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Research article

Process bioengineering applied to BTEX degradation in microaerobic treatment systems

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ABSTRACT

The effect of different microaeration flow rates and dosing points, and of effluent recirculation, on microaerobic BTEX degradation in an anaerobic bioreactor was assessed. Additionally, a sensitivity and recovery analysis for this system was performed during microaeration failure simulations. Under anaerobic conditions, BTEX removal efficiencies between 55 and 82% were achieved depending on the compound, being benzene the most recalcitrant one. Microaeration (0.5–2.0 mL air min^{-1}) ensured high removal efficiencies (> 83%) for all compounds, and the best results were obtained for the flow rate of 1.0 mL air min^{-1}, particularly for benzene, with a 30% increase in its removal efficiency. Effluent recirculation showed to be an important factor to improve mass transfer and, consequently, BTEX removal. Volatilization was negligible even under microaerobic conditions, suggesting that microbial activity was the main removal mechanism. Finally, after microaeration shutdown periods, the bioreactor could recover its prior performance within up to 2 days.

1. Introduction

The compounds benzene, toluene, ethylbenzene and xylenes, usually known as BTEX, are aromatic hydrocarbons commonly found in petroleum products, such as fuels (gasoline), solvents and intermediates of organic compounds synthesis (Bolden et al., 2015; Peng et al., 2015).

Among the forms of groundwater contamination with BTEX, fuel stations are a significant source, either by lubricant or fuel additives spillage, oil separators misuse or, mainly, fuel leakage from underground storage tanks (Alves et al., 2017; Corseuil et al., 2011). Therefore, water contamination with monoaromatic compounds is a serious environmental and public health problem, since these compounds are toxic and potentially carcinogenic to humans (Alves et al., 2017; Corseuil et al., 2011; Cruz et al., 2017; Farhadian et al., 2008; Tsangari et al., 2017).

Aromatic hydrocarbons can be degraded aerobically and anaerobically (Varjani, 2017). However, prior to the 1980s, investigations involving the microbiological removal of these compounds were all carried out under aerobic conditions, in which molecular oxygen is incorporated into hydrocarbon molecule by oxygenases activity as the initial step of the oxidative process. It was not believed that anaerobic organisms could perform a similar reaction (Chakraborty and Coates, 2004). However, current investigations have achieved important findings on BTEX degradation under nitrate-, iron-, manganese-, sulfate-reducing and methanogenic conditions (de Nardi et al., 2005; Firmino et al., 2015a, 2015b; Stasik et al., 2015; Varjani, 2017; Varjani and Upasani, 2017).

Recently, anaerobic systems applied to BTEX removal have been studied in the presence of low oxygen concentrations to improve the removal of these hydrocarbons from contaminated waters (Firmino et al., 2018; Wu et al., 2015), helping the enzymatic process, since BTEX biodegradation involves a number of steps using different enzymes (Varjani, 2017; Varjani and Upasani, 2017). By applying microaeration, it is expected that an oxygen atom will be incorporated into the aromatic hydrocarbon by the enzymatic action of mono-oxygenases, which transfer an oxygen atom to the substrate while reduce the other oxygen atom to the water (Varjani and Upasani, 2017).

Thus, under such microaerobic conditions, some microorganisms use oxygen only to introduce hydroxyl groups into the aromatic ring as in classical aerobic pathways, whereas their cleavage occurs through anaerobic metabolic pathways (Chakraborty and Coates, 2004; Fuchs, 2008). In addition, low concentrations of oxygen suppress the enzymatic activity of dioxygenases, preventing degradation by aerobic respiration, since there is not enough oxygen to act as electron acceptor (Yerushalmi et al., 2001).

Therefore, this configuration seems to be much more attractive than traditional aerobic processes because it is less costly to construct, produce less sludge, which would require a final disposal, and the risks of

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emission of volatile organic compounds (VOCs) are considerably reduced. However, although a recently published study has shown the successful applicability of microaeration (at only one airflow rate and dosing point) to enhance BTEX removal in anaerobic systems, particularly for benzene (with a 30% removal increase) (Firmino et al., 2018), this process, especially with anaerobic inoculum, still needs to be better evaluated and understood in order to subsidize the design of a compact treatment system for ex situ contaminated groundwater bioremediation or even for its application to petrochemical wastewater treatment.

Hence, the present study evaluated the effect of different microaeration flow rates and dosing points, and of effluent recirculation, on microaerobic degradation of BTEX (benzene, toluene, ethylbenzene and xylenes) in a continuous-flow methanogenic bioreactor. In addition, a sensitivity and recovery analysis for the treatment system was performed during microaeration failure simulations.

