Impact of microaeration bioreactor on dissolved sulfide and methane removal from real UASB effluent for sewage treatment

C. S. Cabral, A. L. Sanson, R. J. C. F. Afonso, C. A. L. Chernicharo and J. C. Araújo

ABSTRACT

Two bioreactors were investigated as an alternative for the post-treatment of effluent from an upflow anaerobic sludge blanket (UASB) reactor treating domestic sewage, aiming at dissolved sulfide and methane removal. The bioreactors (R-control and R-air) were operated at different hydraulic retention times (HRT; 6 and 3 h) with or without aeration. Large sulfide and methane removal efficiencies were achieved by the microaerated reactor at HRT of 6 h. At this HRT, sulfide removal efficiencies were equal to 61% and 79%, and methane removal efficiencies were 31% and 55% for R-control and R-air, respectively. At an HRT of 3 h, sulfide removal efficiencies were 22% (R-control) and 33% (R-air) and methane removal did not occur. The complete oxidation of sulfide, with sulfate formation, prevailed in both phases and bioreactors. However, elemental sulfur formation was more predominant at an HRT of 6 h than at an HRT of 3 h. Taken together, the results show that post-treatment improved the anaerobic effluent quality in terms of chemical oxygen demand and solids removal. However, ammoniacal nitrogen was not removed due to either the low concentration of air provided or the absence of microorganisms involved in the nitrogen cycle.

Key words | biological oxidation, dissolved methane, dissolved sulfide, elemental sulfur, microaeration, UASB effluent

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HIGHLIGHTS

- Post-treatment of upflow anaerobic sludge blanket effluent treating real domestic wastewater.
- Biological oxidation of dissolved sulfide and methane.
- Microaeration to improve anaerobic effluent treated.
- Methanotrophic and sulfur (purple and green sulfur) bacteria.
- Microbial community in bioreactors treating anaerobic effluent.

INTRODUCTION

In the tropical climatic zone where most of the developing countries are located, there is significant potential to adopt anaerobic technologies for sewage treatment. In fact, among the anaerobic systems, the use of upflow anaerobic sludge blanket (UASB) reactors for sewage treatment has steadily grown since its introduction in several countries, especially those in Latin America and India (Chernicharo *et al.* 2015). Brazil has the largest park of UASB reactors in the world, considering the technological application for sewage treatment (Chernicharo *et al.* 2018).

The main advantage of UASB technology is the negligible or even zero energy demand, resulting in an up to tenfold drop in operational costs compared to those for activated sludge. However, some limitations remain, such

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as an unpleasant odour due to dissolved sulfide and methane losses in the removal process of the effluent and nutrients (Chernicharo *et al.* 2015).

Therefore, post-treatment units are crucial to improve the quality of the effluent and to reduce environmental impacts. Methods that have been proposed for sulfide removal from anaerobic effluents include membrane photobioreactors and membrane aerated biofilm reactors (MABR) (González *et al.* 2017; Camiloti *et al.* 2018). Dissolved methane removal from anaerobic effluents has also been studied using physical and/or biological methods such as membranes, biofilters, aeration and biological oxidation (Hartley & Lant 2006; Silva-Teira *et al.* 2017; Henares *et al.* 2018; Huete *et al.* 2018).

Microaeration can be an alternative to improve the effluent quality in terms of reduced organic and inorganic species and dissolved methane gas (Khan *et al.* 2011). This is because sulfide-oxidizing bacteria (SOB) are the main group involved in sulfide oxidation under microaerobic conditions, and aerobic methanotrophic bacteria, which are involved in methane removal, can also grow under such conditions. In the biological oxidation of sulfide, the final products (elemental sulfur or sulfate) will depend on the amount of oxygen available for SOB. Briefly, under limited oxygen conditions (microaerobic), elemental sulfur is the main product (Janssen *et al.* 1995). Regarding dissolved methane removal, microaeration conditions can select for methane-oxidizing bacteria such as *Methylomonas*, *Methylomicrobium* and *Methylocystis,* as noted by Matsuura *et al.* (2015). Methane oxidation by methanotrophic bacteria involves four successive oxidation reactions. The first product formed was methanol and then formaldehyde. The formaldehyde can undergo anabolic pathways to synthesize multicarbon cell components or catabolic pathways, comprising the oxidation into formate by formaldehyde dehydrogenase and, finally, the generation of CO_2 (Veillette *et al.* 2012).

In addition, exposure to sunlight favours the growth of photosynthetic bacteria (PSB). This group can utilize various substrates as carbon or nitrogen sources, which are suitable for wastewater treatment (Yang *et al.* 2018).

