Biochemical Engineering Journal 84 (2014) 1–8

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular Article

Hydrogen sulphide removal from air by biotrickling filter using open-pore polyurethane foam as a carrier

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ARTICLE INFO

Article history:
Received 29 November 2012
Received in revised form 24 October 2013
Accepted 25 December 2013
Available online 3 January 2014

Keywords:
Biodesulphurisation
Biofilter
Fixed-bed bioreactors
Aerobic processes
Thiobacillus denitrificans
Open-pore polyurethane foam

ABSTRACT

A study was conducted on H_2S removal in a biotrickling filter packed with open-pore polyurethane foam. *Thiobacillus denitrificans* was used as inoculum and a mixed culture population was developed during the process. The inhibitory effect of sulphate concentration (1.8–16.8 g L^{-1}), pH (6.9–8.6), trickling liquid velocity (TLV, 9.1–22.8 m h^{-1}), H_2S inlet concentration (20–157 ppmv) and the empty bed residence time (EBRT, 9–57 s) on the H_2S removal efficiency (RE) were thoroughly investigated. An increase in pH from 6.9 to 8.5 led to a corresponding increase in H_2S removal. In addition, an inhibitory effect of sulphate concentration was observed from 16.8 g L^{-1} and the maximum elimination capacity was found to be 22 gS m^{-3} h^{-1} (RE 98%). The RE was constant (98.8 ± 0.30%) for EBRT ≥ 16 s, but a decrease in the EBRT from 16 to 9 s led to a corresponding decrease in RE from 98.2 to 89.6% for a TLV of 9.1 m h^{-1} and from 97.9 to 94.9% for a TLV of 22.8 m h^{-1} (inlet load of 11.0 ± 0.2 gS m^{-3} h^{-1}). The sulphur oxidation capacity in the biotrickling filter was not diminished by the presence of other bacteria.

1. Introduction

Hydrogen sulphide (H_2S) is a reduced sulphur compound, which is corrosive, highly toxic and has a strong odour of rotten eggs. The odour threshold of H_2S ranges from 0.5 to 2 ppbv [1] and can be emitted from several industrial activities such as petroleum refining, pulp and paper manufacturing, food processing and wastewater treatment [2]. Strict control of emissions is therefore important to reduce the impact on the local population, to ensure public health and safety and to provide environmental protection.

H_2S can be removed by physical, chemical and biological methods [3], although the first two methods do suffer from significant drawbacks, including higher investment and operating costs (chemical and energy) [1]. The biological technologies do not usually require oxidants or catalysts besides their low energy consumption [4].

The biotrickling filter is a biological technology that consists of a column packed with a synthetic material of high specific surface area on which a biofilm is developed. The contaminated air passes through the column, in parallel or cross-flow, along with a liquid mobile phase (inorganic nutrients). Pollutants are absorbed into the liquid phase and subsequently degraded by microorganisms [4].

The removal of H_2S lies in the biological sulphur cycle, which is associated with a wide variety of sulphur-oxidizing bacteria (SOB) that obtain energy by the oxidation of one or more reduced sulphur compounds. In this respect, H_2S is oxidized to less-polluting compounds such as elemental sulphur (S^0) and sulphate. Reduced inorganic sulphur compounds are exclusively oxidized by prokaryotes. Aerobic SOB belong to genera such as *Acidianus*, *Acidithiobacillus*, *Aquaspirilum*, *Aquifex*, *Bacillus*, *Beggiota*, *Methylobacterium*, *Paracoccus*, *Pseudomonas*, *Starkeya*, *Sulfolobus*, *Thermithiobacillus*, *Thiobacillus* and *Xanthobacter* [5]. Biotrickling filters are usually inoculated with active sludge from wastewater treatment plants or consortia enriched from soil, so as to develop a mixed microbial population. However, the inoculation of the system with pure cultures leads to the shortening – and perhaps even the absence – of the bacterial lag phase, as well as an increase in the efficiency of H_2S removal by biotrickling filters [6]. The genera *Thiobacillus* is one of the most widely studied SOB [7], though the species *denitrificans* has not to date been used in a biotrickling filter. In the study reported here, *Thiobacillus*

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http://dx.doi.org/10.1016/j.bej.2013.12.019

denitrificans was chosen as the inoculum in a biotrickling filter. An indisputably great advantage of the genera *Thiobacillus* lies in the fact that they are autotrophic microorganisms, which use CO_2 from air as a carbon source. Another remarkable advantage is that these microorganisms have a slower biomass growth, thereby minimizing the clogging problems that are typically observed with heterotrophic systems [8,9]. Nevertheless, the elemental sulphur accumulation may likewise potentially result in clogging of the biofilter bed but only under oxygen limited conditions. Moreover, *T. denitrificans* is a facultatively anaerobic bacterium [10] which in the absence of oxygen is capable of using nitrate or nitrite as a terminal electron acceptor. Interestingly, this unique characteristic could be a significant advantage in the case of oxygen depletion and may lead to an improvement in the removal efficiency.