2. Material and methods

2.1. Experimental set-up

The continuous-flow experiment was carried out in a lab-scale UASB (upflow anaerobic sludge blanket) bioreactor (working volume of 2.2 L), made from PVC tubes for sewage, which was inoculated at 10% of its working volume with an anaerobic sludge ($\sim$49 g VSS L^{-1}) from a full-scale UASB bioreactor treating domestic sewage (Fortaleza, Ceará, Brazil).

The bioreactor was operated at room temperature of approximately 27 °C and fed with a synthetic BTEX-contaminated water by a peristaltic pump (Minipuls 3, Gilson, USA). The synthetic contaminated water was an aqueous solution composed of BTEX ($\sim$4.2 mg L^{-1} of each compound), i.e. benzene (99.5%, Dinâmica, Brazil), toluene (99.5%, Vetec, Brazil), ethylbenzene (99.0%, Sigma-Aldrich, USA), o-xylene (98.0%, Fluka, USA), m-xylene (99.0%, Sigma-Aldrich, USA) and p-xylene (99.0%, Sigma-Aldrich, USA), ethanol (99.8%, Dinâmica, Brazil) as co-substrate (1 g COD L^{-1}) and a basal medium prepared according to Firmino et al. (2010). To maintain the pH near 7.0, the solution was buffered with sodium bicarbonate ($NaHCO_3$) in a ratio of 1 g $NaHCO_3$ to each 1 g COD. The feeding was stored at approximately 5 °C in a high-density polyethylene (HDPE) tank, which was kept closed to avoid BTEX loss to atmosphere by volatilization. The pressure balance of the feeding tank was maintained by a hypodermic needle inserted into its lid.

In order to increase the contact between substrate and microorganisms in the sludge blanket, as well as to assist biogas release and avoid biomass loss due to the piston effect caused by entrapped biogas bubbles, effluent was continuously recirculated at an average flow rate of approximately 0.72 L h^{-1} by a metering pump (Concept Plus, ProMinent Dosiertechnik GmbH, Germany). At some operational stages, synthetic air (20% O_2:80% N_2) from a gas cylinder (White Martins, Brazil) was introduced into the bioreactor by using a mass flow controller with adjustment of 0–20 mL min^{-1} (GFC17, Aalborg, USA). The produced biogas by the bioreactor was collected and measured by a previously calibrated gas meter (liquid displacement method).

2.2. Experimental procedure

2.2.1. Anaerobic BTEX removal

At stage I, after inoculation, the bioreactor was fed with the synthetic BTEX-contaminated water and operated under anaerobic conditions at a hydraulic retention time (HRT) of 24 h (Table 1).

2.2.2. Effect of microaeration flow rate on BTEX removal

From stage II to IV, in order to investigate BTEX removal under microaerobic conditions, synthetic air was introduced into the bioreactor at its feeding line, and different microaeration flow rates were tested: 1.0, 0.5 and 2.0 mL air min^{-1} (at 1 atm and 27 °C), which equal to 0.140, 0.068 and 0.274 L O_2 L^{-1} feeding, respectively (Table 1).

Table 1
Operational conditions during anaerobic and microaerobic BTEX removal.

Stage	I	II	III	IV	V	VI	VII
Days of operation	64	49	31	39	28	28	43
Microaeration flow rate[a] (mL air min^{-1})	–	1.0	0.5	2.0	1.0	1.0	1.0
Dose of oxygen[a] (L O_2 L^{-1} feed)	–	0.14	0.07	0.27	0.14	0.14	0.14
Dosing point	–	FL	FL	FL	HS	FL	FL
Recirculation flow rate (L h^{-1})	0.72	0.72	0.72	0.72	0.72	–	0.72
Ethanol (g L^{-1})	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Benzene (mg L^{-1})	4.27	4.16	4.17	4.34	4.26	4.19	4.47
Toluene (mg L^{-1})	4.58	4.28	4.27	4.45	4.42	4.30	4.39
Ethylbenzene (mg L^{-1})	4.38	4.29	4.30	4.11	4.12	4.18	4.20
m,p-Xylenes[b] (mg L^{-1})	9.22	9.15	9.39	9.08	9.28	9.03	8.88
o-Xylene (mg L^{-1})	4.60	4.69	4.49	4.71	4.58	4.56	4.70

FL, feeding line; HS, headspace.
[a] At 1 atm and 27 °C.
[b] The isomers meta- and para-xylenes were quantified together due to the chromatographic method limitation.

2.2.3. Effect of microaeration dosing point on BTEX removal

At stage V, in order to verify the effect of the dosing point, the microaeration was moved from the bioreactor feeding line to its headspace, and the flow rate was set back to 1 mL air min^{-1} (at 1 atm and 27 °C) (Table 1).

