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Microbial community and sulphur behaviour in phototrophic reactors treating UASB effluent under different operational conditions

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ABSTRACT

Two phototrophic bioreactors were applied for sulphide removal from anaerobic effluent. They were operated at different hydraulic retention times (HRTs) and packed with different materials (R1 – polypropylene rings; R2 – polyurethane sponges). The microbial communities in both reactors were investigated by 454 pyrosequencing and results revealed that they were complex and the composition and diversity were influenced by the packing material as well as by the HRT. Sequences of phototrophic purple sulphur bacteria (*Chromatiaceae*) and non-sulphur bacteria (*Rhodopseudomonas*, *Rhodobacter*, *Rhodocista* and *Blastochloris*) were detected in both reactors together with sequences belonging to hydrolytic/fermenting bacteria, methanotrophic, sulphate-reducing bacteria and methanogens. Higher diversity and abundances of sulphur bacteria were observed in R1 than in R2. Whereas higher abundances of non-sulphur bacteria were observed in R2 than in R1. Greater sulphide removal efficiencies were achieved in both reactors at an HRT = 12 h. An almost complete abatement of the dissolved sulphide (>90%) together with a partial conversion of sulphide to sulphur (28%) was achieved in R2-sponge operated at an HRT of 12 h. The results call attention for the potential use of phototrophic reactors for the abatement of dissolved sulphide (and possibly organic matter and methane) from the anaerobic reactors effluents.

1. Introduction

Bioreactors that employ anaerobic process like the upflow anaerobic sludge blanket (UASB) reactor are widely used for domestic wastewater treatment, especially in hot climates countries like Brazil (Chernicharo et al., 2016). Although the UASB reactors provide several advantages in domestic wastewater treatment, significant amounts of hydrogen sulphide (H_2S) remain dissolved in the liquid phase (from 4 to 17 $mgS.l^{-1}$) as a result from the deassimilative reduction of sulphates or thiosulphates (Souza et al., 2012). Therefore, H_2S can be released from the effluent of anaerobic reactors and cause public nuisance because of its odour, as well as cause corrosion of structural materials in WWTPs, such as steel and concrete.

The biological oxidation of sulphide to sulphur and sulphate can be performed by different groups of bacteria, depending on the environmental conditions. Under aerobic conditions, reduced sulphur compounds support the growth of chemoautotrophic bacteria such as *Thiobacillus*, *Thiomicrospira*, *Achromatium*, *Beggiatoa* and *Thermothrix*. Most chemoautotrophs oxidize sulphide to elemental sulphur, which is then deposited inside the cell as granules, as illustrated by the following equation: $H_2S + ½ O_2 \rightarrow S^0 + H_2O$ ($\Delta G = -50$ Kcal) (Maier, 2000). Under anaerobic conditions, photoautotrophic bacteria such as *Chlorobium*, *Chromatium*, *Ectothiorhodospira*, *Thiopedia* and *Rhodopseudomonas* can grow via anoxygenic photosynthesis, using sulphide as an electron donor and carbon monoxide as a carbon source, as illustrated by the following equation: $CO_2 + H_2S \rightarrow S^0 +$ fixed carbon (Maier, 2000). The photoautotrophic oxidation of sulphur is limited to green (GSB)

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and purple sulphur bacteria (PSB). Most PSB stock granules of elemental sulphur inside the cell and perform the complete oxidation to sulphate, while the GSB carry out incomplete oxidation of sulphide and sulphur granules are deposited outside the cell (Tang et al., 2009).

Biological removal of sulphide from effluents has previously been demonstrated (Table 1). However, as shown in Table 1, reactor type, conditions (aerobic or anaerobic, with or without light), type of inoculum, medium (sterile and non-sterile), and sulphide removal efficiencies varied widely between studies. Most of the studies (shown in Table 1) that investigated phototrophic reactors for biological sulphide removal used pure or mixed cultures, which is not a realistic prospect if one thinks in full-scale application of this technology. Moreover, there has been no discussion of ways to select and enrich for the desirable sulphide-oxidizing phototrophs when the reactor is fed with a nonsterile medium, such as wastewater. Therefore, more research on low-cost biotechnological methods for sulphide removal using real wastewater is required. Moreover, the possibility of using microorganisms naturally occurring on the surface of the settler compartment of UASB reactors to remove sulphide from anaerobic effluent has only recently been investigated by our group (Garcia et al., 2015).