T. denitrificans has been inoculated in a continuous stirred tank reactor [11], in a pilot-scale bubble column [12] and in biofilters with peat moss, wood chips, ceramic and granular activated carbon separately as carriers [13]. However, the study of a biotrickling filter inoculated with *T. denitrificans* packed with open-pore polyurethane foam (OPUF) has not yet been reported for the removal of H_2S from air and this happens to be the aim of the work described here. The effect of sulphate concentration, pH, trickling liquid velocity (TLV), H_2S inlet concentration and empty bed residence time (EBRT) on the H_2S removal efficiency (RE) was studied. As it is quite difficult to maintain the system without establishing other populations, the bacterial community was characterized using denaturing gradient gel electrophoresis (DGGE).

2. Materials and methods

2.1. Microorganism and medium preparation

The pure culture strain of the *T. denitrificans* used in this work was obtained from the German Collection of Microorganisms and Cell Culture (DSM12475).

The microorganism was propagated according to the instructions of DSMZ for storage in an autotrophic medium (Medium DSMZ113): Solution A: 2.0 g KH_2PO_4, 2.0 g KNO_3, 1.0 g NH_4Cl, 0.8 g $MgSO_4{\cdot}7H_2O$, 2.0 mL of trace elements solution SL-4 (according to the DSMZ procedure, medium 14) and 940.0 mL of distilled water (pH adjusted to 7.0 with NaOH). Solution B: 5.0 g $Na_2S_2O_3{\cdot}5H_2O$ and 40.0 mL of distilled water. Solution C: 1.0 g $NaHCO_3$ and 20.0 mL of distilled water. Solution D: 0.002 g $FeSO_4{\cdot}7H_2O$ and 1.0 mL of H_2SO_4 (0.1 N). Solutions A, B and D were sterilized by autoclaving at 121 °C for 15 min. Solution C was sterilized by filtration (membrane pore size of 0.22 μm). Following sterilization, the four solutions were then mixed together. The medium used for H_2S removal studies contained all compounds but $Na_2S_2O_3.5H_2O$ (referred to as DSMZ113S).

2.2. Carrier material

OPUF cubes (8 cm^3) were used as carrier material for the biomass immobilization (Filtren TM25450, Recticel Iberica, Spain). This support material is characterized by a high specific surface area (600 $m^2\ m^{-3}$), low density (20–24 $kg\ m^{-3}$) and compression resistance ranging between 2.5 and 4.5 kPa.

2.3. Experimental set-up

A schematic diagram of the system used in this work is shown in Fig. 1. The system consisted of a gas-generating system and a biotrickling filter operated in an up-flow air and counter-current mode. The biotrickling filter was a cylindrical packed bed reactor made of transparent PVC with an inner diameter of 105.6 mm and packed height of 317 mm (working volume of 2.8 L). The column contained sampling ports distributed along the biofilter to aid the RE analysis of H_2S. The pH was controlled by the addition of sterile NaOH (0.5 N) solution in the trickling medium using a pH controller (Crison M44 Multimeter, Spain), an electrode (Crison 5303, Spain) and a peristaltic pump (Cole Parmer 7542–30, USA). The trickling medium DSMZ113S was continuously recirculated over the packed bed using a centrifugal magnetic pump (Selecta MP15R, Spain) jointly with a flow metre (ABB 10A1197, Spain).