2.2.4. Effect of effluent recirculation on BTEX removal

At stage VI, the air dosage was moved back to the feeding line as at previous stages (II to IV). However, although the airflow rate was kept at 1 mL air min^{-1} (0.140 L O_2 L^{-1} feeding at 1 atm and 27 °C), effluent recirculation was turned off in order to assess its impact on BTEX removal efficiency (Table 1). Finally, at stage VII, effluent recirculation was reestablished, i.e. the operational conditions were the same as at stage II (Table 1).

2.2.5. Sensitivity analysis and recovery time in microaeration failures

After stage VII, the impact of a microaeration shutdown (MS1) (a simulated operational failure) on the bioreactor BTEX removal performance was assessed. For this purpose, the microaeration (1 mL air min^{-1} at 1 atm and 27 °C) was turned off for 7 days and then turned on again. In order to verify the reproducibility of the system response, two more 7-day shutdowns (MS2 and MS3) were carried out after the bioreactor reached effluent concentrations similar to those obtained before the simulated failures (MS1 and MS2, respectively). The other bioreactor operational parameters during this experiment were similar to those of stage VII (Table 1).

The impact of the microaeration shutdowns was assessed by the parameters sensitivity index (SI) and recovery time (RT) (Cai et al., 2009). Although these parameters were originally used to evaluate the effect of load shocks on the performance of treatment systems (Cai et al., 2009), they were used in this experiment because they were adequate to visualize the impact of such failures on effluent quality for the different compounds tested.

Therefore, SI was calculated using Equation (1):

$$SI = \frac{O_{max} - O_n}{O_n}, \quad (1)$$

in which O_{max} is the maximum compound concentration observed in the effluent during the operational failure period, and O_n is the concentration normally observed in the effluent before the failure.

RT was defined as the time required for the system to reach values similar to those obtained in the pre-failure period after reestablishing the microaeration.

2.3. Chemical and chromatographic analysis

COD and pH were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

BTEX compounds were determined in water samples by static headspace extraction (Triplus HS, Thermo Scientific, USA) followed by gas chromatography-flame ionization detection (HS-GC-FID) (Trace GC Ultra, Thermo Scientific, USA) according to Carneiro et al. (2014). All samples were diluted with ultrapure water (Milli-Q system, EMD Millipore, USA) to a final volume of 10 mL directly into borosilicate glass vials (20 mL) for headspace extraction (Supelco, USA), which were then sealed with PTFE/silicone septa and aluminum seals (Supelco, USA) (Firmino et al., 2015a).

In order to verify BTEX volatilization, these hydrocarbons were also determined in biogas by gas chromatography-flame ionization detection (GC-FID) (Trace GC Ultra, Thermo Scientific, USA). 0.5-mL biogas samples were collected using a Gastight syringe, manually injected in splitless mode, and analyzed at the same aforementioned chromatographic conditions (Carneiro et al., 2014).

The biogas characterization was performed in terms of air (O_2 + N_2), CO_2 and CH_4, by gas chromatography-thermal conductivity detection (GC-TCD) (GC-17A, Shimadzu Corporation, Japan) as described by Firmino et al. (2015b).

2.4. Statistical analysis

The non-parametric Mann-Whitney and Kruskal-Wallis tests, which do not require a specific data distribution, were used for the statistical analysis of the data in order to compare the bioreactor performance during different experimental stages at a 95.0% confidence level (Firmino et al., 2015b).

3. Results and discussion

3.1. Anaerobic BTEX removal

After inoculation, the system was fed with ethanol and BTEX as carbon source under anaerobic conditions (stage I). The highest removal efficiencies were obtained for m,p-xylenes, followed by ethylbenzene, o-xylene and toluene, respectively, whereas benzene was the most recalcitrant compound, showing an average efficiency of approximately 55% (Table 2).

Similar results were found by Firmino et al. (2015a), who used a UASB bioreactor (HRT = 48 h) to treat a water contaminated with BTEX (~3.0 mg L^{-1} of each compound) and ethanol (~1.8 g COD L^{-1}). These authors also reported that BTEX removal occurred in the same order as in the current study, in which the highest average efficiency were achieved for m,p-xylenes (~87%), and the lowest one, for benzene (53%).

As BTEX compounds are volatile and, consequently, can be transferred from liquid to air, they were also monitored in biogas. Very low load values (~1 μg day^{-1}) were found for all compounds (Table S1), which represented a loss of less than 0.01% over the influent load. Therefore, BTEX volatilization was negligible under anaerobic conditions, which suggests that biological activity was the main removal mechanism.