Previous studies have shown that bioreactors exposed to sunlight can improve the quality of effluent from the UASB reactor treating domestic sewage (Garcia *et al.* 2017; Azevedo *et al.* 2018). The microbial communities in the bioreactors were investigated and showed a complex and diverse community including hydrolytic/fermenting bacteria, sulfur bacteria, non-sulfur bacteria, methanogens, sulfate-reducing bacteria and methanotrophic bacteria. Significant sulfide removal was observed with an almost complete abatement in some conditions with elemental sulfur formation (Garcia *et al.* 2017). Despite the detection of methanotrophic bacteria in the system, methane removal was not investigated.

Therefore, this study aims to improve the quality of the anaerobic effluent by post-treating UASB reactor effluent in two bioreactors without inoculation exposed to sunlight and microaeration as an alternative for sulfide and methane removal. The overall performance of the reactors in terms of nitrogen bioconversion, organic matter and solid removal was evaluated.

MATERIALS AND METHODS

Experimental set-up

The treatment system consisted of a pilot-scale UASB reactor (340 L) built in fibreglass followed by two identical acrylic bioreactors (volume = 25 L, inner diameter = 35 cm, h = 50 cm), designed with a shape similar to the settler compartment of a UASB reactor, operating in parallel and under conditions next to the real scale (Figure 1). The UASB reactor treated real domestic wastewater after preliminary treatment, and the bioreactors post-treated the anaerobic effluent.

Both bioreactors were operated in two phases with a hydraulic retention time (HRT) of 6 and 3 h, corresponding to 120 L d^{-1} and 240 L d^{-1}, respectively, and were monitored over 13 months. The bioreactors were packed with polypropylene rings exposed to sunlight and were not inoculated. Air was continuously supplied to only one reactor (R-air) with an airflow of 0.010 L L^{-1} h^{-1} (240 L m^{-3} d^{-1}). The other reactor was operated without aeration as a control reactor (R-control). The microaeration apparatus consisted of an aquarium pump and a perforated tube to distribute small bubbles at the base of the bioreactor (Figure 1). The systems were installed at the Centre for Research and Training in Sanitation (CePTS) UFMG/COPASA, located at the Arrudas wastewater treatment plant (design flow rate of 4.5 m^3 s^{-1}) in Belo Horizonte, Brazil.

Dissolved gases

Influent and effluent samples of the bioreactors were collected twice a week for analysis of dissolved sulfide and methane. The procedure for liquid sample collection and preservation of sulfide (S^{2-}) followed the recommendations of *Standard Methods* (APHA 2012). An adaptation of the methodology described by Plas *et al.* (1992) was used for the analysis of dissolved sulfide. The analytical procedure for dissolved methane (CH_4) was adapted from the methods

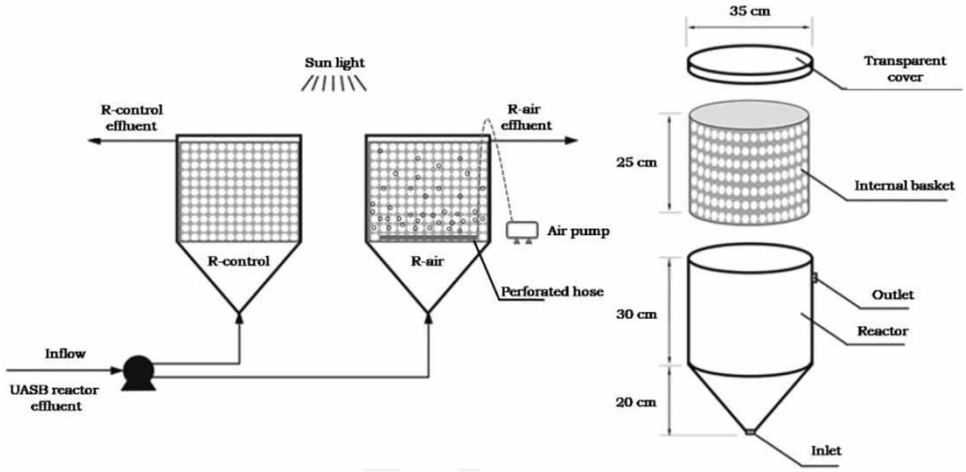

Figure 1 | Schematic representation and dimensions of the bioreactors. Adapted from Garcia *et al.* (2017).

described by Alberto *et al.* (2000) and Hartley & Lant (2006), based on headspace gas samples and gas chromatography (Perkin Elmer).

