowledge, the effect of the variation in the microaeration flow rate on the removal/biotransformation of OMPs in anaerobic systems remains unknown.

Hence, this work aimed to assess the effect of increasing microaeration flow rates on the removal/biotransformation of seven OMPs frequently found in wastewaters, namely the hormones estrone (E1), 17β-estradiol (E2), and 17α-ethinylestradiol (EE2), the xenoestrogen bisphenol A (BPA), and the pharmaceuticals diclofenac (DCF), sulfamethoxazole (SMX), and trimethoprim (TMP), in a UASB reactor. Additionally, the operational stability of the system and the evolution of its microbial community under microaerobic conditions were evaluated.

2. Material and methods

2.1. Synthetic wastewater

The synthetic wastewater consisted of an aqueous solution containing the OMPs (~200 μg L^{-1} each) E1 (natural hormone, 99%, Sigma-Aldrich, USA), E2 (natural hormone, 98%, Sigma-Aldrich, USA), EE2 (synthetic hormone, 100%, Sigma-Aldrich, USA), BPA (xenoestrogen, 99%, Sigma-Aldrich, USA), DCF (anti-inflammatory, 98.5%, Sigma-Aldrich, USA), SMX (antibiotic, 99%, Sigma-Aldrich, USA), and TMP (antibiotic, 98%, 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. All reagents were used as purchased without further purification.

2.2. Experimental set-up

The continuous-flow experiment was carried out in an upflow anaerobic sludge blanket (UASB) reactor with a working volume of 3.5 L and an HRT of 7.4 h. The reactor was inoculated with mesophilic anaerobic sludge (~50 g VSS L^{-1}) from a UASB reactor of a domestic wastewater treatment plant (WWTP) located in Fortaleza, Ceará, Brazil.

The reactor was fed with the synthetic wastewater (described in section 2.1) by a peristaltic pump (Minipuls 3, Gilson, USA) and operated at room temperature of approximately 28 °C. In some experimental periods, the reactor was microaerated at the feeding line with synthetic air (80% N_2:20% O_2, White Martins, Brazil), whose flow rate was controlled 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.

2.3. Experimental procedure

The experiment with the OMP-containing wastewater was carried out in seven periods (Table 1). Initially, in period I, the removal of the different OMPs was evaluated under anaerobic conditions (control period). Then, to investigate the effect of microaeration on the removal/biotransformation of such compounds, different flow rates of synthetic air (1, 2, 4, and 6 mL min^{-1} at 28 °C and 1 atm, equivalent to 0.025, 0.051, 0.101, and 0.152 L O_2 L^{-1} feed, respectively) were tested from period II to V. Subsequently, in period VI, 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 VII, to reinforce the oxygen effect and eliminate the hypothesis of microbiota adaptation to the OMPs throughout the experiment, the reactor was again operated under anaerobic conditions. The experimental periods were changed after verifying system stability.

2.4. Chemical analysis

For the quantification of the OMPs, the samples (500 mL) were previously filtered (0.45 μm) and acidified with HCl (pH 2.5–3). Then, they were percolated through Strata-X® cartridges (500 mg, 6 mL) (Phenomenex®, USA) for the solid phase extraction (SPE) of the OMPs, which were eluted with HPLC/UV grade methanol (4 mL) (99.8%, Neon, Brazil). The eluate (20 μL) was then analyzed by an LC-20A Prominence high-performance liquid chromatograph (HPLC) equipped with a Shimpack CLC-ODS(M)® C18 column (4.6 × 150 mm, 5 μm) and a UV–Vis SPD-20A detector (258 nm) (Shimadzu Corporation, Japan). The elution was performed by mobile phase composed of HPLC/UV grade acetonitrile (99.9%, Sigma-Aldrich, Germany) and 0.1% HCl solution with the following gradient: 10%–80% increase in acetonitrile in 10 min, returning to 10% in 4 min. The flow rate was initially 1.0 mL min^{-1} and, after 5 min of run, it was increased to 2.0 mL min^{-1}. The oven temperature 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 biogas was characterized in terms of CH_4, CO_2, O_2, and N_2. CH_4 and CO_2 were quantified by gas chromatography with barrier-discharge ionization detection (GC-BID)

Table 1
Operational conditions of the reactor throughout the experiment.

Period	I	II	III	IV	V	VI	VII
End of period (day)	106	155	263	290	318	355	372
Microaeration (mL min^{-1})	–	1	2	4	6	1	–
Dose of oxygen (L O_2 L^{-1} feed)	–	0.025	0.051	0.101	0.152	0.025	–
HRT (h)	7.4	7.4	7.4	7.4	7.4	7.4	7.4
COD (g L^{-1})	1.0	1.0	1.0	1.0	1.0	1.0	1.0
E1 (μg L^{-1})	221	199	200	213	212	202	219
E2 (μg L^{-1})	230	213	198	198	197	205	200
EE2 (μg L^{-1})	213	223	196	204	216	202	196
BPA (μg L^{-1})	208	210	206	211	202	207	212
DCF (μg L^{-1})	232	203	201	204	205	200	217
SMX (μg L^{-1})	203	205	200	209	207	205	216
TMP (μg L^{-1})	230	205	196	207	210	207	214

BPA, bisphenol A; COD, chemical oxygen demand; DCF, diclofenac; E1, estrone; E2, 17β-estradiol; EE2, 17α-ethinylestradiol; HRT, hydraulic retention time; SMX, sulfamethoxazole; TMP, trimethoprim.