3.2. Effect of microaeration flow on BTEX removal

After the bioreactor started to be microaerated at a flow rate of only 1.0 mL air min^{-1} (at 1 atm and 27 °C) (stage II), the removal efficiencies of benzene and toluene increased from approximately 55 and 67% to 84 and 89%, respectively (Table 2). Similarly, the other compounds, although with a lower increase (7.5–11%), had their removal efficiencies increased to values greater than 85% (Table 2).

These results are in accordance with those obtained by Firmino et al. (2018), who operated a similar UASB bioreactor (HRT = 48 h) to treat a BTEX-contaminated water (~3.0 mg L^{-1} of each compound) under microaerobic conditions. When an airflow rate of 1.0 mL min^{-1} was applied to the bioreactor at the feeding line, removal efficiencies above 80% were achieved for all compounds, especially for benzene (with a 30% removal increase).

In fact, oxygen is a key reagent at the first step of the aromatic ring cleavage, which facilitates the following microbial degradation of by-products with lower molecular weights (Liu, 2015). Thus, under microaerobic conditions, microorganisms can use mono-oxygenase enzymes to convert monoaromatics to phenols by inserting a hydroxyl group into the aromatic ring, therefore reducing their toxicity (Fuchs, 2008; Yerushalmi et al., 2001). For instance, the subsequent aerobic benzene mineralization would require high saturation of oxygen in the liquid medium (8–12 mg O_2 L^{-1} for the oxidation of 5–8 mg L^{-1} benzene). Conversely, under microaerobic conditions, after the activation of the aromatic ring of this hydrocarbon by the mono-oxygenase, the by-product (phenol) can be biodegraded anaerobically (Yerushalmi et al., 2001). Therefore, in the present study, it is probable that microaeration assisted the initial activation of BTEX compounds, which is

Table 2
Average influent and effluent concentrations of benzene (B), toluene (T), ethylbenzene (E), m,p-xylenes (m,p-X) and o-xylene (o-X), and their respective average removal efficiencies during the different operational conditions.

Stage		I	II	III	IV	V	VI	VII
Dosing point		–	FL	FL	FL	HS	FL	FL
Microaeration (mL air min^{-1})		–	1.0	0.5	2.0	1.0	1.0	1.0
Recirculation (L h^{-1})		0.72	0.72	0.72	0.72	0.72	–	0.72
B	Influent (mg L^{-1})	4.27 ± 0.17	4.16 ± 0.20	4.17 ± 0.18	4.34 ± 0.24	4.26 ± 0.20	4.19 ± 0.17	4.47 ± 0.26
	Effluent (mg L^{-1})	1.93 ± 0.09	0.67 ± 0.05	1.00 ± 0.05	0.73 ± 0.08	1.71 ± 0.12	1.14 ± 0.12	0.75 ± 0.04
	Efficiency (%)	54.9 ± 1.21	83.9 ± 1.41	75.9 ± 0.89	83.1 ± 2.29	59.8 ± 2.29	72.7 ± 3.62	83.1 ± 1.30
T	Influent (mg L^{-1})	4.58 ± 0.18	4.28 ± 0.14	4.27 ± 0.14	4.45 ± 0.34	4.42 ± 0.18	4.30 ± 0.13	4.39 ± 0.28
	Effluent (mg L^{-1})	1.50 ± 0.13	0.49 ± 0.08	0.81 ± 0.03	0.46 ± 0.11	0.86 ± 0.08	0.44 ± 0.07	0.29 ± 0.13
	Efficiency (%)	67.3 ± 2.39	88.6 ± 2.00	81.1 ± 0.95	89.9 ± 1.80	80.6 ± 1.71	89.8 ± 1.92	93.3 ± 3.03
E	Influent (mg L^{-1})	4.38 ± 0.24	4.29 ± 0.22	4.30 ± 0.17	4.11 ± 0.28	4.12 ± 0.23	4.18 ± 0.19	4.20 ± 0.18
	Effluent (mg L^{-1})	0.81 ± 0.04	0.40 ± 0.06	0.65 ± 0.03	0.50 ± 0.07	0.84 ± 0.04	0.42 ± 0.11	0.39 ± 0.05
	Efficiency (%)	81.4 ± 1.11	90.6 ± 1.28	85.0 ± 0.92	87.9 ± 1.44	79.6 ± 1.20	89.1 ± 3.30	90.6 ± 1.27
m,p-X	Influent (mg L^{-1})	9.22 ± 0.36	9.15 ± 0.26	9.39 ± 0.36	9.08 ± 0.39	9.28 ± 0.33	9.03 ± 0.19	8.88 ± 0.46
	Effluent (mg L^{-1})	1.65 ± 0.12	0.95 ± 0.12	1.00 ± 0.06	1.11 ± 0.13	1.88 ± 0.21	2.14 ± 0.10	0.93 ± 0.08
	Efficiency (%)	82.1 ± 1.27	89.6 ± 1.49	89.4 ± 0.67	87.8 ± 1.44	79.7 ± 2.26	76.1 ± 1.10	89.5 ± 1.02
o-X	Influent (mg L^{-1})	4.60 ± 0.25	4.69 ± 0.12	4.49 ± 0.31	4.71 ± 0.19	4.58 ± 0.16	4.56 ± 0.13	4.70 ± 0.16
	Effluent (mg L^{-1})	1.14 ± 0.07	0.64 ± 0.08	0.73 ± 0.04	0.80 ± 0.05	1.14 ± 0.13	1.17 ± 0.04	0.63 ± 0.02
	Efficiency (%)	75.2 ± 2.09	86.3 ± 1.71	83.5 ± 1.81	83.0 ± 1.45	75.2 ± 2.73	74.0 ± 1.53	86.5 ± 0.84