Performance parameters

To evaluate treatment performance, other parameters besides dissolved gases were measured. Chemical oxygen demand (COD), total suspended solids, volatile suspended solids, sulfate (SO_4^{2-}), ammonium and nitrite were measured according to *Standard Methods* (APHA 2012). Nitrate was evaluated using the Nitraver 5 nitrate reagent (Hach). Elemental sulfur (S^0) in the influent and effluent samples was measured by extraction with chloroform and determined by high performance liquid chromatography (HPLC) using a PRP-1 reverse phase HPLC column (dimensions: 15 cm length × 4.1 mm internal diametre) as described by Henshaw *et al.* (1998). Temperature, dissolved oxygen (DO), pH and oxidation reduction potential (ORP) within the bioreactors were determined using a multiparameter probe (HACH HQ40D).

Identification of the microbial community

Samples of attached biomass from polypropylene rings and sludge present at the bottom of both bioreactors were collected at the end of each phase. Total DNA was extracted from 0.5 g of biomass using a Power KitSoil (MO Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocols. DNA quantification was carried out using a NanoDrop2000 spectrophotometer (Thermo Scientific, USA). The DNA was stored at −20 °C and subjected to amplicon sequencing. Sequencing was performed at McGill University and Genome Quebec Innovation Centre (Canada) on the Illumina MiSeq System (PE300), using universal primers 926f-modified (5′-AAACTYAAAKGAATWGRCGG-3′) and 1392r-modified (5′-ACGGGCGGTGWGTRC-3′), targeting the V6–V8 variable region of the 16S rRNA gene from bacteria and archaea. Sequencing reads were analysed with the QIIME package (qiime2-2019.4) (Quantitative Insights Into Microbial Ecology, Caporaso *et al.* 2010), by joining forward and reverse reads, removing low-quality sequences, removing chimeric sequences and identifying operational taxonomic units at 97% similarity (method usearch61, Edgar 2010) using the reference database SILVA (Silva 132_99_926F1392R_CLASSIFIER_2019_4).

Statistical analyses

Statistical analysis was performed using Statistica Software. To compare influent and effluent concentrations and removal efficiency of dissolved gases in both phases, the Mann–Whitney test for independent samples was applied at a significance level of 5%.

RESULTS AND DISCUSSION

Sulfide removal in bioreactors

Dissolved sulfide concentrations were evaluated in the influent and effluent samples of the bioreactors. The results showed that a higher amount of dissolved sulfide was removed by the bioreactor supplied with air (R-air) in both HRTs (Figure 2) than in the control reactor. By reducing the HRT from 6 h to 3 h, the sulfide loading rate increased from 52 g S^{2-} m^{-3} d^{-1} to 152 g S^{2-} m^{-3} d^{-1}. This decrease

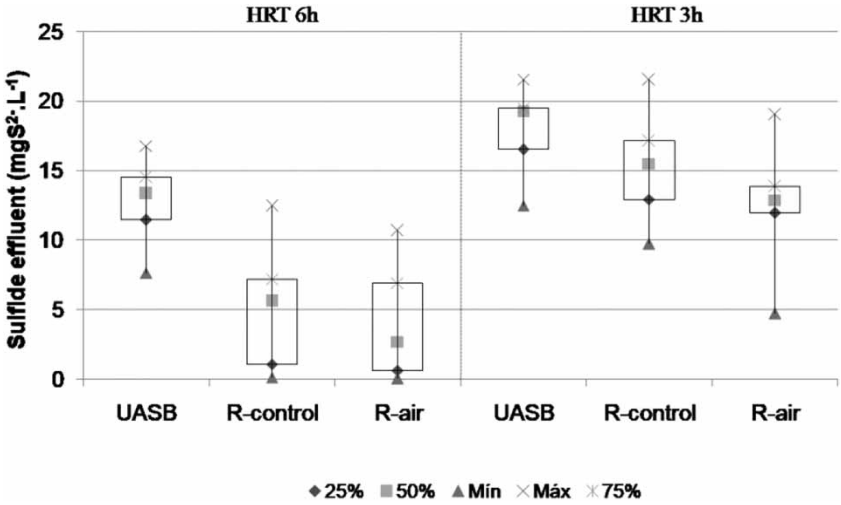

Figure 2 | Box plot of sulfide concentrations (influent and effluent) of two bioreactors in both HRT (6 and 3 h).

in HRT coupled with low oxygen concentrations reduced sulfide removal and the sulfide effluent remained high (median concentrations of 15.5 and 13.0 mg L^{-1} for R-control and R-air, respectively). The optimal scenario was observed at an HRT of 6 h with 61% sulfide removal efficiency in R-control and 79% in R-air. A statistical difference was observed between the bioreactors (R-control and R-air) and the UASB effluent, but not between the bioreactors.