(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}. O_2 and N_2 were quantified 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 II (1 mL air min^{-1}), V (6 mL air min^{-1}), and VI (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 OMPs under anaerobic conditions

Under anaerobic conditions (period I), the removal efficiencies (REs) of all OMPs were considerably low (~20%) (Fig. 1), with the lowest mean value achieved for BPA (Table 2). Actually, apart from this xenoestrogen, no significant difference was found among the REs of the other studied OMPs ($p = 0.440$).

These results are in agreement with those reported in previous studies (Buarque et al., 2019; Vassalle et al., 2020). Buarque et al. (2019), for instance, using a lab-scale mesophilic UASB reactor (28 °C) with a 7-h HRT to treat synthetic wastewater containing the same mixture of the OMPs used in the present investigation (~230 μg L^{-1} of each compound) and ethanol (1 g COD L^{-1}) as co-substrate, obtained very low mean REs (4–9%). Vassalle et al. (2020), assessing the removal of OMPs (20–80 ng L^{-1}), such as BPA, DCF, E1, E2, EE2, and others, from domestic wastewater (~0.5 g COD L^{-1}) in a full-scale mesophilic UASB reactor (22 °C and HRT of 7 h), despite observing mean REs of hormones as high as 84%, also reported low mean REs of pharmaceuticals and xenoestrogens (<22%), particularly of BPA (~1.5%). Therefore, even at concentrations much lower than those used in the current study, the REs of most OMPs were low, which shows the recalcitrance of such compounds under anaerobic conditions.

In fact, according to the literature, despite some exceptions, such as the antibiotics SMX and TMP, OMPs are typically removed more efficiently under aerobic conditions than under anaerobic conditions (Alvarino et al., 2014, 2018, 2018; Brandt et al., 2013; Harb et al., 2019). However, in aerobic systems, adsorption on sludge may be a relevant removal mechanism, accounting for up to 30% of the influent load. On the other hand, in anaerobic systems, due to the long solid retention times (SRTs) (>70 d), the aforementioned mechanism may be negligible in the long term (up to 3% of the influent load), as the sludge blanket tends to saturate very quickly (up to 1 week depending on the pollutant concentration). Therefore, although anaerobic systems are usually less efficient, biotransformation is their main removal mechanism of OMPs (Harb et al., 2019).

The recalcitrance of OMPs under anaerobic conditions is probably related to their (poly)aromatic structure, which confers to them high stability in oxygen-free environments (Aquino et al., 2013). Hence, in general, oxygen may play an essential role in the biotransformation of several OMPs, in which the hydroxylation reaction is reported as an important step (Chen et al., 2018; Jewell et al., 2016; Poirier-Larabie et al., 2016; Yu et al., 2013; Zhang et al., 2013). Although the hydroxylation of aromatic compounds (e.g., benzene) can also occur anaerobically, it is much more favorable in the presence of oxygen, which is used by oxygenase enzymes and inserted into the molecules as hydroxyl groups (Foght, 2008; Fuchs et al., 2011).

Furthermore, it is worth mentioning that the REs of OMPs in anaerobic systems depend not only on the physicochemical properties of the compounds but also on the operational parameters of the reactors, mainly the HRT, since it is directly related to the substrate-microorganisms contact time (Harb et al., 2019). Therefore, anaerobic reactors with longer HRTs (19–24 h) tend to reach higher REs of some OMPs (e.g., >80–100% for SMX and TMP, and 60–80% for E2) than those obtained in the present work (Alvarino et al., 2014, 2019, 2019; Arias et al., 2018).

However, for low-strength wastewaters, such as domestic wastewater, using long HRTs (>10 h) is not viable (Harb et al., 2019). Additionally, the longer the HRT, the greater the reactor volume and, therefore, the investment costs (capital expenditures, CAPEX). Thus, other strategies to improve the removal/biotransformation of OMPs in anaerobic systems operated at short HRTs (6–8 h) should be used. Besides increasing the retention of the compounds inside the reactor by using adsorbents or membranes (Harb et al., 2019), one of those strategies would be the injection of small amounts of oxygen (microaeration) (up to 1 L O_2 L^{-1} feed) (Krayzelova et al., 2015) into the anaerobic system to facilitate the initial biotransformation of OMPs without compromising its overall performance and stability (Buarque et al., 2019), especially methanogenesis, an important step for anaerobic biotransformation of OMPs (Gonzalez-Gil et al., 2018), as it will be discussed below.

3.2. Removal of OMPs under microaerobic conditions

In period II, with the application of microaeration (1 mL air min^{-1}) (0.025 L O_2 L^{-1} feed), although the fluctuation in the RE values has remained (Fig. 1), the mean REs of all compounds were higher than 50%, except for DCF and SMX, which presented lower values ($p < 0.050$) (Table 2). Therefore, it was evident that the microaerobic conditions significantly favored the removal/biotransformation of OMPs compared to the anaerobic conditions (period I) ($p < 0.001$). In general, hydrophobic OMPs ($\log D_{pH\ 8} > 3.2$) present higher REs than hydrophilic ones ($\log D_{pH\ 8} < 3.2$) due to sorption mechanism. However, their biodegradability seems to be associated with their chemical structures, i.e., the presence of electron-donating groups (EDGs), such as chloro, carboxyl, and amide, and electron-withdrawing groups (EWGs), such as hydroxyl, alkyl, and amine, in the compound molecule (Tadkaew et al., 2011; Wijekoon et al., 2013, 2015). Therefore, the lower RE of DCF ($\log D_{pH\ 8} = 1.06$) may be justified by the presence of strong EWGs (chloro), which confer to it a higher persistence. On the other hand, despite possessing EDGs (amine and alkyl), SMX ($\log D_{pH\ 8} = -0.96$) was not so efficiently removed as expected.