FL, feeding line; HS, headspace.

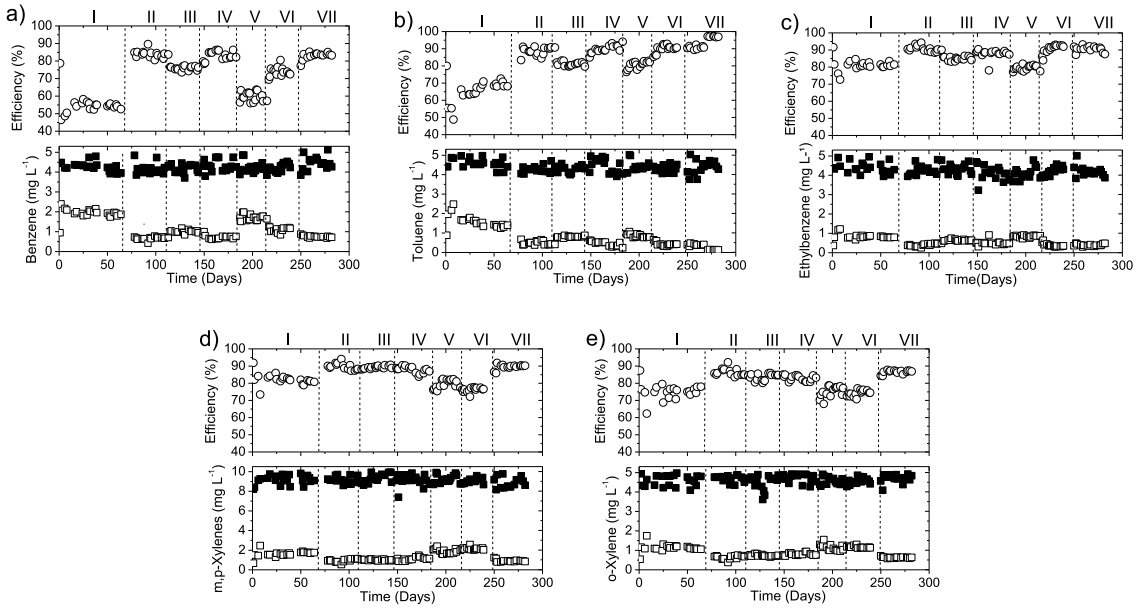

Fig. 1. Influent (filled squares) and effluent (empty squares) concentrations of benzene (a), toluene (b), ethylbenzene (c), m,p-xylenes (d) and o-xylene (e), and their respective removal efficiencies (empty circles) over the different operational stages.

usually considered the limiting step of the anaerobic degradation process, mainly for benzene (Liu, 2015).

At stage III, after reducing the microaeration flow rate to 0.5 mL air min^{-1} (at 1 atm and 27 °C), benzene had its efficiency decreased to approximately 76% (Table 2), being the only compound with efficiencies below 80%, whereas m,p-xylenes removal remained similar to that of stage II (Fig. 1). The other compounds had a small reduction in their efficiency values (7.5%, 5.7% and 2.7% for toluene, ethylbenzene and o-xylene, respectively) (Table 2).

At stage IV, with the increase of the airflow rate to 2.0 mL min^{-1} (at 1 atm and 27 °C), it was expected an improvement in BTEX removal in relation to stage II (1.0 mL air min^{-1}). However, even with a twofold higher airflow rate, the removal efficiencies of these compounds presented values very close to those obtained at stage II (Fig. 1). In fact, there was no significant difference between both effluent concentrations and removal efficiencies obtained at stages II and IV for the compounds benzene, toluene and o-xylene (Table 2). Therefore, in the present work, 1 mL air min^{-1} was considered the best airflow rate in terms of BTEX removal efficiency, mainly for benzene removal, the most recalcitrant compound under anaerobic conditions (stage I).

