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Technical Article

Enhanced removal of emerging micropollutants by applying microaeration to an anaerobic reactor

Remoção acelerada de micropoluentes emergentes pela aplicação de microaeração em reator anaeróbio

Patrícia Marques Carneiro Buarque[1]*, Ricardo Bruno Pinheiro de Lima[2], Carla Bastos Vidal[2], Hugo Leonardo de Brito Buarque[1], Paulo Igor Milen Firmino[2], André Bezerra dos Santos[2]

ABSTRACT

The present paper aimed to evaluate the impact of microaeration on both the removal performance of some emerging micropollutants (pharmaceuticals, hormones, and bisphenol A) and the microbial community structure of an anaerobic reactor treating synthetic wastewater. Under anaerobic conditions, the removal efficiencies of the micropollutants were very low (< 10%). However, the microaeration (1.0 mL air·min^{-1} at 27 °C and 1 atm, equivalent to a Q_{AIR}/Q_{INF} ratio of 0.1) expressively improved the removal efficiencies of all compounds (> 50%). Therefore, supplementing anaerobic reactors with low amounts of oxygen seems to be an interesting strategy to enhance the removal of the micropollutants tested. However, further studies should be carried out with other compounds in order to evaluate the wide applicability of microaeration to different classes of micropollutants in lab- and full-scale treatment systems. Concerning the microbiota structure, both bacterial and archaeal communities were not compromised by the different operational conditions and preserved their functional organization with high richness during the whole experiment.

Keywords: pharmaceuticals; hormones; bisphenol A; anaerobic treatment; microaerobic treatment.

RESUMO

O presente trabalho teve como objetivo avaliar o impacto da microaeração tanto no desempenho de remoção de alguns micropoluentes emergentes (fármacos, hormônios e bisfenol A) quanto na estrutura da comunidade microbiana de um reator anaeróbio tratando uma água residuária sintética. Sob condições anaeróbias, as eficiências de remoção dos micropoluentes foram muito baixas (< 10%). Entretanto, a microaeração (1,0 mL de ar·min^{-1} a 27 °C e 1 atm, equivalente a uma relação Q_{AR}/Q_{AF} de 0,1) melhorou expressivamente as eficiências de remoção de todos os compostos (> 50%). Portanto, a suplementação de reatores anaeróbios com baixas quantidades de oxigênio parece ser uma estratégia interessante para melhorar a remoção dos micropoluentes testados. Entretanto, mais estudos devem ser realizados com outros compostos para avaliar a ampla aplicabilidade da microaeração a diferentes classes de micropoluentes em sistemas de tratamento em escala laboratorial e real. Com relação à estrutura da microbiota, tanto as comunidades de bactérias quanto as de arqueias não foram comprometidas pelas diferentes condições operacionais e preservaram sua organização funcional com elevada riqueza durante todo o experimento.

Palavras-chave: fármacos; hormônios; bisfenol A; tratamento anaeróbio; tratamento microaeróbio.

INTRODUCTION

Several emerging micropollutants from different classes (*e.g.* pharmaceuticals and hormones) are consumed every year worldwide. Such pharmaceutical compounds include antipyretics, analgesics, lipid regulators, antibiotics, antidepressants, chemotherapeutics, contraceptives, among others (YANG *et al.*, 2017). In addition, some compounds, such as bisphenol A (BPA), which is mainly used in the production of polycarbonate plastics and epoxy resins, also have estrogenic activity (ZIELIŃSKA *et al.*, 2014). Therefore, the occurrence of these micropollutants in aquatic environments has brought impacts on fauna, flora, and human health to light (TAMBOSI *et al.*, 2010).

Adverse effects caused by these emerging micropollutants include aquatic toxicity, increase in pathogenic bacteria resistance, genotoxicity, increase in breast and prostate cancer incidence, endometriosis, and other endocrine disorders (AQUINO; BRANDT; CHERNICHARO, 2013; KÜMMERER, 2010). Thus, the development of processes that can promote the effective removal of micropollutants, along with

[1]Instituto Federal de Educação, Ciência e Tecnologia do Ceará – Quixadá (CE), Brazil.
[2]Universidade Federal do Ceará – Fortaleza (CE), Brazil.
***Corresponding author:** pmcbuarque@gmail.com
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other priority pollutants, is an emerging issue in science and environmental engineering. These processes need to reach certain goals, such as higher efficiency, compliance with environmental requirements, more compact units that operate with greater flexibility and efficiency, and lower installation and operational costs (AQUINO; BRANDT; CHERNICHARO, 2013).