H_2S gas was generated by the chemical reaction between Na_2S and HCl [2]. The two solutions were fed into the system from the top of a PVC column with a diameter of 63 mm and packed with 5 mm glass spheres to a height of 175 mm. Air supplied by an industrial compressor was used as the carrier gas for desorption of the formed H_2S. The different H_2S concentrations were obtained by changing the Na_2S and HCl concentrations and/or the air flow rate. The air stream containing H_2S was diluted with a humid air stream in an expansion tank (2.0 L), in order to homogenize the input stream. Prior to mixing the streams, a portion of the compressed air was bubbled through a distilled water column (diameter of 63 mm and height of 450 mm) to humidify the inlet stream until saturation. The

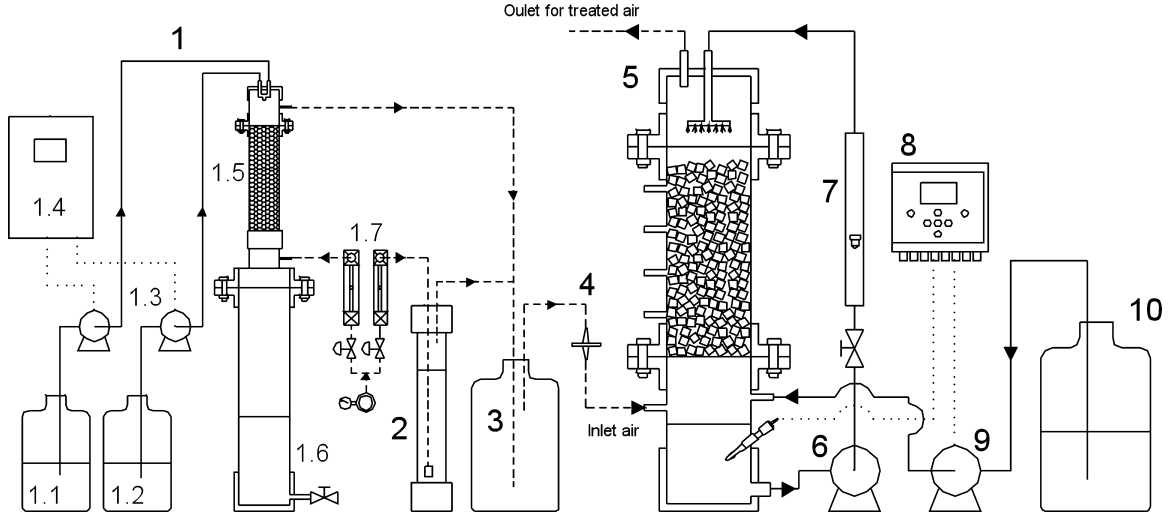

Fig. 1. Schematic representation of the experimental system. 1. H_2S generation system; 1.1. Na_2S container; 1.2. HCl container; 1.3. Peristaltic pumps; 1.4. Control panel pumps; 1.5. PVC column packed with glass spheres; 1.6. Residues reservoir; 1.7. Flow metre; 2. Humidifier; 3. Expansion tank; 4. Filter; 5. Biotrickling filter; 6. Recirculating nutrient pump; 7. Recirculating nutrient flow metre; 8. pH controller; 9. NaOH pump; 10. NaOH container.

non-humidified air stream used for the saturation contamination was a small proportion of the whole influent air and as such it did not significantly alter the moisture of the stream entering the system. The input current was filtered (0.2 μm filter) in order to protect the aseptic biofilter. The flow rates of the streams were controlled using a flow metre (Cole Parmer 1-800-323, USA: 0–25 L min^{-1}).

2.4. Immobilization and adaptation of the microorganism

The biotrickling filter was inoculated with 2.0 L of DSMZ113 medium with 20% (v/v) of *T. denitrificans* culture (exponential growth phase). The suspension of *T. denitrificans* cells was fed onto the top of the column at a constant flow rate of 80 L h^{-1} (TLV of 9.1 m h^{-1}). Following the depletion of the energy source (thiosulphate), 50% of the trickling medium was drawn off in the first two cycles (100% in subsequent cycles) and fresh DSMZ113 medium was then added. Six cycles were performed with a total duration of 350 h. The pH was maintained at 7.0 and the system was kept at room temperature. The thiosulphate concentration and biomass in suspension were duly measured. The substrate consumption rate was the indicator of the degree of immobilization [2].

After immobilization, the biofilm was adapted to the H_2S. From this point onwards, DSMZ113S medium was used as the trickling solution. The procedure was carried out taking the following into consideration: the EBRT being 37 s, the flow rate of the trickling medium being 80 L h^{-1} (TLV of 9.1 m h^{-1}) and the inlet load (IL) 5.6 ± 0.4 gS m^{-3} h^{-1} (41 ± 3 ppmv).

2.5. Biofiltration experiments

The operational conditions used in the experiments are summarized in Table 1. The maximum nitrite concentration was 25.5 mg L^{-1} in all the experiments (data not shown).