These results corroborate those by Buarque et al. (2019), who

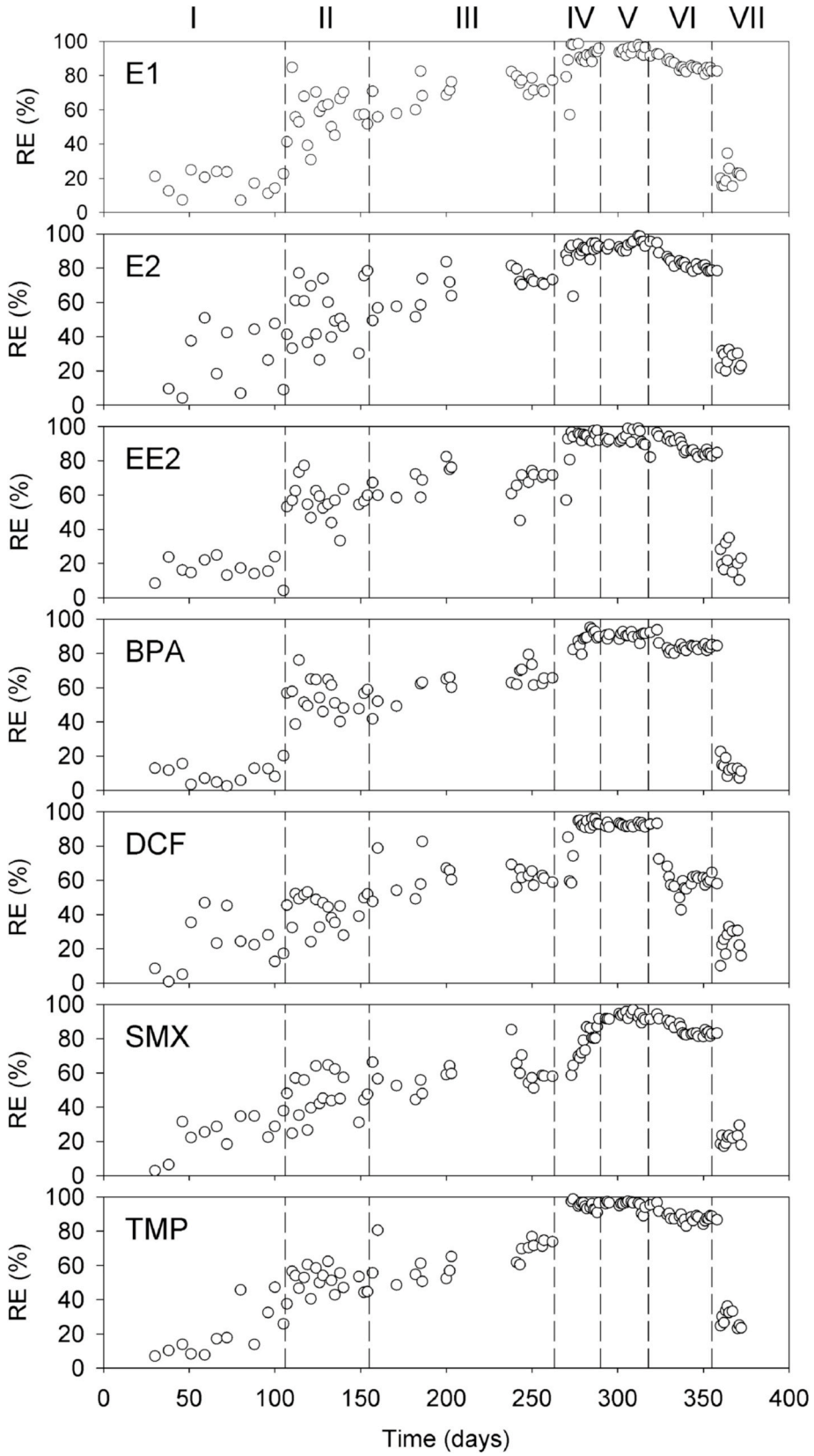

Fig. 1. Removal efficiencies (REs) of estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2), bisphenol A (BPA), diclofenac (DCF), sulfamethoxazole (SMX), and trimethoprim (TMP) throughout the experiment.

Table 2
Mean influent and effluent concentrations and removal efficiencies (REs) of the organic micropollutants throughout the experiment.