In a previous study, Garcia et al. (2015) designed and tested two bioreactors exposed to sunlight for the abatement of sulphide from the effluent of a UASB reactor treating domestic wastewater. The reactors were not inoculated with pure cultures; instead, bacteria naturally present in the anaerobic effluent developed in the reactors and colonized the support material inside the reactors (R1 was packed with polypropylene rings whereas R2 was filled with sponge). The performance of the reactors was measured at two different hydraulic retention times (HRTs), and sulphide removal efficiencies ranging from 30 to 65% were achieved at HRT of 24 h, whereas removal efficiencies of 90% were obtained at HRT of 12 h. These results indicated that by reducing the HRT (from 24h to 12 h), the sulphide loading rate (S^{2-} LR) increased (from 3.5 to 7.0 $mgS \cdot l^{-1} \cdot d^{-1}$) and consequently might have selected and enriched for sulphide oxidizing bacteria. The bacterial community diversity was investigated by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and this analysis revealed that GSB and PSB were present in these reactors and were likely responsible for the efficient sulphide removal observed. However, PCR-DGGE has limited resolving power and can only detect the dominant members of a bacterial community (Van Elsas and Boersma, 2011). Therefore, information obtained in the previous work, regarding microbial community diversity and the metabolic processes occurring in these reactors, were limited.

Second-generation sequencing technologies, due to their high coverage, provide more comprehensive information about microbial communities than the tradition Sanger-based methods (Pereira et al., 2014). Pyrosequencing can detect organisms at low abundance. Since the biomass is crucial for the efficiency of the biological sulphide removal and maintenance of the process, it is important to investigate in detail the composition and behaviour of microbial communities relative to increasing S^{2-} LR. Therefore, the aims of this study were: (i) to investigate the microbial community diversity in the phototrophic reactors developed to remove sulphide from anaerobic effluent by 454 pyrosequencing under different S^{2-} LR; (ii) to evaluate the influence of the packing material on composition of the microbial communities and sulphide conversions. To the best of our knowledge, this is the first time that

Table 1
Performances reported in the literature of different bioreactors for the dissolved sulphide abatement.

Reactor type	Inoculum used	Energy demand[a]	Type of wastewater and sulphide conc. ($mgS\ l^{-1}$)	Sulphide volumetric loading rate ($mgS\ l^{-1}h^{-1}$)	Sulphide removal efficiency (%)	Elemental sulphur formation (%)	Reference
Photosynthetic column and phototube	Enrichment culture of phototrophic bacteria (*Chlorobium* and *Rhodopseudomo-nas*)	yes	Effluent from anaerobic filter (6.0–36.0)	0.56–83.3	95	0–10[b]	Kobayashi et al. (1983)
Photosynthetic continuous stirred tank reactor	*Chlorobium limicola*	yes	Synthetic Na_2S medium (90–550)	2.1–5.6	100	100[c]	Henshaw et al. (1998)
Tubular photo-reactor	*Chlorobium thiosulfatophi-lum*	yes	Synthetic industrial wastewater	111–286	82–99	75–95[d]	Henshaw and Zhu (2001)
Tubular photo-reactor	*Chlorobium limicola*	yes	Synthetic industrial wastewater	83–1451	Aprox 100	–	Syed and Henshaw (2003)
Illuminated packed column reactors	*Chlorobium limicola*; natural sediment sample	yes	Synthetic sulphide-containing medium (32.1–112.2)	Not mentioned	99.5	Not mentioned	Ferrera et al. (2004)
Fed-Batch airlift reactor	Mixed culture of *Thiobacilli*	yes	Synthetic Na_2S medium (60131.3)	38.5–200.4	100	0–73[e] depending on O_2 conc.	Janssen et al. (1995)
Reverse fluidized loop reactor	Lab culture of *Thiobacillus denitrificans*	No	Synthetic wastewater (250)	250.0–312.2	90–100	65–90[c]	Krishnakumar et al. (2005)
Airlift bioreactor	activated sludge	yes	Synthetic medium (90-500)	33.3–170.8	90	5–93[d]	Lohwacharin and Annachhatre (2010)
Phototrophic reactor packed with polyurethane foam	Microorganisms present in the UASB effluent	No	Effluent from UASB reactor (1.0–6.0)	0.15–0.58	70–90	10–28[b]	This study
Phototrophic reactor packed with polypropylene rings	Microorganisms present in the UASB effluent	No	Effluent from UASB reactor (1.0–6.0)	0.15–0.58	50–90	4–15[b]	This study

[a] Energy demand for lighting or aeration.
[b] $S^0_{formed\ (g)}/Total\ S_{in}$.
[c] $S^0_{formed\ (g)}/S^{2-}_{removed}$.
[d] $S^0_{out\ (g)}/S^{2-}_{in}$.
[e] Used calculations were not informed.

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