In Brazil, the sulfide discharge standard established by the National Environmental Council is 1.0 mg L^{-1} (CONAMA 2011). Thus, considering the median sulfide concentration values obtained in the effluents from reactors R-control and R-air at HRT of 6 h (5.6 mg S^{2-} L^{-1} and 2.7 mg S^{2-} L^{-1}, respectively), we can conclude that under the operational conditions employed in this study, both reactors were not completely successful in removing sulfide and in complying with the Brazilian discharge standard. At an HRT of 3 h, the median values were even higher (as previously mentioned).

By using anaerobic bioreactors exposed to sunlight to treat real domestic wastewater and using different packing materials (polypropylene rings and polyurethane sponge), Garcia *et al.* (2017) obtained low sulfide concentrations at an HRT of 6 h. Sulfide concentrations in the effluent of bioreactors packed with polypropylene rings and polyurethane sponge were 1.6 and 0.8 mg S^{2-} L^{-1}, corresponding to 52% and 69% removal efficiency, respectively. The lower sulfide concentrations observed by Garcia *et al.* (2017) might be due to the considerably lower sulfide loading rate (14 g S^{2-} m^{-3} d^{-1}) applied compared to the present study. As both studies (Garcia *et al.* 2017 and the present study) used real domestic wastewater in which sulfide concentrations and loads varied between studies, the sulfide removal efficiency values are more reliable than sulfide concentrations when comparing these studies. With respect to the sulfide removal efficiency, the results of the present study indicated that microaeration improved sulfide removal from anaerobic effluent.

Sunlight can be an important parameter in wastewater treatment because it favours the growth of PSB. These bacteria can perform wastewater treatment and utilize nutrients simultaneously. Different PSB organisms have several characteristics and metabolisms. They have the ability to use carbon, nitrogen, sulfur, hydrogen and/or phosphorus compounds. In relation to oxygen demand, they are active in aerobic, anaerobic and/or microaerobic conditions, in addition to light (Lu *et al.* 2019). Sulfide removal was observed in the post-treatment of UASB effluent with sunlight due to the presence of green and purple sulfur bacteria and purple non-sulfur bacteria (Garcia *et al.* 2015, 2017; Azevedo *et al.* 2018).

The route of partial sulfide oxidation to elemental sulfur or complete sulfide oxidation to sulfate was evaluated using elemental sulfur (S^0) and sulfate (SO_4^{2-}) analysis, respectively. In the influent, the forms of sulfur measured were SO_4^{2-} and sulfide (S^{2-}), whereas for the effluent, three forms of sulfur (S^0, SO_4^{2-} and S^{2-}) were measured (Figure 3). The fractions of sulfur precipitated or adsorbed in the sludge biomass were not measured or the thiosulfate in the effluent. The sulfur mass balance difference observed between the R-control and R-air at each phase may be due to sulfur forms and compounds not measured in the present study, such as thiosulfate. The sulfur content of the UASB effluent was different between phase 1 (HRT 6 h) and phase 2 (HRT

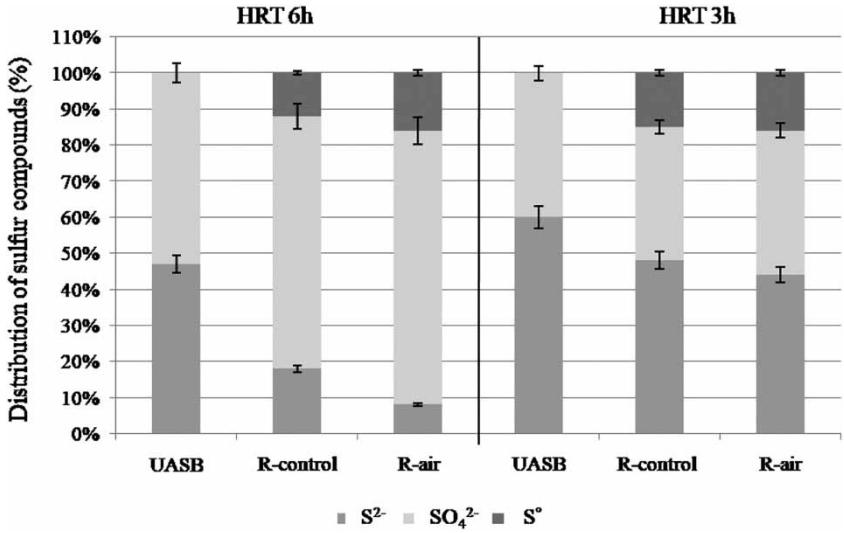

Figure 3 | Sulfur species mass balance in UASB, R-control and R-air effluent in both phases (HRT 6 h and 3 h).