Period		I	II	III	IV	V	VI	VII
Microaeration (mL min^{-1})		–	1	2	4	6	1	–
E1	Influent ($\mu g\ L^{-1}$)	221 ± 20	199 ± 24	200 ± 17	213 ± 11	212 ± 10	202 ± 8	219 ± 8
	Effluent ($\mu g\ L^{-1}$)	183 ± 16	85 ± 25	56 ± 16	21 ± 21	12 ± 4	31 ± 6	172 ± 11
	RE (%)	17 ± 7	57 ± 13	72 ± 8	92 ± 5	94 ± 2	85 ± 3	21 ± 6
E2	Influent ($\mu g\ L^{-1}$)	230 ± 24	213 ± 17	198 ± 16	198 ± 10	197 ± 11	205 ± 8	200 ± 12
	Effluent ($\mu g\ L^{-1}$)	174 ± 20	101 ± 40	61 ± 17	21 ± 14	12 ± 5	38 ± 6	147 ± 13
	RE (%)	27 ± 18	53 ± 17	69 ± 10	90 ± 7	94 ± 3	82 ± 3	26 ± 5
EE2	Influent ($\mu g\ L^{-1}$)	213 ± 24	223 ± 27	196 ± 17	204 ± 15	216 ± 8	202 ± 11	196 ± 12
	Effluent ($\mu g\ L^{-1}$)	177 ± 17	96 ± 19	62 ± 14	18 ± 19	14 ± 10	25 ± 8	146 ± 20
	RE (%)	16 ± 6	57 ± 10	68 ± 8	92 ± 9	93 ± 5	87 ± 4	22 ± 8
BPA	Influent ($\mu g\ L^{-1}$)	208 ± 22	210 ± 27	206 ± 25	211 ± 13	202 ± 16	207 ± 9	212 ± 18
	Effluent ($\mu g\ L^{-1}$)	188 ± 24	94 ± 20	75 ± 12	23 ± 9	19 ± 4	34 ± 3	183 ± 13
	RE (%)	10 ± 5	55 ± 9	63 ± 9	89 ± 4	91 ± 2	83 ± 2	13 ± 5
DCF	Influent ($\mu g\ L^{-1}$)	232 ± 27	203 ± 16	201 ± 14	204 ± 15	205 ± 10	200 ± 12	217 ± 12
	Effluent ($\mu g\ L^{-1}$)	179 ± 37	116 ± 14	76 ± 15	24 ± 22	16 ± 2	82 ± 11	167 ± 19
	RE (%)	22 ± 15	43 ± 9	62 ± 9	88 ± 11	92 ± 1	59 ± 6	23 ± 7
SMX	Influent ($\mu g\ L^{-1}$)	203 ± 15	205 ± 26	200 ± 25	209 ± 13	207 ± 8	205 ± 11	216 ± 9
	Effluent ($\mu g\ L^{-1}$)	153 ± 24	109 ± 22	81 ± 19	40 ± 18	14 ± 4	31 ± 6	169 ± 11
	RE (%)	24 ± 11	46 ± 12	59 ± 9	80 ± 10	93 ± 2	85 ± 3	22 ± 4
TMP	Influent ($\mu g\ L^{-1}$)	230 ± 32	205 ± 16	196 ± 16	207 ± 11	210 ± 11	207 ± 8	214 ± 13
	Effluent ($\mu g\ L^{-1}$)	181 ± 35	101 ± 14	70 ± 19	10 ± 5	10 ± 5	26 ± 4	152 ± 9
	RE (%)	21 ± 14	51 ± 7	64 ± 10	95 ± 2	95 ± 3	88 ± 2	29 ± 5

BPA, bisphenol A; DCF, diclofenac; E1, estrone; E2, 17β-estradiol; EE2, 17α-ethinylestradiol; SMX, sulfamethoxazole; TMP, trimethoprim.

observed a similar remarkable increase in the mean REs of the same OMPs (from less than 10% to more than 50%) after microaerating a lab-scale UASB reactor (HRT of 7 h) at its feeding line, with 1 mL min^{-1} of synthetic air (80% N_2:20% O_2) at 27 °C and 1 atm (0.021 L O_2 L^{-1} feed), in the presence of ethanol (1 g COD L^{-1}) as co-substrate.

According to the literature, cometabolism is, most likely, the main process of biotransformation of OMPs, as their concentrations are very low, thus preventing the use of these compounds as a growth substrate. Actually, this process can occur due to the activity of some non-specific enzymes, mainly ammonia monooxygenase in nitrifying systems (Alvarino et al., 2018; Fernandez-Fontaina et al., 2016; Fischer and Majewsky, 2014). However, under microaerobic conditions, nitrification does not occur because the concentration of dissolved oxygen is usually extremely low (<0.1 mg L^{-1}). Accordingly, in the present study, since nitrification did not happened (data not shown), most likely, ammonia monooxygenase could not be produced/activated. Therefore, the presence of small amounts of oxygen may have stimulated the production of other non-specific monooxygenases enzymes able to catalyze the removal/biotransformation of OMPs.

It is worth mentioning that, under microaerobic conditions, oxygen is not used as the terminal electron acceptor as in aerobic respiration. It is only used by the monooxygenases for the initial biotransformation of the compounds through hydroxylation, and then their intermediates can be anaerobically degraded (Fuchs, 2008; Siqueira et al., 2018). For instance, according to Yerushalmi et al. (2001), under microaerobic conditions, benzene is initially hydroxylated into phenol by benzene monooxygenase, and then phenol is anaerobically degraded through the benzoate pathway.

Concerning residual oxygen, less than 10% of the amount added to the system was found in the biogas (data not shown). Therefore, both the solubilization of oxygen and its consumption in the medium were efficient. Then, in periods III, IV, and V, the effect of increasing the microaeration flow rate on the removal of OMPs was evaluated.

With 2 mL air min^{-1} (0.051 L O_2 L^{-1} feed) (period III), the REs of all OMPs increased progressively over the period and tended to stabilize at the end of it (Fig. 1), thus ensuring a significant improvement in their mean REs compared to period II ($p < 0.001$) (Table 2). However, the hormones (E1, E2, and EE2) were more easily removed than the other compounds ($p < 0.001$). As E1, E2, and EE2 are hydrophobic (log $D_{pH\ 8} > 3.2$) and contain strong EDGs (hydroxyl) (Tadkaew et al., 2011; Wijekoon et al., 2013), it may justify their higher REs, mainly in the presence of oxygen, since hydroxylation is an important step in the biotransformation of hormones (Chen et al., 2018; Yu et al., 2013).

With the increase in the microaeration flow rate to 4 mL air min^{-1} (0.101 L O_2 L^{-1} feed) (period IV), the mean REs of all OMPs were considerably higher than those observed in period III (2 mL air min^{-1}) ($p < 0.001$) (Table 2). The most significant increase was observed for the antibiotic TMP, which had an increase of 31% in its mean RE, reaching the highest value (95%) among all compounds evaluated. Probably, the presence of five EDGs in the TMP molecule (two amine groups and three ether groups) may have facilitated the microaerobic biotransformation of this antibiotic (Tadkaew et al., 2011; Wijekoon et al., 2013) through hydroxylation, reported to be a key step in the process (Jewell et al., 2016). On the other hand, the lowest mean RE was observed for the other antibiotic, SMX (80%). However, it is worth mentioning that, noticeably for this compound, the REs increased gradually, reaching values above 90% only at the end of period IV (last seven days) (Fig. 1).