There are biological and non-biological processes (physical, chemical, and physicochemical) for removing these compounds from environmental water matrices. The non-biological methods include advanced oxidation processes, ozonation, nanofiltration, reverse osmosis, and adsorption on zeolite or activated carbon (DE LA CRUZ *et al.*, 2012; VIDAL *et al.*, 2015). However, these techniques incur high installation and operational costs. Furthermore, non-destructive techniques (*e.g.* physical) require auxiliary processes intended to adsorb, degrade, or dispose of the pollutants previously extracted (AQUINO; BRANDT; CHERNICHARO, 2013; PESSOA *et al.*, 2014).

Some investigations into micropollutants removal by anaerobic reactors have been carried out, but their removal efficiencies are much lower than those of aerobic treatment systems (ALVARINO *et al.*, 2016; DE GRAAFF *et al.*, 2011; JOSS *et al.*, 2004).

Recent studies have shown that adding low oxygen concentrations (microaeration) to anaerobic systems could improve the initial degradation of recalcitrant compounds, such as monoaromatic hydrocarbons (BTEX) (FIRMINO *et al.*, 2018; SIQUEIRA *et al.*, 2018). Nevertheless, to the best of the authors' knowledge, there has been no investigation into microaeration of anaerobic reactors for micropollutants removal.

Hence, the present paper aimed to assess the impact of microaeration on both the removal performance of some emerging micropollutants (the natural estrogens estrone (E1) and estradiol (E2), the synthetic estrogen ethinylestradiol (EE2), the anti-inflammatory diclofenac (DCF), the antibiotics sulfamethoxazole (SMX) and trimethoprim (TMP), and the xenoestrogen bisphenol A (BPA)) and the microbial community structure of an anaerobic reactor treating synthetic wastewater.

MATERIAL AND METHODS

Experimental set-up

The continuous flow experiment was carried out in an upflow anaerobic sludge blanket (UASB) reactor, with a working volume of 3.7 L, made from PVC tubes and connections for sewage. The reactor was inoculated with anaerobic sludge (~60 g SSV·L^{-1}) from a mesophilic internal circulation (IC) reactor of a brewery (Horizonte, Ceará, Brazil).

The influent was kept under refrigeration throughout the experiment (~5 °C) to avoid degradation, and the reactor was fed by a peristaltic pump (Minipuls 3, Gilson, USA) through a Tygon® flexible tubing (Cole-Parmer, USA), at an average flow rate of 14 L·d^{-1} (TDH ≈ 7 h). A dosing pump (Concept ProMinent Dosiertechnik GmbH, Germany) was used to recirculate the effluent (at 0.7 L·h^{-1}) in order to improve mass transfer, avoid preferential paths, and facilitate the release of biogas bubbles, thus preventing biomass loss due to the piston effect.

The microaeration was introduced into the reactor at its feeding line from a gas cylinder containing synthetic air (20% O_2:80% N_2), by using a mass flow controller (Cole Parmer, USA), at an airflow rate of 1.0 mL·min^{-1} (at 27 °C and 1 atm). This airflow rate corresponds to a microaeration rate (MR) of approximately 0.10, which is calculated as the ratio between the airflow rate and the influent flow rate of the reactor (Q_{AIR}/Q_{INF}).

The produced biogas was collected and quantified by the liquid displacement method and characterized by gas chromatography as specified in the chemical and chromatographic analyses section.

Synthetic wastewater composition

The synthetic wastewater was prepared weekly by dissolving in potable tap water a mixture of the following micropollutants (~230 µg·L^{-1} each): E1 (99.0%, Sigma-Aldrich, USA), E2 (98.0%, Sigma-Aldrich, USA), EE2 (100%, Sigma-Aldrich, USA), DCF (98.5%, Sigma-Aldrich, USA), SMX (99%, Sigma-Aldrich, USA), TMP (98%, Sigma-Aldrich, USA), BPA (99%, Sigma-Aldrich, USA), ethanol (99.8%, Dynamics, Brazil) as co-substrate (1 g COD·L^{-1}), macro and micronutrients (FIRMINO *et al.*, 2010), and sodium bicarbonate (1 g COD·L^{-1}) as buffer (to maintain pH 7.0).