3 h) (Figure 3). The operation of the UASB was similar in both phases; therefore, this difference might be related to the influent sulfate concentration (corresponding to raw domestic sewage).

At an HRT of 6 h, the mass of sulfate in the effluents of both the bioreactors increased compared to that in the UASB effluent, indicating that the sulfide influent was completely oxidized to sulfate during the biological post-treatment (Figure 3).

The ratio of S^0 formed/S^{2-} influent shows the proportion of elemental sulfur formed to the sulfide loading rate applied in the influent. This ratio indicates that although the amount of elemental sulfur formed was higher in both bioreactors in phase 2 (at HRT of 3 h, with S^{2-} loading rate equal to 152 g S^{2-} m^{-3} d^{-1}) than in phase 1 (at HRT of 6 h, with S^{2-} loading rate equal to 52 g S^{2-} m^{-3} d^{-1}), in the bioreactor supplied with air (R-air) at an HRT of 6 h, the elemental sulfur formation, in relation to sulfide influent, was proportionally higher (Table 1).

In both HRT, although S^{2-} was totally oxidized to SO_4^{2-} instead of partially to S^0, the problems that could occur due to the presence of sulfide in anaerobic effluent, as highlighted by Krayzelova *et al.* (2015), such as corrosion of water transport systems, emissions of unpleasant odours and toxicity to humans, could all be solved. It is important to highlight that the sulfide concentration effluent must meet the Brazilian standard as mentioned earlier.

Camiloti *et al.* (2018) observed a high rate of sulfide removal (90%) from anaerobic effluent when using a UASB reactor combined with an MABR to treat synthetic effluent with pure oxygen (flow of 2.4 L h^{-1}). They observed that microaeration improved sulfate removal and decreased sulfide effluent and that complete re-oxidation to sulfate did not occur or oxidation to thiosulfate or sulfite. In microaerobic conditions, elemental sulfur was formed, but was not removed and analysed because it was attached to the membrane. Most of the studies found in the literature (including the study of Camiloti *et al.* 2018) used synthetic effluent with known concentrations of sulfur compounds. This is associated with microaeration, applied to improve the efficiency of the systems, and allows better control of sulfur conversions when compared to the use of real domestic wastewater; thus, high sulfur formation from anaerobic effluent is possible.

Table 1 | Sulfide loading rate, sulfide removal efficiency (median values) and the ratio of elemental sulfur and sulfate formed to influent sulfide

Phases	Bioreactor	Sulfide loading rate (g S^{2-} m^{-3} d^{-1})	Sulfide removal (%)	$S^0_{formed}/S^{2-}_{influent}$ (%)[a]	$SO_4^{2-}{}_{formed}/S^{2-}_{influent}$ (%)[b]
1-HRT 6 h	R-control	52	61	28	56
	R-air		79	41	66
2-HRT 3 h	R-control	152	22	24	20
	R-air		32	25	20

[a]Ratio between the S^0 mass in the effluent and S^{2-} in the influent.
[b]Ratio between the S mass of SO_4^{2-} in the effluent and S^{2-} in the influent.

Sousa *et al.* (2017) evaluated the biological oxidation of S^{2-} in anaerobic reactors. The top of a hybrid reactor (UASB unit and anaerobic filter) was open to the air, allowing gaseous exchange and the ingress of light. These conditions allowed the oxidation of S^{2-} in the anaerobic filter unit, which was mediated by suspended cells of photoautotrophic and/or photoheterotrophic bacteria. As a consequence, sulfide concentration decreased from 18.97 mg S L^{-1} in the UASB effluent to 0.86 mg S L^{-1} in the anaerobic filter effluent and was in accordance with the Brazilian standard discharge limits for sulfide (1 mg L^{-1}). Similar to the present study, light allowed the growth of phototrophic microorganisms and consequently aided sulfide removal. Thus, the unpleasant odour that is typical of anaerobic effluents was substantially reduced. The sulfide loading rate was approximately 16 g Sm^{-3} d^{-1}, which may justify the lower concentration of sulfide in the effluent, in contrast to the present study. The concentration of SO_4^{2-} (12.46 mg L^{-1}) was high in the effluent, thus maintaining the complete oxidation of S^{2-} to SO_4^{2-} (Sousa *et al.* 2017).