The strategy of using air for microaeration causes a lower toxicity to strict anaerobic microorganisms present in anaerobic sludge than using pure oxygen (Krayzelova et al., 2015). However, increasing the airflow rate in a system will not always favor a better removal of a compound. A higher microaeration flow rate leads to an increased oxygen flow rate in the biofilm matrix and, as a result, deeper oxygen penetration, which may inhibit strict anaerobic microorganisms, such as acetoclastic bacteria and methanogenic archaea (Khongsumran et al., 2014; Wu et al., 2015).

Stephenson et al. (1999) used two UASB bioreactors continuously operated at a 24-h HRT and 35 °C. The oxygenation rate was varied, reaching a maximum dissolved oxygen (DO) concentration of 2.9 mg L^{-1}. Methane was formed even for the highest DO concentration, although in a smaller proportion. However, in the present study, there was no significant decrease in BTEX removal at stage IV when compared to the previous stages, with values similar to those of stage II (Fig. 1). Possibly, for the established operational conditions, the saturation of BTEX biodegradation was reached, not being possible an improvement in efficiency. Thus, the use of a lower microaeration flow rate without a significant change in the bioreactor performance represents lower operational costs.

Microaeration was also successful and contributed to increase the removal efficiency of BTEX and phenol in a research with petrochemical wastewater from a plant located in China (Wu et al., 2015). The authors compared a fully anaerobic bioreactor with another anaerobic system with limited aeration, presenting dissolved oxygen concentration from 0.2 to 0.3 mg L^{-1}. The bioreactors were inoculated with aerobic sludge (activated sludge system). The authors reported that even in the presence of considerable concentrations of BTEX and phenol (~45 mg L^{-1}), the microaerated bioreactor obtained an efficiency of 82.1%. In contrast, the efficiency found in the anaerobic bioreactor was only 38.7%.

Regarding the hydrocarbons volatilization, it was expected that, by microaerating the bioreactor (stage II), BTEX load in biogas would be higher than at stage I (anaerobic conditions) since a larger fraction of these monoaromatics might have been removed by stripping. However, the average values were even smaller than the registered ones at the previous stage (Table S1). Therefore, these results corroborate the hypothesis that the introduction of low amounts of oxygen into the system might have stimulated the production of mono-oxygenases by the bioreactor microbiota, which was, then, able to biodegrade BTEX more effectively.

By reducing the microaeration to 0.5 mL air min^{-1} at stage III, most of the compounds had their load in biogas increased (Table S1). Apparently, the activity of the mono-oxigenases were reduced, which decreased BTEX removal efficiencies as mentioned above (Table 2) and allowed the increment of these compounds in biogas. When the airflow rate was increased to 2.0 mL min^{-1} (stage IV), it was expected that the monoaromatics load in biogas would be at least as low as it was at stage II (1.0 mL air min^{-1}). However, except for m,p-xylenes, the amount of all hydrocarbons were higher than at stage II, and some of them were even higher than at stage III, when the airflow rate was only 0.5 mL min^{-1} (Table S1). Although BTEX removal efficiencies of stages II and IV were similar (Table 2), a microaeration flow rate above 1.0 mL air min^{-1} seemed to increase slightly the turbulence inside the bioreactor and, therefore, favor BTEX transfer from liquid to gaseous phase, probably by stripping. Nevertheless, the removal of these compounds by volatilization was still negligible, reinforcing that microbial activity played a major role in BTEX removal, which was not a mere physical process.

3.3. Effect of microaeration dosing point on BTEX removal

At stage V, the bioreactor started to be microaerated directly into its headspace (1.0 mL air min^{-1} at 1 atm and 27 °C), and a decrease in the removal efficiencies of the monoaromatic compounds was observed (Fig. 1). Practically, the average efficiencies of all compounds returned to those obtained under anaerobic conditions (stage I), except toluene, whose removal efficiency was approximately 13% greater than at the first stage (Table 2).

Studies on microaerobic H_2S removal in anaerobic bioreactors indicate that the headspace, specifically at the gas-liquid interface, is the best microaeration dosing point for removal of this compound. By applying air into the bioreactor headspace, oxygen can react directly with the gaseous hydrogen sulfide, and, therefore, the amount of air required by each given amount of hydrogen sulfide is minimized. The results of microbial analyses revealed that populations of sulfide oxidizing microorganisms grow mainly on the walls of the headspace or at the gas-liquid interface, suggesting that biological sulfide oxidation occurs at these places (Díaz et al., 2011; Krayzelova et al., 2015; Ramos et al., 2014).