2.6. Molecular biology techniques

Three samples were analyzed in order to determine whether the biotrickling filter had been contaminated: biofilm (sample 1, day 180), 100 mL of the trickling medium (sample 2, day 180) and 2 mL of pure culture of *T. denitrificans* (sample 3).

Four OPUF cubes were selected randomly from the biotrickling filter on day 180. The OPUF was sonicated for 15 min in DSMZ113S medium (25 mL) using an Ultrasons-H sonicator operating at 40 kHz in order to allow the total desorption of immobilized microorganisms. Then the OPUF cubes were removed and the liquid medium was labelled sample 1. The three samples were centrifuged at 10,000 × *g* for 15 min. The resulting pellets were collected and these samples were used for total DNA extraction (stored frozen at −20 °C).

Total DNA was extracted using the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories Inc., USA) according to the manufacturer's instructions. The V3–V5 region (586 bps) of the bacterial 16S rRNA gene was amplified by PCR using the forward primer GC-338F and the reverse primer 907R as described by Ramírez et al. [14].

PCRs were performed with an MJ Mini Gradient Thermal Cycler (BioRad, USA). The PCR included an initial denaturation period of 2 min at 95 °C; 10 'touchdown' cycles of 94 °C for 30 s, 56–51 °C for 30 s with a ramp rate of 0.5 °C per cycle, 68 °C for 1 min and 25 cycles of normal PCR of 94 °C for 30 s, 51 °C for 30 s and 68 °C for 1 min. The PCR samples were stored at −20 °C.

The DNA fragments obtained by PCR were used for the DGGE analysis in the Dcode™ Universal Mutation Detection System (BioRad, USA). The PCR products were loaded onto 6% (w/v) polyacrylamide gels and run in 1X TAE (40 mM Tris, pH 8.0, 20 mM acetic acid, 1 mM EDTA). The polyacrylamide gels were made with a denaturing gradient from 30 to 60% (where 100% denaturant contained 7 M urea and 40% formamide). Electrophoresis was performed at 60 °C and 75 V for 17 h. After electrophoresis, the gel was stained with Ethidium Bromide. The Gel Documentation Systems ImagenQuant 100 GE Healthcare® was used to document the gels.

2.7. Analytical techniques

The H_2S concentration in the gas phase was measured using a specific sensor (Industrial Scientific Corporation Gasbadge Pro 15071, USA). Sulphate concentration was determined by a standard turbidimetric method (4500-SO_4^{2-} E) [15]. The thiosulphate and dissolved sulphur species ($H_2S/HS^-/S^{2-}$) in the trickling medium were analyzed by iodometric titration in line with Rodier's methodology [16]. The nitrate and nitrite concentrations were analyzed by an ultraviolet spectrophometric screening method (4500-NO_3^- B) and a colorimetric method (4500-NO_2^- B), respectively [15]. The total biomass immobilized on the OPUF

Table 1
Operational conditions.

Experiment	pH	EBRT (s)	Cin (ppmv)	IL (gS m^{-3} h^{-1})	TLV (m h^{-1})	[SO_4^{2-}] (g L^{-1})	Time (days)
Adaptation	7.0 ± 0.1	37	44 ± 3	5.6 ± 0.4	9.1	<10	0–17
pH	6.9–8.6	37	54 ± 1	6.8 ± 0.2	9.1	<10	27–59
Sulphate concentration	7.9 ± 0.1	37	49–145	6.2–18	9.1	1.8–16.8	59–91
TLV and EBRT	7.5 ± 0.1	57	138 ± 1	11.0 ± 0.2	9.1	<10	115–117
	7.5 ± 0.1	57	138 ± 1	11.0 ± 0.2	13.7	<10	133–136
	7.5 ± 0.1	57	138 ± 1	11.0 ± 0.2	18.3	<10	136–139
	7.5 ± 0.1	57	138 ± 1	11.0 ± 0.2	22.8	<10	139–141
	7.5 ± 0.1	32	71 ± 1	11.0 ± 0.2	9.1	<10	141–143
	7.5 ± 0.1	32	71 ± 1	11.0 ± 0.2	13.7	<10	143–146
	7.5 ± 0.1	32	71 ± 1	11.0 ± 0.2	18.3	<10	146–148
	7.5 ± 0.1	32	71 ± 1	11.0 ± 0.2	22.8	<10	148–150
	7.5 ± 0.1	16	38 ± 1	11.0 ± 0.2	9.1	<10	151–153
	7.5 ± 0.1	16	38 ± 1	11.0 ± 0.2	13.7	<10	153–155
	7.5 ± 0.1	16	38 ± 1	11.0 ± 0.2	18.3	<10	155–157
	7.5 ± 0.1	16	38 ± 1	11.0 ± 0.2	22.8	<10	157–160
	7.5 ± 0.1	9	20 ± 1	11.0 ± 0.2	9.1	<10	160–162
	7.5 ± 0.1	9	20 ± 1	11.0 ± 0.2	13.7	<10	162–165
	7.5 ± 0.1	9	20 ± 1	11.0 ± 0.2	18.3	<10	165–167
	7.5 ± 0.1	9	20 ± 1	11.0 ± 0.2	22.8	<10	167–169
H_2S inlet concentration	7.5 ± 0.1	34	73 ± 1	10 ± 0.1	18.3	<10	169–171
	7.5 ± 0.1	34	97 ± 1	13 ± 0.1	18.3	<10	172–174
	7.5 ± 0.1	34	132 ± 1	18 ± 0.2	18.3	<10	175–176
	7.5 ± 0.1	34	157 ± 1	22 ± 0.8	18.3	<10	195