The feasibility of using an internal silicone membrane reactor to treat dissolved sulfide produced in anaerobic reactors was evaluated by Valdés *et al.* (2016). The reactor was inoculated with granular sludge from a full-scale UASB reactor used for poultry slaughterhouse wastewater treatment and fed with synthetic wastewater. The sulfide removal efficiencies reached 96% in a combined anaerobic/microaerobic reactor, and significant sulfate production did not occur. The reactor configuration allowed the development of SOB under microaerobic conditions (airflow of 4.5 L h^{-1}) and improved sulfide conversion to elemental sulfur. Higher sulfide oxidation was achieved by the authors with low sulfate production, even with higher air flow in comparison to the present study. These results could be as a result of the reactor configuration, the use of permeable membranes and the diffusion of oxygen to treat the synthetic wastewater, which allowed the colonization of SOB.

Dissolved sulfide removal from anaerobic effluent by biological methods has lower operational costs due to a reduced or absent utilization of chemicals. In addition, the biological sulfide removal process displays a high removal rate and generates elemental sulfur, thus recovering mineral resources from waste (Cai *et al.* 2017). The final products of biological oxidation depend on the amount of oxygen available for SOB (Pokasoowan *et al.* 2009; Tang *et al.* 2009). Under oxygen-limited conditions (dissolved oxygen less than 0.1 mg L^{-1}), sulfur is the major end-product of sulfide oxidation, while sulfate is formed under the conditions of limited sulfide (Pokasoowan *et al.* 2009). However, the reports on the different oxygen amounts applied to the anaerobic process vary widely; there is no set of parameters or general indicators to compare the results of different studies objectively (Guerrero *et al.* 2016). It is important to highlight that most studies involving dissolved sulfide removal used synthetic effluent and an inoculation of biomass; however, the present study was conducted with real domestic sewage and without inoculation.

Methane removal

Figure 4 shows the results of dissolved methane from treated domestic wastewater in the UASB effluent and in the bioreactors effluent. High concentrations of methane in the UASB effluent were observed with median values equal to 20 and 18 mg CH_4 L^{-1}, at HRT 6 h and 3 h, respectively. Souza *et al.* (2011) reported concentrations in the range of 18 to 22 mg L^{-1} in the effluent of pilot-, demo-, and full-scale UASB reactors treating domestic wastewater. The methane losses in the effluent from these reactors accounted for 36–41% of the total methane produced (Souza *et al.* 2011). Silva-Teira *et al.* (2017) observed 29 mg CH_4 L^{-1} in UASB effluent treating synthetic wastewater, corresponding to 20–30% of the overall methane generated in the UASB reactor.

The post-treatment of UASB effluent in bioreactors exposed to sunlight at an HRT of 6 h reduced the dissolved methane concentrations in the effluent to 14 mg L^{-1} and 9 mg L^{-1} in R-control and R-air, respectively (Figure 4). These differences were not significant, as determined by the Mann–Whitney test ($\alpha = 5\%$), but between the UASB effluent and each bioreactor effluent, a statistical difference was observed. Methane removal efficiencies in phase 1 (at HRT 6 h) were 31% in R-control and 55% in R-air. At an HRT of 3 h, the removal of dissolved methane in the post-treatment did not occur, as shown in Figure 4.

Silva-Teira *et al.* (2017) studied how the post-treatment of the UASB effluent in a membrane bioreactor simultaneously removes methane and nitrogen. The system was fed with synthetic low-strength wastewater. Methane removal efficiencies of approximately 80% and 95% were observed, but nitrogen was only partially removed. The DO in the aerated compartment was much higher (average DO concentration was 2.8 mg O_2 L^{-1}) than in the present study. This condition and inoculation may have been favourable for a high methane removal efficiency.

A study by Matsuura *et al.* (2015) used a two-stage closed downflow hanging sponge (DHS) system, which resulted in