However, for BTEX removal, the contact between microorganisms and substrates is crucial because the initial step of (aliphatic and aromatic) hydrocarbons degradation is generally mediated by oxidation reactions catalyzed by oxygenases associated with cell surface. Therefore, BTEX removal occurs in the sludge blanket, starting with the aromatic ring rupture (Varjani, 2017; Varjani and Upasani, 2017).

For the system studied, it was expected that the transfer of oxygen to the liquid would not be efficient since, most likely, the residence time of the air bubbles injected into the bioreactor would not be higher than 2 s (Firmino et al., 2018). Therefore, in microaerobic bioreactors, oxygen might be dissolved into the liquid principally from the air stored in the system headspace (gas-liquid interface), being the biogas residence time in this compartment an important parameter (Firmino et al., 2018; Lopes, 2010).

Hence, it was expected that the oxygen present in synthetic air, inserted directly into the headspace, would be dissolved into the liquid (in the bioreactor sedimentation compartment) and, assisted by effluent recirculation, would reach the microorganisms in the sludge blanket (in the bioreactor digestion compartment). However, probably, this did not occur. Mass transfer from a gaseous to a liquid phase can be controlled by the turbulence of the liquid (Herlina and Jirka, 2008), deformations of the gas-liquid interface caused by the gas movement (Turney and Banerjee, 2013), and, ultimately, molecular diffusion into this interface (Herlina and Jirka, 2008). Therefore, most likely, the absence of some turbulence at the gas-liquid interface (stage V), caused previously by the release of air bubbles from the liquid to the headspace (as at stages II to IV), might have hindered oxygen dissolution at this region.

The injection of air into the headspace considerably reduced BTEX loads in biogas, reaching values below the limit of detection, such as for toluene and ethylbenzene (Table S1). This operational condition might have favored the growth of a microbiota in this compartment capable of consuming the remaining hydrocarbons in biogas as verified in the aforementioned investigations involving H_2S degradation.

3.4. Effect of effluent recirculation on BTEX removal

At stage VI, the microaeration (1.0 mL air min^{-1} at 1 atm and 27 °C) was applied again at the feeding line and, to evaluate the impact of effluent recirculation on BTEX removal, the recirculation pump was turned off. The use of effluent recirculation can aid in the distribution of the liquid at the inlet of the treatment system, reducing the concentration gradient and toxicity of the substrate along the bioreactor and providing a more efficient mass transfer between substrate and microbiota, which, consequently, could influence positively the conversion of substrate into biogas (Mohan et al., 2007; Zuo et al., 2014). However, depending on the composition of the wastewater, the configuration of the treatment system and the recirculated flow rate, the results may be quite different (Díez-Montero et al., 2016; Giustinianovich et al., 2015; Ramakrishnan and Gupta, 2008).

Comparing the hydrocarbon efficiencies of the current stage with stage II (microaeration and recirculation), only toluene and ethylbenzene did not have their efficiencies altered by the absence of recirculation (Fig. 1). However, the efficiencies of the other compounds decreased considerably (11–14%) (Table 2). It is worth mentioning that the average removal efficiencies of xylenes were even lower than those of stage I (anaerobic conditions), being the lowest values recorded for these compounds throughout the experiment (Table 2).

Thus, the results indicate that recirculation seemed to play a fundamental role in microaerobic BTEX removal since it can increase the solubilization of oxygen in liquid (Stephenson et al., 1999). In the present work, the absence of liquid recirculation decreased the liquid-air, substrate-air and substrate-microorganism contacts, reducing the removal efficiencies of benzene and xylenes (Fig. 1). Additionally, according to Thaveesri et al. (1994), both recirculation and high upflow velocities improve mass transfer and reaction rates.

In relation to BTEX fraction in biogas, all hydrocarbons presented reduced values, with the exception of m,p-xylenes, for which the highest load was recorded during the whole experiment (2.49 μg day^{-1}) (Table S1). Nevertheless, it represents a loss of less than 0.02% over the pollutant load removed.

Although there were variations in some of its operational parameters, the bioreactor remained stable throughout the experiment. In fact, by returning to the same operational conditions as those of stage II (stage VII), which presented the best results for BTEX removal, similar efficiencies were obtained for all compounds except for toluene, which presented an increase of almost 5% in its average efficiency at stage VII (Table 2). This observation reinforces the importance of recirculation in microaerobic BTEX removal and indicates that the differences observed in the bioreactor performance during the different experimental stages were due to the operational changes made in the system and not a probable adaptation of the microbiota over time.