Experimental procedure

The experiment was run in three periods (Table 1). In period I (acclimatization), the reactor was operated under anaerobic conditions and fed

Table 1 – Operational conditions in the experimental periods.

Period	I	II	III
Operation time (days)	48	132	200
HRT (h)	7	7	7
COD (mg·L^{-1})	890	994	954
Bisphenol A (BPA) (µg·L^{-1})	-	268	225
Diclofenac (DCF) (µg·L^{-1})	-	251	235
Estrone (E1) (µg·L^{-1})	-	264	239
Estradiol (E2) (µg·L^{-1})	-	260	231
Ethinylestradiol (EE2) (µg·L^{-1})	-	227	229
Trimethoprim (TMP) (µg·L^{-1})	-	225	238
Sulfamethoxazole (SMX) (µg·L^{-1})	-	216	227
Recirculation (L·h^{-1})	0.7	0.7	0.7
Microaeration (mL·min^{-1})	-	-	1.0
Q_{AIR}/Q_{INF} ratio	-	-	0.1

HRT: hydraulic retention time; COD: chemical oxygen demand; Q_{AIR}/Q_{INF}: ratio between the airflow rate and the influent flow rate.

with micropollutant-free wastewater. Therefore, ethanol (1.0 g COD·L^{-1}) was the only carbon and energy source. In period II, the micropollutants were added to the synthetic wastewater in order to assess the removal performance of the reactor under anaerobic conditions. The concentration of the micropollutants (~230 µg·L^{-1} of each compound) used in this study was in accordance with that observed in domestic wastewaters (PESSOA *et al.*, 2014). Finally, in period III, a microaeration flow rate of 1.0 mL·min^{-1} of synthetic air (at 27 °C and 1 atm) was introduced into the reactor at its feeding line (Q_{AIR}/Q_{INF} = 0.1) to evaluate the micropollutants removal performance under microaerobic conditions. This airflow rate was set based on previous studies on microaerobic BTEX removal (FIRMINO *et al.*, 2018; SIQUEIRA *et al.*, 2018).

The transition between experimental periods occurred after checking the stability of effluent chemical oxygen demand (COD) and micropollutants concentrations in the last five data (variation up to 10%).

Chemical and chromatographic analyses

COD and pH were determined according to APHA (2005), whereas the pharmaceuticals and hormones were determined by high-performance liquid chromatography with diode array detection (HPLC-DAD) according to Vidal *et al.* (2015). The biogas was characterized in terms of air (O_2 + N_2), CH_4, and CO_2 by gas chromatography with thermal conductivity detection (GC-TCD) (GC-17A, Shimadzu Corporation, Japan) according to Firmino *et al.* (2015).

Microbial community analysis

To evaluate the microbial community structure (functional organization, richness, and diversity), sludge samples, including the inoculum, were withdrawn from the reactor at the end of all experimental periods and frozen at -20 °C, until the genomic DNA was extracted using a fast extraction kit (Biomedicals, USA) following the manufacturer protocol. The DNA concentration (0.2 to 2 mg·L^{-1}) was determined by spectrophotometry with a Nanodrop 2000 (Thermo Fisher Scientific, USA).

Bacterial and archaeal community structure was analyzed by polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR-DGGE) according to Sousa *et al.* (2016), as follows. The 16S rRNA gene hypervariable regions V6-V8 of Bacteria and V2-V3 of Archaea were amplified by polymerase chain reaction (PCR) using the universal bacterial primers 1401-R and 968-F, and the archaeal primers A 109(T)-F and 515-R (IDT, USA). Primers 968-F and 515-R included a 40 pb GC-clamp at the 5' end (5'-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGG-3').