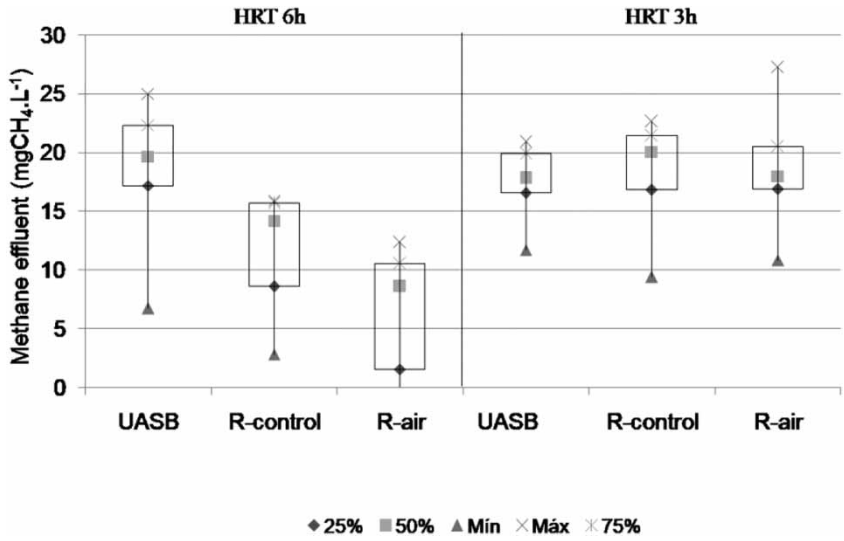

Figure 4 | Box plot of methane concentrations (influent and effluent) of two bioreactors in both HRT (6 and 3 h).

the removal of more than 99% of the dissolved methane during the experimental period. Air (2,500 L m^{-3} d^{-1}) was introduced in the second DHS to oxidize the residual dissolved methane from the first DHS. The authors reported that the air flow rate was also a crucial factor in determining the treatment performance of the DHS reactors. The almost complete abatement of dissolved methane reported by Matsuura *et al.* (2015) in comparison to other studies, including the present study, could be related to the air flow applied and the type of reactor used. Under these conditions, dissolved methane was recovered and a low amount of methane was emitted into the atmosphere.

The presence of microorganisms involved in methane oxidation is imperative for methane removal in the post-treatment. Silva-Teira *et al.* (2017) detected methanotropic bacteria and denitrifying anaerobic methane oxidation microorganisms. Garcia *et al.* (2017) observed sequences related to *Methylomonas* (methanotrophic bacteria) in bioreactors treating UASB effluent, probably due to the presence of methane dissolved in the effluent. In another study, Matsuura *et al.* (2015) reported the presence of methane-oxidizing bacteria. In the present study, the conditions investigated favoured the growth of methanotrophic bacteria in the bioreactors (2.1% of the total sequences were related to methanotrophs, according to the amplicon sequencing results). Therefore, part of the methane removed could be related to biological oxidation processes. Nevertheless, the inoculation of methanotrophic microorganisms (bioaugmentation), in order to increase the abundance of these microorganisms in the post-treatment reactor, could be an option to improve methane removal efficiencies.

Occurrence and removal of other contaminants

Microaeration did not affect the anoxic condition because the ORP values inside the reactors were still negative (R-air ranged from −78 mV to −88 mV and R-control from −86 mV to −95 mV). The median pH values in the UASB effluent were approximately 7.0. For the bioreactors, median pH values were between 7.0 and 7.1 in both phases. The organic matter removal efficiency was not affected by the microaeration as observed in other studies (Camiloti *et al.* 2018). The median COD concentrations of the R-air effluent equalled 110 mg COD L^{-1} (HRT of 6 h) and 85 mg COD L^{-1} (HRT of 3 h), which are slightly lower than the UASB effluent values of 124 mg COD L^{-1} and 94 mg COD L^{-1}, for HRTs of 6 h and 3 h, respectively. Nitrification did not occur in the bioreactors in both phases. The median ammonium concentrations observed in the UASB effluent were 41 mg NH_4–N^+L^{-1} (both HRT), 44 mg NH_4–N^+L^{-1}, and 43 mg NH_4–N^+L^{-1} for R-control and R-air at HRT 6 h, respectively. During phase 2 (HRT of 3 h), the median ammonium concentration values post-treatment were 46 and 43 mgL^{-1} for R-control and R-air, respectively. Nitrite and nitrate were absent, thus confirming that nitrification did not occur. These results could indicate the low availability of oxygen and/or the absence of microorganisms in the nitrogen cycle.

Microbial community

The microbial community composition in both reactors was investigated by 16S rRNA gene amplicon sequencing. The

investigation revealed the presence of methanotrophic bacteria as well as phototrophic bacteria (green and purple sulfur bacteria) related to methane and sulfide oxidation, respectively (Table 2).