The positive impact of the increase in the microaeration flow rate on the removal/biotransformation of the studied OMPs is evident, i.e., the greater availability of oxygen in the medium, most likely, enabled a higher enzymatic synthesis and accelerated the hydroxylation reactions. Therefore, a more significant removal/biotransformation of the recalcitrant compounds was achieved. Additionally, the residual oxygen in the biogas remained low (~12% of the added amount) even at a fourfold higher airflow rate, thus indicating that mass transfer was not limited, and oxygen remained promptly available in the medium to be used.

In period V (6 mL air min^{-1}) (0.152 L O_2 L^{-1} feed), very high and stable REs were achieved (Fig. 1), ensuring mean values above 90% for all OMPs (Table 2). Except for BPA, no statistically significant difference was observed among the mean REs of the compounds ($p = 0.118$). Therefore, with greater availability of oxygen, especially in periods IV and V, this difference decreased.

Compared to period IV (4 mL air min^{-1}), the increase in the airflow rate to 6 mL min^{-1} had a significant impact only on the removal of E2 ($p = 0.003$) and SMX ($p < 0.001$), with increases in the mean RE of 4% and 13%, respectively (Table 2). However, considering only the final data of period IV, when the REs were very stable (~92% for both E2 and SMX), this difference found between periods IV and V no longer exists ($p > 0.050$).

Therefore, saturation in the removal/biotransformation capacity of OMPs in the microaerobic system may have likely occurred due to biochemical limitations and not to a mass transfer problem (oxygen solubilization), since the residual oxygen detected in the biogas was only 17% of the amount provided by microaeration. Hence, in general, among the microaeration flow rates tested in the present study, the most relevant results of removal of the evaluated OMPs were obtained with 4 mL air min^{-1} (period IV) because, above this flow rate, the efficiency

increase was negligible.

According to Siqueira et al. (2018), increasing the availability of oxygen in a microaerobic system do not always improve the removal of a compound because oxygen may diffuse more deeply into the anaerobic sludge granule and inhibit obligate anaerobic microorganisms that live in the granule core, namely acetogenic bacteria and methanogenic archaea. Consequently, as methanogenesis plays an important role in the biotransformation of some OMPs during anaerobic digestion (Gonzalez-Gil et al., 2018), the inhibition of this step may hinder the removal process of these compounds.

In period VI, with the decrease in the microaeration flow rate to 1 mL min^{-1} (0.025 L O_2 L^{-1} feed), as expected, all OMPs had their REs reduced (Fig. 1). However, except for DCF, which presented a mean RE below 60%, the mean REs of the other compounds remained considerably high (>80%) (Table 2). Surprisingly, compared to period II, in which the same airflow was applied, the REs of all compounds were much higher ($p < 0.001$). For instance, the increase in the mean RE of the hormones E1, E2, and EE2 was almost 30%, and that of SMX and TMP, approximately 40% (Table 2). Therefore, it is likely that the enzymes (probably monooxygenases) that were increasingly produced throughout the periods II to V remained present and active even when a lower microaeration flow rate was applied, which guaranteed higher REs than in period II, i.e., the available oxygen may have been used more efficiently in the hydroxylation reactions due to the probable larger quantity of enzymes in period VI. However, further studies are necessary to verify if the REs would reach the same values as in period II due to a decrease in the number of enzymes in the long term, caused by the lesser availability of oxygen.

Finally, in period VII, the reactor was again operated under anaerobic conditions as in period I. Consequently, the REs of OMPs decreased significantly ($p < 0.001$) (Fig. 1), achieving a similar performance as in period I ($p > 0.050$) (Table 2). Therefore, these results strongly reinforce the key role of oxygen, even at low concentrations (microaeration), in the removal/biotransformation of OMPs, i.e., the enhanced reactor performance was not a result of a mere adaptation of the microbiota to the compounds throughout the experiment.

3.3. Operational stability of the system

In period I, under anaerobic conditions, the system was stable and showed a high mean COD RE (89%) and a methane content in the biogas above 75% (Table 3). Therefore, apparently, the OMPs did not affect the methanogenic microorganisms. In period II, with the addition of microaeration (1 mL air min^{-1}, equivalent to 0.025 L O_2 L^{-1} feed), despite the statistic difference ($p = 0.003$), the mean COD RE remained high and very close to that of period I (only 1% lower). Conversely, no significant change in the methane content was observed (Table 3). Therefore, as observed in the aforementioned investigation by Buarque et al. (2019), the addition of 1 mL air min^{-1} did not cause a significant impact on the reactor performance either in terms of organic matter removal or methane dilution/molar production.