was measured by taking one OPUF cube from the middle of the biofilter and placing it into a flask with a known amount of sterile distilled water. The sample was then sonicated for 10 min, to allow the total desorption of immobilized microorganisms. The quantity of immobilized biomass was obtained by the bacterial count in a Neubauer chamber per gram of dry carrier at 80 °C [17].

3. Results and discussion

The biotrickling filter performance with time (195 days) is shown in Fig. 2. The duration of each experimental study (Table 1) is denoted by dotted lines as can be seen therein.

3.1. Biomass immobilization

The immobilization of *T. denitrificans* on OPUF was achieved after 6 immobilization cycles with a total duration of 350 h. It is noteworthy that the DGGE profile (see Section 3.6) showed the development of a mixed culture. However, as the band resultant from *T. denitrificans* was present at the end of the experiment, it can thus be asserted that *T. denitrificans* developed properly in the biofilm. The concentration of the immobilized biomass on the OPUF after 180 days was $8.2 \pm 0.14 \times 10^9$ cells g^{-1} dry carrier, indicating that the microorganisms had been immobilized successfully. This value is based on the bacterial count of only one sample. OPUF has previously been used as a carrier with activated sludge as inoculum [1,18]. An unopened-pore polyurethane foam was used in a similar immobilization procedure and biomass immobilizations of 8.1×10^{10} cells g^{-1} dry carrier of *T. thioparus* [2] and 1.6×10^{10} cells g^{-1} of *A. thiooxidans* were obtained in an ex situ immobilization procedure (Erlenmeyer flask) [19]. Ma et al. [13] studied the immobilization of *T. denitrificans* on other packing materials such as granular activated carbon where they reported attaining a biomass immobilization of 4.0×10^8 cells g^{-1}. This leads us to the conclusion that OPUF is therefore a highly suitable media for *T. denitrificans* immobilization.

3.2. Effect of pH

The pH value plays a fundamental role in the process under investigation. The study of pH is seen to be intrinsically vital owing to the fact that each microorganism grows at an optimum pH value. Furthermore, the pH is known to exert influence over the sulphide concentration in the trickling medium. *T. denitrificans* has an optimum pH for growth in the range 6.8–7.4 [10], but the microorganisms in the biofilm are likely to be exposed to a pH slightly different from the bulk liquid. However, the pH was measured using microelectrode in biofilms of nitrate-reducing, sulphide-oxidizing bacteria in a bioreactor for sulphide removal, with a vertical pH microprofile obtained from pH 8.0 inside the biofilm to 8.2 at the surface [20].

The effect of pH on the H_2S removal was examined in the pH range 6.9–8.6 (Table 1 and Experiment 2). Upon increasing the pH from 6.9 to 7.6, an increase was observed in the RE from 83% to 97%. The optimum pH range was found to be between 7.6 and 8.5 (RE 97–98%). The concentration of dissolved sulphur species ($H_2S/HS^-/S^{2-}$) in the trickling medium rose from 0.6 to 3.9 mg L^{-1} at a pH above 8.5 (Fig. 2).