In Table 2, sulfur bacteria groups were composed of sequences related to *Allochromatium* (*Chromatiales*), *Chlorobaculum* (*Chlorobiales*) and *Paracoccus* (*Rhodobacterales*) corresponding to purple sulfur-bacteria, green sulfur-bacteria and sulfur-oxidizing bacteria, respectively, as well as sequences of *Caldisericum* (*Caldisericales*), a thiosulfate-reducing bacterium, and *Tepidimonas* (*Betaproteobacteriales*), a chemoheterotrophic bacterium able to oxidize thiosulfate and tetrathionate to sulfate. *Blastochloris* (*Rhizobiales*), *Rhodobacter* (*Rhodobacterales*), *Rhodoblastus* (*Rhizobiales*), *Rhodocista* (*Azospirillales*), *Rhodoplanes* (*Rhizobiales*) and *Rhodopseudomonas* (*Rhizobiales*) were considered in regards to purple non-sulfur bacteria sequences and *Chloroflexus* (*Chloroflexales*) was considered in regards to green non-sulfur bacteria. Sulfate-reducing bacteria were composed of *Desulfovibrio* (*Desulfobacterales*) and *Smithella* (*Syntrophobacterales*) sequences. Methanotrophic bacteria were composed of sequences related to *Methylococcus* (*Methylococcales*) and *Methylocystis* (*Rhizobiales*). All results are presented as the percentage of sequences related to the total reads in a sample.

The results showed that the relative abundance of methanotrophic bacteria (Table 2), such as *Methylocystis* (2.0%) and *Methylococcus* (0.13%), were higher in R-air than in R-control. During phase 1 (HRT of 6 h), *Methylococcus* (0.5%), and *Methylocystis* (0.7%) were identified as well as phototrophic bacteria of the genera *Blastochloris* (27%), *Rhodopseudomonas* (27%) and *Rhodoplanes* (9%). These genera were dominant in both bioreactors at an HRT of 6 h. In phase 2 (at HRT of 3 h), the most dominant genus in the R-control was *Chloroflexus* (62.0%); whereas in the R-air, the most dominant genera were *Blastochloris* (27.0%), *Rhodoplanes* (8.4%) and *Rhodopseudomonas* (4.0%). These results confirm the evidence of methane and sulfide biological oxidation in both reactors, thus corroborating the physico-chemical removal data.

CONCLUSIONS

Two bioreactors were applied for sulfide and methane removal from anaerobic effluent. They were operated at different HRT (6 and 3 h) with and without aeration. The highest removal efficiencies were obtained (79% for sulfide and 55% for methane), under microaerobic conditions at an HRT of 6 h. The support material was important to retain the microorganisms inside the reactor, thus allowing biofilm formation. At an HRT of 3 h, the high sulfide loading rate reduced the sulfide removal. At this HRT, the air flow rate and the methane load applied to the R-air might not have been appropriate for the methanotrophs because the lowest abundances of these bacteria were observed in this condition. In both phases, complete oxidation of sulfide to sulfate was observed. Nevertheless, some elemental sulfur was formed and the ratio of S^0 formed/S^{2-} influent at HRT 6 h was higher than at an HRT of 3 h. Further research will be focused on the optimization and control of the amount of air dosed in order to improve sulfide removal and ensure elemental sulfur formation.

Microbial community analysis confirmed that the bacteria involved in the biological removal of sulfide and methane were present in the bioreactors. Global performance was not affected by small quantities of air supplied to the bioreactor.

The system could be improved by seeding the reactor with the bacteria involved in the nitrogen cycle and by controlling the aeration to allow nitrogen removal and thus improve effluent quality. The dose of air supplied to

Table 2 | Relative abundance of bacteria involved in sulfide and methane oxidation detected by 16S rRNA gene amplicon sequencing in the bioreactors

	R-control				R-air			
	Biomass		Sludge		Biomass		Sludge	
Abundance of bacteria (%)/phase (HRT)	6 h	3 h	6 h	3 h	6 h	3 h	6 h	3 h
Sulfur bacteria	0.58	3.15	0.88	7.2	0.82	5.18	1.28	0.73
Non-sulfur bacteria[a]	64.92	69.95	35.54	17.67	62.84	40.28	11.02	48.25
Sulfate-reducing bacteria	0.19	0.10	0.20	1.68	0.14	1.69	0.33	0.17
Methanotrophic bacteria	1.2	0.42	0.64	0.14	2.18	0.20	0.14	0.05

[a]This group comprises of sequences of phototrophic bacteria, including genera involved in sulfur oxidation (Garcia *et al.* 2017), organic acids and COD removal (Kim *et al.* 2004).

the bioreactors is still a challenge because there is no consensus in the scientific literature on the most effective concentration of DO to use or what airflow rate to use. In addition, most studies used synthetic effluent with controlled conditions and known influent compound concentrations. Finally, the wastewater treatment system described herein represents a feasible and low-cost alternative for the anaerobic treatment of domestic sewage by biological methods.

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