The PCR mixture (50 µL) contained 10 µL of reaction buffer (5×), 5 µL of $MgCl_2$ (25 mM), 0.25 µL of Taq polymerase (5 µL) (Promega, USA), 1 µL of deoxynucleotide triphosphates (10 mM), 1 µL of the extracted DNA, 1 µL of PCR primers (10 µM), and nuclease-free water (Promega, USA) up to a final volume of 50 µL. PCR was conducted in a T100 Thermal Cycler instrument (Bio-Rad Laboratories, USA). The PCR thermal cycling program for bacterial amplification consisted of 2 min of predenaturation at 95 °C, 32 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 45 s, and elongation at 72 °C for 1 min, with a final 30-min elongation at 72 °C. Archaeal amplification consisted of 2 min of predenaturation at 95 °C, 35 cycles of denaturation at 95 °C for 40 s, annealing at 53 °C for 40 s, and elongation at 72 °C for 1 min, with a final 30-min elongation at 72 °C. PCR products were verified in 1.7% agarose (w/v) gel electrophoresis, using the 1kb DNA Ladder (Promega, USA) as a molecular weight marker. The gels were stained with SYBR Green I nucleic acid gel stain (Sigma-Aldrich, USA) for 40 min, and the result was analyzed in a Universal Hood II Transilluminator (Bio-Rad Laboratories, USA).

The double gradient DGGE analysis was performed in a D-Code Universal Mutation Detection System (Bio-Rad Laboratories, USA) using polyacrylamide gels with a urea/formamide denaturing gradient of 42-67% for bacterial community analysis and 30–60% for archaeal community analysis, superimposed with a porous gradient of acrylamide/bisacrylamide (6–10%). Electrophoresis was performed in 0.5× TAE buffer at 60 °C and 85 V for 16 h for bacterial amplicons and 60 °C and 65 V for 18 h for archaeal amplicons. The gels were stained with SYBR Green I nucleic acid stain (Sigma-Aldrich, USA) for 40 min.

The gel images were processed by using the BioNumerics software (Applied Maths BVBA, Sint-Martens-Latem, Belgium) to score the band patterns. These patterns were used to calculate the following ecological parameters: evenness/functional organization (Fo), which indicates the ability of the community to organize in an adequate distribution of dominant microorganisms and resilient ones, and range-weighted richness (Rr), which indicates the richness and genetic diversity within a bacterial community, according to Marzorati *et al.* (2008).

Statistical methods

In order to compare the reactor performance in the three periods, non-parametric tests were used (Mann-Whitney and Kruskal-Wallis). The results were considered statistically different when $p \leq 0.05$.

RESULTS AND DISCUSSION

Removal of emerging micropollutants

After the acclimatization (period I), the reactor started to be fed with the micropollutant-containing wastewater (period II). As expected, under anaerobic conditions, the average removal efficiencies of all compounds were very low (< 10%) (Table 2). Therefore, adsorption is not an important mechanism for removal of micropollutants under

anaerobic conditions (HARB *et al.*, 2019). Among the estrogens, E1 presented the highest average removal efficiency (9%), whereas E2 and EE2 showed average values near 5% (Table 2).

According to Joss *et al.* (2004) and De Mes *et al.* (2007), E2 presents lower removal efficiencies than E1 in anaerobic treatment systems because low redox potential values favor the reduction of E1 to E2. EE2 is more recalcitrant due to steric hindrance, i.e., the ethinyl group at position 17 does not allow the formation of a ketone (as observed in E2), thus its removal efficiency is lower than that of E2 (CZAJKA; LONDRY, 2006).

As to the pharmaceuticals and the xenoestrogen BPA, TMP was the most recalcitrant, showing a negative average removal efficiency (Table 2). In fact, this behavior had already been reported in the literature (AQUINO; BRANDT; CHERNICHARO, 2013; GULKOWSKA *et al.*, 2008). The possible causes for negative removals are deconjugation of the metabolites during the treatment process and change in the behavior of the possible adsorption of the analytes on the particles in the treatment process, affecting the influent/effluent concentration ratio (LINDBERG *et al.*, 2005).

According to Aquino, Brandt, and Chernicharo (2013), the low anaerobic biodegradability of emerging micropollutants is probably due to the presence of phenolic aromatic rings in their structures, which are more difficult to degrade in the absence of dissolved oxygen.

In period III, microaeration was applied to the reactor at its feeding line, and the average removal efficiencies of all compounds increased from below 10% to above 50%. This improvement is significant when compared to the anaerobic period (Table 2). Therefore, supplementing anaerobic reactors with low amounts of oxygen seems to be an interesting strategy to enhance the removal of the micropollutants tested. However, further studies should be carried out with other compounds in order to evaluate the wide applicability of microaeration to different classes of micropollutants in lab- and full-scale treatment systems.