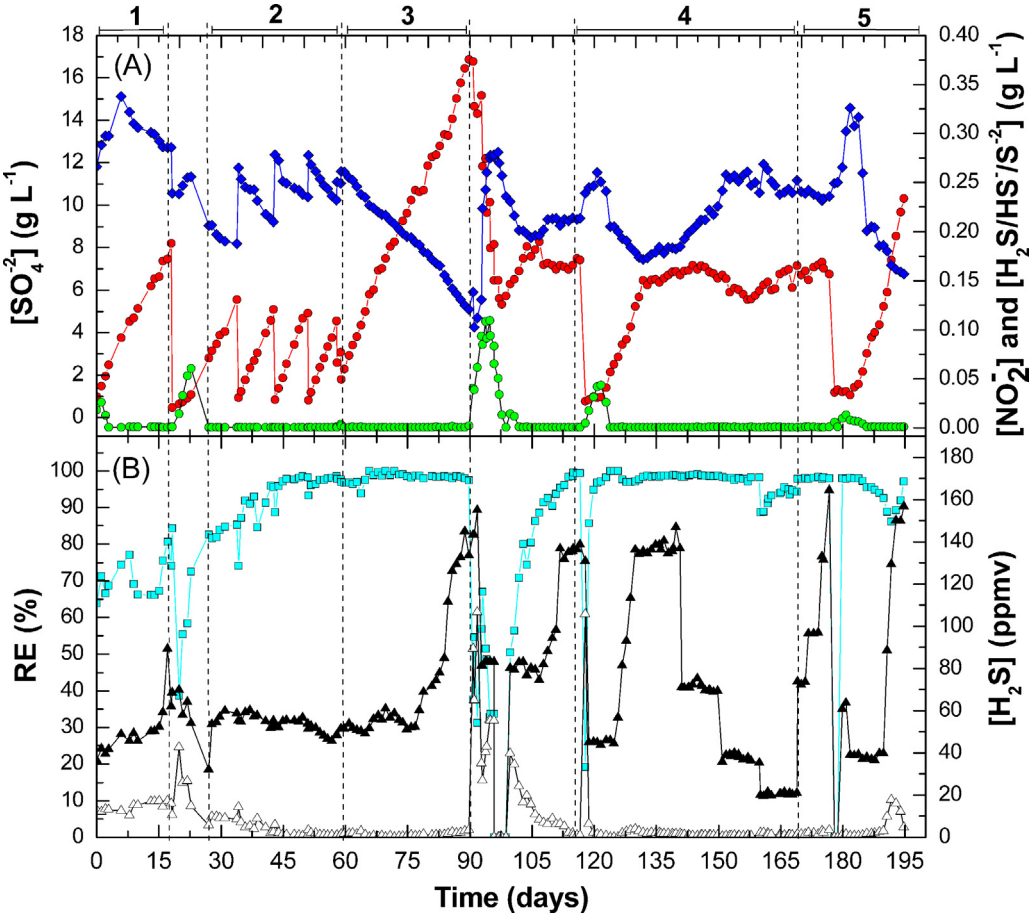

Fig. 2. Concentrations of (A) sulphate (●), nitrate (♦), and $H_2S/HS^-/S^{2-}$ in the trickling medium (●), and (B) H_2S concentration at inlet (▲) and outlet (△) and RE (■). Period 1. Adaptation; 2. Effect of pH; 3. Effect of sulphate concentration; 4. Effect of EBRT and TLV; and 5. Effect of H_2S IL.

Microorganisms that support alkaline pH values are of paramount interest because the amount of H_2S absorbed in the liquid medium is found to be greater under these conditions. However, the determination of the optimum pH value is essential owing to the fact that the accumulation of sulphide in the liquid phase is toxic to chemolithoautotrophic denitrifying microorganisms [21] and as such the sulphide concentration should therefore be maintained below inhibitory levels.

3.3. Effect of sulphate concentration

H_2S is oxidized by SOB to S^0 (partial oxidation, Eq. (1)) and/or sulphate (total oxidation, Eq. (2)) [7]:

$$H_2S + O_2 \rightarrow 2S + 2H_2O \quad (1)$$

$$S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \quad (2)$$

To aid our study of the effect of sulphate concentration, fresh medium was not added to the biotrickling filter for a period of 32 days (Table 1 and Experiment 3), during which time the sulphate concentration increased from 1.8 to 16.8 g L^{-1}. The oxidation of H_2S by means of SOB was inhibited at sulphate concentrations of 16.8 g L^{-1} where a decrease was observed in the RE from 97 to 38% (Fig. 2).