In periods III, IV, and V, the microaeration flow rates were elevated to 2, 4, and 6 mL air min^{-1} (0.051, 0.101, and 0.152 L O_2 L^{-1} feed), respectively. The COD removal remained quite stable, and no significant differences were found among these periods ($p = 0.099$) (Table 3). Similarly, Siqueira et al. (2018), who tested different microaeration flow rates (0.5–2.0 mL air min^{-1} at 27 °C and 1 atm, equivalent to 0.07–0.14 L O_2 L^{-1} feed) to improve anaerobic BTEX biodegradation in a UASB reactor (HRT of 24 h), also observed a very stable mean COD RE throughout their experiment (~80.5%). On the other hand, in the present study, the methane content sharply decreased (from 57% to 22%) throughout them, with values well below those obtained in periods I and II (~78%) (Table 3). However, no significant change in methane productivity was observed ($p = 0.464$) (data not shown). Therefore, the increase in airflow rates did not compromised methanogenesis, and the decrease in the methane content resulted from biogas dilution by the high nitrogen content (80%) of microaeration source (synthetic air). The same behavior was observed by do Nascimento et al. (2021) when the airflow rate of a microaerated UASB reactor (HRT of 8 h) treating paraben-containing wastewater was increased from 1 to 2 and then to 4 mL min^{-1} at 28 °C and 1 atm (equivalent to 0.027, 0.055, and 0.110 L O_2 L^{-1} feed, respectively). When the biogas is intended to be used in combined heat and power plants, a minimum methane content of 40% is required (Haubrichs and Widmann, 2006). Accordingly, the two highest microaeration flow rates tested in the current work (4 and 6 mL air min^{-1}) impair the use of biogas in such facilities. Hence, the use of pure oxygen instead of air as the microaeration source should be considered, as dilution problem can be effectively mitigated.

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

Period	I	II	III	IV	V	VI	VII
Microaeration (mL min^{-1})	–	1	2	4	6	1	–
Influent COD (mg L^{-1})	1017 ± 73	1041 ± 60	1053 ± 84	1016 ± 82	1011 ± 42	1020 ± 63	998 ± 30
Effluent COD (mg L^{-1})	109 ± 49	124 ± 13	106 ± 19	118 ± 25	113 ± 17	114 ± 13	130 ± 17
COD RE (%)	89 ± 5	88 ± 2	90 ± 2	88 ± 3	89 ± 2	89 ± 2	87 ± 2
Biogas production (L d^{-1})	1.8 ± 0.4	3.8 ± 0.2	4.9 ± 0.1	7.3 ± 0.3	11.0 ± 0.2	4.0 ± 0.1	2.1 ± 0.2
CH_4 in the biogas (%)	77 ± 4	78 ± 3	57 ± 3	33 ± 3	22 ± 2	81 ± 1	82 ± 2
pH	7.3 ± 0.1	7.2 ± 0.1	7.1 ± 0.2	7.2 ± 0.2	7.1 ± 0.1	6.9 ± 0.3	7.2 ± 0.2
TA (mg L^{-1})	641 ± 31	640 ± 50	656 ± 56	670 ± 34	655 ± 27	684 ± 33	723 ± 45
VFA (mg L^{-1})	409 ± 72	310 ± 62	282 ± 36	285 ± 30	298 ± 31	301 ± 28	310 ± 62
VFA/TA	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1

COD, chemical oxygen demand; RE, removal efficiency; TA, total alkalinity; VFA, volatile fatty acids.

In periods VI and VII, the reactor was operated under the same operational conditions as in periods II and I, respectively, and there were no significant differences in both organic matter removal and methane content ($p > 0.050$) (Table 3). Finally, the reactor remained stable during the whole experiment, as there was no significant variation in pH and accumulation of volatile fatty acids (Table 3).

3.4. Dynamics of the microbial community of the system

Comparing the sludge sample from period II (1 mL air min^{-1}) with the inoculum (anaerobic), the ecological indices Chao1 and inverse Simpson indicate that both microbial richness and diversity increased expressively (Table 4). Accordingly, not only the amount of observed species, represented by the number of operational taxonomic units (OTUs), increased but also the community became more even, i.e., without dominance of only some species. In fact, the microbial

Table 4
Ecological indices of richness (Chao1) and diversity (inverse Simpson) for the inoculum and samples collected at the end of periods II (1 mL air min^{-1}), V (6 mL air min^{-1}), and VI (1 mL air min^{-1}).

Sample	OTUs[a]	Chao1	Inverse Simpson
Inoculum	1196	2042	7.68
II	1782	3413	16.67
V	1117	1995	13.43
VI	1961	3319	14.43

[a] Number of operational taxonomic units.

community in period II was very different from that of the inoculum at genus level (63% dissimilarity) (Fig. 2). Therefore, as also observed by Buarque et al. (2019) and Firmino et al. (2018), microaeration at a flow rate as low as 1 mL air min^{-1} may have played a key role in increasing the microbial diversity, thus probably stimulating the growth of monooxygenases-producing species.

On the other hand, increasing the airflow rate to 6 mL min^{-1} (period V) negatively affected the community richness (41.5% decrease), reaching a value close to that of the inoculum. However, the diversity/evenness remained rather similar (less than 20% lower than in period II) (Table 4). As a result, the sample of period V was much more similar to that of period II than to the inoculum (Fig. 2). Therefore, the higher availability of oxygen in the medium may have imposed a greater selection pressure on the microbiota, impairing the survival of less aerotolerant species in the outer zones of the anaerobic sludge granule.

When the microaeration flow rate was reduced back to 1 mL air min^{-1} (period VI), the microbial richness increased again, presenting a Chao1 index quite similar to that obtained in period II, when the same airflow rate was used. Moreover, although the number of OTUs increased, the community evenness was maintained (comparable inverse Simpson index) (Table 4). Nevertheless, at genus level, the sample of period VI was more similar to that of period V (6 mL air min^{-1}) than to that of period II (1 mL air min^{-1}) (Fig. 2). Therefore, due to continued exposure to microaerobic conditions, a gradual evolution of the microbiota seemed to occur over time, which may explain why the removal of OMPs in period VI was better than in period II (Table 2).