De Mes *et al.* (2007) identified an increase of up to 40% in the estrogens removal efficiency (E1, E2, and EE2) when a downflow hanging sponge (DHS) reactor was operated as a microaerobic post-treatment for blackwater anaerobically treated by a UASB reactor. According to the authors, the microaeration of the post-treatment intensified the degradation of these compounds.

Joss *et al.* (2004) demonstrated that the biological removal of some estrogens depended on the biomass activity and the redox potential in the treatment systems, i.e., the presence of oxygen is an important factor for the removal of these compounds.

Firmino *et al.* (2018) verified that microaeration remarkably enhanced BTEX removal, especially for benzene, which is usually very recalcitrant under anaerobic conditions. They found that small amounts of oxygen favored the initial degradation of these compounds, probably by activating monooxygenase enzymes of some microorganisms. Hence, they might have converted the aromatic hydrocarbons into less recalcitrant phenolic intermediates, whose degradation process could occur anaerobically. Another possibility is that oxygen might favor the cometabolic reactions involved in micropollutants removal.

Thus, the dissolved oxygen concentration in the environment is an important factor for the biological removal of pharmaceuticals and, especially, hormones, which are more easily degraded under aerobic conditions (VIRKUTYTE; VARMA; JEGATHEESAN, 2010).

Finally, it is noteworthy that COD removal efficiencies were higher than 90% during the whole experiment (Table 3). Despite the slight reduction in the average values of COD removal efficiency and methane production in period III when compared to the previous periods, there is no statistically significant difference among all experimental periods (Table 3). Therefore, microaeration did not alter the organic matter removal capacity of the reactor. These results corroborate those by Siqueira *et al.* (2018), who did not found any significant

Table 2 – Average influent and effluent micropollutants concentrations and their respective removal efficiencies.

Micropollutant	Period					
	II			III		
	IC ($\mu g \cdot L^{-1}$)	EC ($\mu g \cdot L^{-1}$)	RE (%)	IC ($\mu g \cdot L^{-1}$)	EC ($\mu g \cdot L^{-1}$)	RE (%)
BPA	274 (21)	250 (28)	8 (10)	224 (14)	94 (9)	58 (4)
DCF	251 (23)	236 (24)	6 (3)	233 (19)	110 (19)	53 (7)
E1	264 (28)	239 (27)	9 (11)	239 (17)	111 (25)	54 (10)
E2	260 (40)	243 (30)	6 (3)	232 (14)	109 (17)	53 (8)
EE2	228 (31)	220 (24)	4 (3)	229 (15)	101 (14)	56 (7)
SMX	216 (27)	206 (23)	5 (3)	226 (11)	101 (13)	55 (6)
TMP	225 (19)	233 (34)	-4 (19)	238 (21)	112 (15)	53 (6)

IC: influent concentration; EC: effluent concentration; RE: removal efficiency; BPA: bisphenol A; DCF: diclofenac; E1: estrone; E2: estradiol; EE2: ethinylestradiol; SMX: sulfamethoxazole; TMP: trimethoprim.
The standard deviation is shown in parentheses.

difference in COD removal (~80.5%) after microaerating, at different airflow rates (0.5–2 mL·min^{-1}), an anaerobic reactor fed with BTEX-contaminated water.

Microbial community structure

The effect of the different operational conditions on the bacterial and archaeal communities of the reactor can be observed in their corresponding DGGE profiles (Figure 1), from which the ecological parameters Rr and Fo were calculated (Table 4) to evaluate the changes in the microbiota.

After period I, when ethanol was the only carbon source, the richness of the bacterial community increased expressively (from 8, low Rr, to 91, high Rr), whereas its evenness remained high (Fo < 30%) (Table 4). These parameters indicate the development of a community formed by groups of generalist organisms without specific dominance, which might have been due to the carbon source used (ethanol), a simple and easily degradable substrate.

The introduction of the micropollutants (period II) reduced both Rr and Fo of the bacterial community (Table 4). Nevertheless, its richness and evenness remained high according to Marzorati *et al.* (2008). Therefore, the environment maintained a broad carrying capacity, being considered very habitable, as the microbial diversity remained high (MARZORATI *et al.*, 2008).

In period III, Rr increased considerably, whereas Fo presented a slight decrease (Table 4). Therefore, the microaeration might have positively affected the bacterial community, increasing its diversity, i.e., some monooxygenase-producing populations might have grown, favoring the micropollutants biotransformation as observed in previous investigations into microaerobic BTEX removal (FIRMINO *et al.*, 2018; SIQUEIRA *et al.*, 2018). However, no specific group showed dominance, as the community evenness remained high (Fo < 30%) (Table 4).