According to Ongcharit et al. [22], the inhibitory effect of sulphate concentration on *T. denitrificans* occurs above 200–250 mM (19–24 g L^{-1}) and it is said to be related to the increase in the ionic strength of the medium. The same authors arbitrarily chose a maximum sulphate concentration of 125 mM (12 g L^{-1}) in a reactor with *T. denitrificans* immobilized using coculture with floc-forming heterotrophs. In these experiments, it was assumed that concentrations below 10 g L^{-1} were low enough to maintain the stability of the bacterial population immobilized on OPUF since concentrations above 16.8 g L^{-1} are not recommendable. This value is higher than that obtained for *T. thioparus* according to Ramírez et al. [2], who reported an optimum sulphate concentration below 5 g L^{-1}. However *A. thiooxidans* is found to show a high RE without inhibition until sulphate concentrations of 97 g L^{-1} are reached [19]. An autotrophic H_2S-degrading culture obtained from the activated sludge of the wastewater treatment plant of a resin-producing industry was inhibited by sulphate concentrations of around 1.9 g L^{-1}. In this case, however, the pH of the trickling medium left uncontrolled was found to decrease from 6.8 to 2.0 during sulphate production. As a result, the biological activity was inhibited by the sulphate content besides the low pH value [9].

3.4. Effect of EBRT and TLV

The effect of EBRT values of 57, 32, 16 and 9 s (gas velocity of 11, 20, 36 and 127 m h^{-1}) and TLV values of 9.1, 13.7, 18.3 and 22.8 m h^{-1} was studied at a constant H_2S IL of 11.0 ± 0.2 g $S\,m^{-3}\,h^{-1}$ (Table 1 and Experiment 4). As shown in Fig. 3, a high RE of about 99% was achieved with EBRTs in the range of 16–57 s, whereas lower RE values were obtained for an EBRT of 9 s. The TLV was found to exert no effect on the biofilter performance in the range of 16–57 s.

The effect of TLV has been studied by other authors. The external mass transfer depends on the concentration gradient across the gas–liquid interface [9]:

$$\text{Mass flux} = K_L\left(\frac{C_G}{H} - C_L\right) \quad (3)$$

where K_L is the mass transfer coefficient, C_G the H_2S gas concentration, C_L the H_2S liquid concentration and H being the dimensionless Henry's constant.

Kim and Deshusses [23] concluded that the TLV did not affect the H_2S RE at 4000 m h^{-1}, which is around the upper limit of air velocity at which external mass transfer limitation was thought to occur.

In the work described here, despite the fact that all of the experiments occur under external mass transfer limitation (maximum air velocity of 127 m h^{-1}), a small decrease in RE was observed following the use of a lower EBTR of 9 s. This finding can be explained considering that, at this EBRT, the inlet concentration (20 ppmv), the gradient concentration and mass flux were all lower. In addition, for a constant EBRT of 9 s, the RE declined from 94.2 to 89.6% while the TLV underwent a decrease from 18.3 to 9.1 m h^{-1}, probably due to decreases in the wetted specific surface and mass transfer coefficient K_L.

3.5. Effect of H_2S IL

The inlet H_2S load was elevated from 10 ± 0.1 to 22 ± 0.8 g $S\,m^{-3}\,h^{-1}$ (Table 1 and Experiment 5) and the H_2S concentration was measured throughout the bed at heights of 82, 116, 149, 181 and 234 mm. The RE was found to reach about 98.5% in the first tier (bed height of 82 mm, 35% of the total bed height) for H_2S IL up to 18 ± 0.2 g $S\,m^{-3}\,h^{-1}$ while it remained constant throughout the rest of the bed, except for the H_2S IL of 22 ± 0.8 g $S\,m^{-3}\,h^{-1}$. In this case, the RE increased from 86 to 97% from a bed height of 82 mm to 181 mm (data not shown).