It is worth mentioning that methanogenesis was not compromised throughout the experiment because microaeration did not harm the archaeal community. Actually, the relative abundance of the phylum Euryarchaeota remained above 50% and even increased when a higher airflow rate was applied (period V) (Fig. 3a). At genus level, compared to the inoculum, there was a remarkable increase in the relative abundance of *Methanosaeta* (exclusively acetoclastic methanogens) (Fig. 3b).

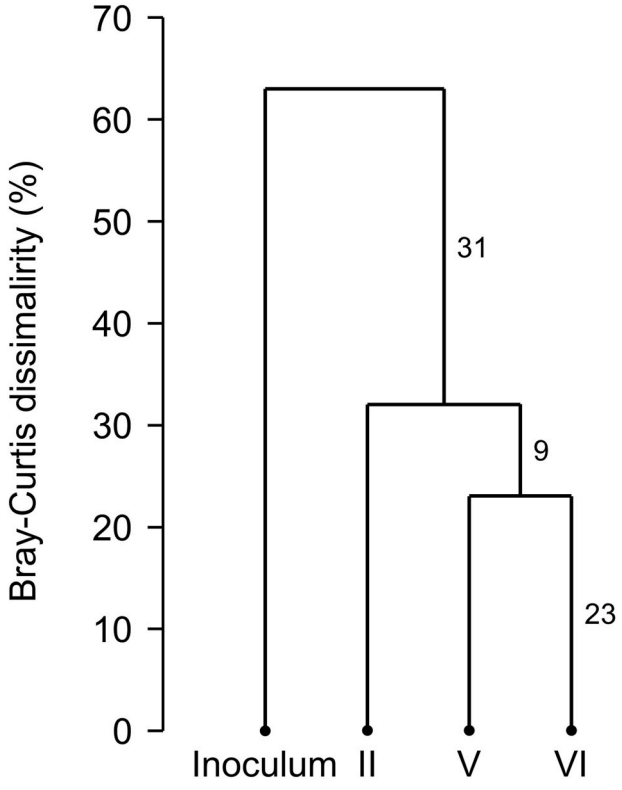

Fig. 2. UPGMA cluster analysis at genus level based on Bray-Curtis dissimilarity index for the inoculum and samples collected at the end of periods II (1 mL air min^{-1}), V (6 mL air min^{-1}), and VI (1 mL air min^{-1}).

Probably, it resulted from the used carbon source (ethanol), which is converted into acetate and hydrogen by syntrophic acetogenic bacteria, whose found genera (*Syntrophomonas*, *Syntrophobacter*, and *Sytrophorhabdus*) kept their relative abundance rather constant in the microaerated periods (Fig. 3b).

The maintenance of the methanogenic activity under microaerobic conditions is possible due to the layered structure of granular sludge, in which obligate anaerobes (e.g., acetogenic bacteria and methanogenic archaea) are mostly found in the inner layers (core of the granule) and protected by facultative species that grow in the outer layers (Baloch et al., 2008; Picioreanu et al., 2005).

Hence, as acetoclastic methanogenesis may be involved in the cometabolic biotransformation of some OMPs (e.g., DCF and BPA) due to the activity of the acetate kinase enzyme (Gonzalez-Gil et al., 2017), the preservation of the archaeal community, as well as of the acetogenic bacteria, may have also played a role in the removal of the tested compounds. Furthermore, although syntrophy between acetogens and methanogens has been hardly reported to be directly related to anaerobic biotransformation of OMPs (Carneiro et al., 2020; Wolfson et al., 2018), the importance of this ecological relationship for anaerobic degradation of aromatic compounds is widely accepted (Gieg et al., 2014; Qiu et al., 2008). For instance, *Syntrophorhabdus aromaticivorans* is one of these species able to degrade syntrophically phenol to acetate (Qiu et al., 2008).

Concerning other genera that seemed to be positively affected by microaeration, *Geobacter* and *Leptolinea* were the most evident, reaching relative abundances of 9.5% and 8.1% in period V (6 mL air min^{-1}), respectively, i.e., the most abundant bacterial genera (Fig. 3b). Despite being classified as obligate anaerobes, some *Geobacter* and *Leptolinea* species can tolerate low oxygen concentrations and even grow under microaerobic conditions (Lovley et al., 2011; Ward et al., 2015). However, whereas *Leptolinea* was even more abundant (12.2%) at the end of the long microaerobic term (249 days), unexpectedly, *Geobacter* practically vanished (0.7%) after the reduction in the microaeration flow rate to 1 mL air min^{-1} (period VI) (Fig. 3b).

The genus *Longilinea*, which is very similar to *Leptolinea*, since they belong to the same family of strictly anaerobic bacteria (Anaerolineaceae) (Yamada et al., 2007), despite keeping relative abundance below 2% in the microaerobic periods, presented the same increasing tendency over time as *Leptolinea* (Fig. 3b).

To the best of the authors' knowledge, the aforementioned genera have not been associated with the biotransformation of OMPs. However, several *Geobacter* species can degrade aromatic compounds independently or with syntrophic partners, mainly *Methanosaeta* species, under anaerobic conditions (Lovley et al., 2011). Additionally, *Longilinea* was associated with aromatic ring cleavage in the presence of oxygen, probably by oxygenases (Zhu et al., 2018). In contrast, no studies on degradation of aromatics by the genus *Leptolinea* were found. Nevertheless, due to its high similarity to *Longilinea*, *Leptolinea* may have the same ability. Therefore, as some *Geobacter*, *Leptolinea*, and *Longilinea* species are microaerophilic, they may be capable of producing oxygenases that could have cometabolized the OMPs studied in the current work.