As for the archaeal community, no remarkable changes were found among the inoculum and all experimental periods, in which richness and evenness remained very high (MARZORATI *et al.*, 2008). Consequently, the operational changes did not cause sufficient stress to modify the microbiota structure, and its functionality was preserved during the whole experiment. Probably, the structural configuration of granular sludge, in which the facultative or microaerophilic bacteria (in the outer layers) protect the strictly anaerobic archaea (in the granule core) from oxygen, and the low retention time of oxygen in the sludge blanket might have contributed to maintain the archaeal community (FIRMINO *et al.*, 2018).

In general, the introduction of the micropollutants and microaeration (periods II and III, respectively) did not compromise the structure of the bacterial and archaeal communities, as, according to Marzorati *et al.* (2008), evenness and richness remained high during the 200 days of operation (Table 4).

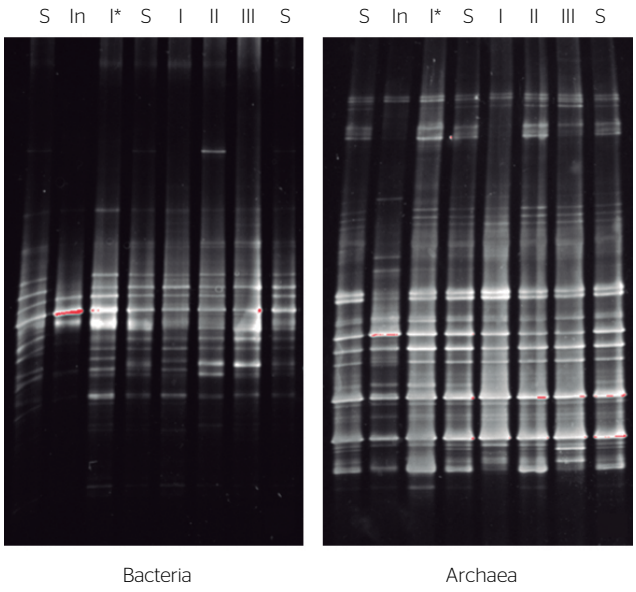
CONCLUSIONS

Under anaerobic conditions, the removal efficiencies of the emerging micropollutants were very low (< 10%). However, the microaeration expressively improved the removal efficiencies of all compounds (> 50%). Therefore, supplementing anaerobic reactors with low amounts of oxygen seems to be an interesting strategy to enhance the removal of the micropollutants tested.

Table 3 – COD removal and methane production.

Period		I	II	III
COD	Influent (mg·L^{-1})	888 (96)	892 (131)	949 (233)
	Effluent (mg·L^{-1})	41 (13)	35 (13)	60 (32)
	Efficiency (%)	95 (1)	96 (2)	93 (4)
CH_4	(mL·g COD_{rem}^{-1})	459 (18)	448 (41)	415 (21)

COD: chemical oxygen demand; COD_{rem}: chemical oxygen demand removed.
The standard deviation is shown in parentheses.



S: standard; In: inoculum; I*: sample withdrawn one week after inoculation; I, II, and III: samples withdrawn at the end of each experimental period.

Figure 1 – Bacterial and archaeal denaturing gradient gel electrophoresis profiles.

Table 4 – Functional organization (Fo) and range-weighted richness (Rr) for the bacterial and archaeal communities.

Parameters	Inoculum	Period I	Period II	Period III
Bacteria				
Fo (%)	25	26	21	17
Rr	8	91	53	70
Archaea				
Fo (%)	30	25	28	31
Rr	216	241	228	200

Fo: low Fo/high evenness (< 30%), medium Fo and evenness (30-70%), high Fo/low evenness (> 70%) (MARZORATI et al., 2008).
Rr: low (< 10), medium (10-30), high (> 30) (MARZORATI et al., 2008).

However, further studies should be carried out with other compounds in order to evaluate the wide applicability of microaeration to different classes of micropollutants in lab- and full-scale treatment systems.

Concerning the microbiota structure, both bacterial and archaeal communities were not compromised by the different conditions and preserved their functional organization with high richness during the whole experiment.

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