Surprisingly, at stage VII, the load values of all BTEX compounds in biogas were much lower than those observed at stage II, except for the m,p-xylenes isomers, which were twofold higher (1.82 μg day^{-1}) (Table S1). These results reinforces the hypothesis of growth of microorganisms in the headspace after the stage at which the microaeration was applied at this dosing point (stage V), which might have been responsible for decreasing even more the already low BTEX load in biogas.

Finally, it is worth mentioning that the bioreactor showed a good stability during the whole experiment (stages I to VII), in which COD removal efficiency and effluent pH remained approximately 80.5% and 7.7, respectively.

3.5. Sensitivity analysis and recovery time in microaeration failures

The sensitivity of a treatment system or microorganisms in the presence of BTEX can be verified by increasing the applied load, which can cause a decrease in the removal efficiency (Mohammad et al., 2017; Rajamanickam et al., 2017). In the present study, as microaeration showed an increase in BTEX removal efficiency, the sensitivity of the system was tested during microaeration shutdowns, evaluating the efficiency recovery after reestablishment of air injection (Fig. S1).

Prior to the first microaeration shutdown period (MS1), the average effluent concentrations of the benzene, toluene, ethylbenzene, m,p-xylenes and o-xylene compounds were approximately 0.75, 0.13, 0.44, 0.95, and 0.65 mg L^{-1}, respectively (Table 3). During MS1, the effluent concentrations of all aromatic hydrocarbons increased (Fig. S1), noticeably benzene, which presented the highest sensitivity index (SI), followed by ethylbenzene and m,p-xylenes (Table 3). Although toluene had a SI similar to those of the other hydrocarbons, it can be considered a different case (Table 3). The maximum effluent concentration of

Table 3
Sensitivity index and recovery time of each BTEX compound during microaeration shutdown periods.

Period	MS1			MS2			MS3		
Compound	EC (mg L^{-1})	SI	RT (d)	EC (mg L^{-1})	SI	RT (d)	EC (mg L^{-1})	SI	RT (d)
Benzene	0.75	1.86	2	0.86	1.53	2	0.33	1.44	2
Toluene	0.13	1.07	> 2	0.14	0.97	2	0.13	1.08	2
Ethylbenzene	0.44	1.42	2	0.60	1.05	2	0.60	1.16	2
m,p-Xylenes	0.95	1.15	> 2	1.18	0.64	2	1.16	0.67	2
o-Xylene	0.65	0.66	2	0.77	0.37	1	0.85	0.25	1

MS, microaeration shutdown; EC, effluent concentration before the shutdown period; SI, sensitivity index; RT, recovery time.

toluene during MS1 (0.28 mg L^{-1}) was considerably lower than the maximum values of the other compounds (Fig. S1). After the microaeration reestablishment, the recovery time (RT) for all BTEX compounds was between 2 and 3 days (Table 3).

In order to verify the reproducibility of the system response, the microaeration was turned off again for another 7-day period (MS2). The SI of all compounds reduced when compared to MS1, particularly for the isomers of xylene (~50% reduction), and the RT was up to 2 days (Table 3). At the third shutdown period (MS3), the SI and RT values were similar to those at MS2 (Table 3), showing a good reproducibility.

In general, the xylenes isomers were the least sensitive compound to microaeration shutdown, whereas benzene was the most sensitive one (Table 3). In fact, when the microaeration was turned off, benzene effluent concentrations achieved values similar to those obtained at stage I (> 2 mg L^{-1}), when the bioreactor was operated under anaerobic conditions (Fig. S1), reinforcing that oxygen plays a major role in the initial activation of this aromatic hydrocarbon.

Similarly, in biogas, BTEX daily load increased when the microaeration was turned off and decreased after the air was injected back into the system (Fig. S1), confirming that BTEX removal was mainly a biological process and not only a physical transfer from liquid to biogas (stripping) when the bioreactor was microaerated.

4. Conclusions

Microaeration (0.5–2.0 mL air min^{-1}) ensured high removal efficiencies (> 83%) for all compounds, and the best results were obtained for the flow rate of 1.0 mL air min^{-1}, particularly for benzene, the most recalcitrant compound, with a 30% increase in its removal efficiency.

Effluent recirculation showed to be an important factor to improve mass transfer and, consequently, BTEX removal.

BTEX removal by stripping was negligible even under microaerobic conditions, suggesting that microbial activity was the main removal mechanism.

Finally, after microaeration shutdown periods, during which effluent concentrations increased, the bioreactor could recover its prior performance within up to 2 days.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2018.06.066.

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