Finally, other genera that also drew attention for having become more abundant throughout the microaerobic term were *Methylocystis* and *Mycobacterium* (1.7% and 4.5% in period VI, respectively) (Fig. 3b). *Mycobacterium* is an aerobic genus that contains some dioxygenase-producing strains able to degrade polycyclic aromatic hydrocarbons (Guo et al., 2010). *Methylocystis* species are aerobic methanotrophs, but some of them can grow under microaerobic conditions (Vecherskaya et al., 2009). Additionally, methane monooxygenase, the key enzyme for methane oxidation by methanotrophic bacteria, was demonstrated to degrade cometabolically SMX and benzotriazole (Benner et al., 2015). Hence, both genera may have participated in the biotransformation of the tested compounds, particularly in hydroxylation reactions.

Considering that these five supposed microaerophilic genera were

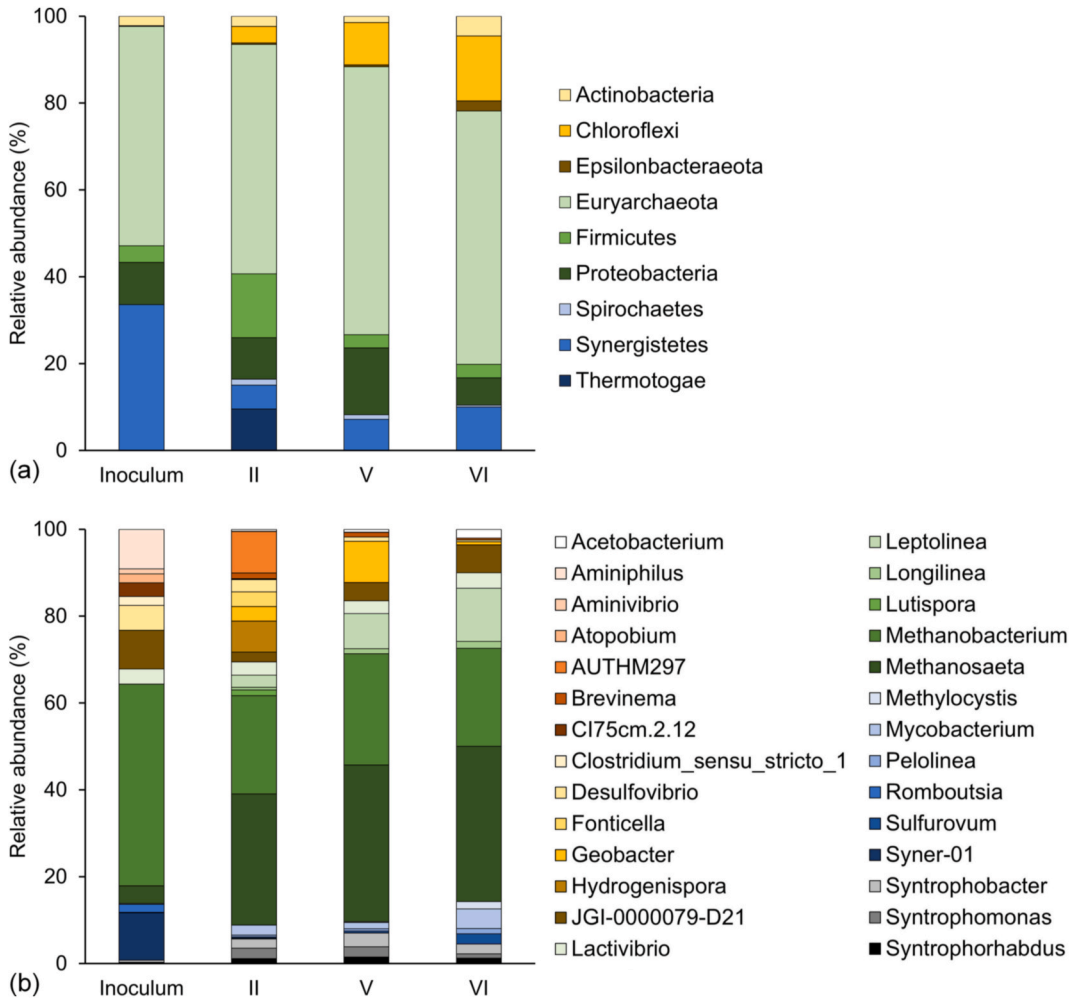

Fig. 3. Microbial diversity at phylum (a) and genus (b) levels of the inoculum and samples collected at the end of periods II (1 mL air min^{-1}), V (6 mL air min^{-1}), and VI (1 mL air min^{-1}).

someway involved in the biotransformation of OMPs, especially in the first steps, the sum of their relative abundances were much higher in period VI (20.7%) than in period II (9.1%), although the same airflow rate was used (1 mL air min^{-1}). Therefore, this could justify the better removal of OMPs in period VI, as there was some microbial enrichment throughout the microaerobic periods.

4. Conclusions

Microaeration was demonstrated to be an effective strategy to improve the limited removal/biotransformation of the evaluated OMPs in short-HRT anaerobic wastewater treatment systems.

The rise in the airflow rate (1–6 mL min^{-1}, i.e., 0.025–0.152 L O_2 L^{-1} feed) considerably increased the REs of all OMPs. However, there seems to be a saturation limit for the biochemical reactions. Then, the best results were obtained with 4 mL air min^{-1} (0.101 L O_2 L^{-1} feed) (~90%) because, above this flow rate, the efficiency increase was negligible.

The long-term exposure to microaerobic conditions (249 days) led the microbiota to a gradual evolution. Consequently, there was some enrichment with species potentially associated with the biotransformation of OMPs, which may explain the better performance at the end of the microaerobic term even with the lowest airflow rate tested.

Credit author statement

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111313.

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