An overview of the EC vs. IL for all experiments is shown in Fig. 4 along with the critical conditions. High RE values (98%) were observed up to IL values of 22 g $S\,m^{-3}\,h^{-1}$. Despite the lack of information in the literature about *T. denitrificans* in biotrickling filters, studies involving bioreactors have been reported. In a pilot-scale bubble column the maximum removal was 12.8 g $S\,m^{-3}\,h^{-1}$ [12]. This value was found to be significantly lower than that obtained in the experiment reported here. Other species, including *T. thioparus* and *A. thiooxidans*, have been studied under similar conditions to those described here. For instance, Ramírez et al. [2] studied the removal of H_2S by *T. thioparus* immobilized on polyurethane foam (not open-pore) in a biotrickling filter, where they obtained a critical elimination capacity (EC) of 14.9 g $S\,m^{-3}\,h^{-1}$ (RE of 99.8%). In the case of *A. thiooxidans*, the critical EC was 58.7 g $S\,m^{-3}\,h^{-1}$ (RE of 98%) [14]. For an autotrophic H_2S-degrading culture obtained from the activated sludge of the wastewater treatment plant of a resin-producing industry, the critical EC was 21.2 g $S\,m^{-3}\,h^{-1}$ [9]. For a biotrickling filter inoculated with activated sludge derived from an urban wastewater treatment plant and operated under extremely acidic conditions, the maximum EC obtained was 70 g $m^{-3}\,h^{-1}$ [24].

The RE for pH < 7.6 was maintained between 64–96% and for pH > 7.6 the maximum RE was reached, as described above. At an EBRT of 9 s, the RE was found to be more sensitive to the variation of the TLV and ranged from 89 to 96%. A sulphate concentration higher than 16.8 g L^{-1} clearly affected the removal efficiency (RE = 38%).

3.6. Sulphur mass balance

The sulphur mass balance was obtained in periods without replacement of the trickling medium. The amount of S^0 was estimated by assuming exclusive S^0 and sulphate formation. In the periods between the following days from 34–51 and 59–90, the system was running at low EC (6.6 ± 0.5 g $S\,m^{-3}\,h^{-1}$) and S^0 production was 17 ± 4%. At low EC, the system is under sulphide-limiting conditions and therefore sulphate was the major end-product of the sulphide oxidation [25] (Eq. (2)), while S^0 was mainly formed under oxygen-limiting conditions (Eq. (1)). S^0 production hit 52% when the EC was elevated to 16.1 ± 1.3 g $S\,m^{-3}\,h^{-1}$ (days 59–87), hence the ratio between dissolved oxygen and sulphide concentration decreased, which in turn led to an increase in the S^0 production.

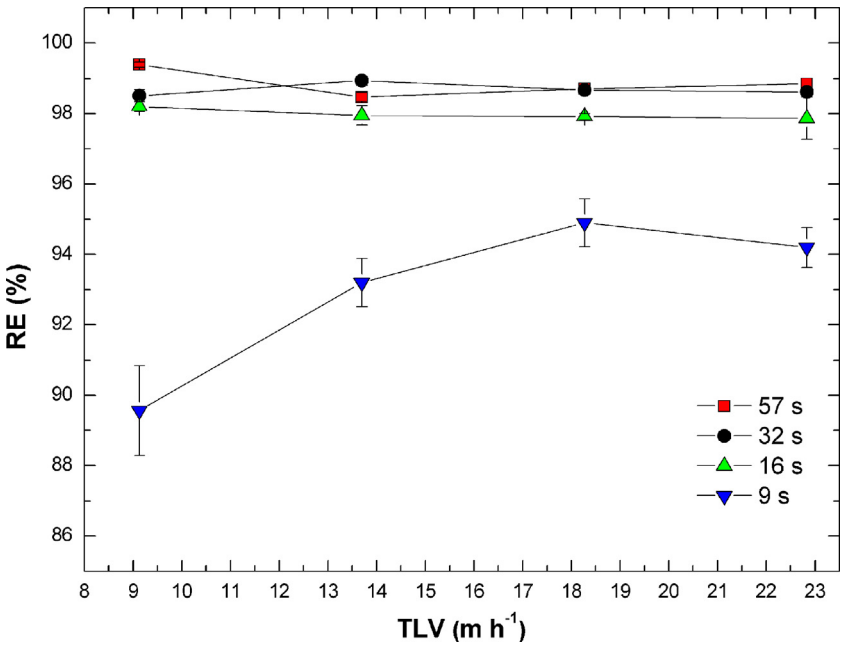

Fig. 3. Effect of the EBRT and TLV on the H_2S RE.

Stefess et al. [26] have reported about sulphide oxidation conversion to S^0 of 20 and 40% for *Thiobacillus* o and *T. neapolitanus*, respectively, at 50% of air saturation and 50 and 60% at 0% of air saturation.

The nitrate and sulphide consumption rates were 0.045 ± 0.005 mmol N-NO_3^- h^{-1} and 98.6 ± 17 mmol S h^{-1}, respectively, and the periods selected to calculate the rates were between the following days: 34–43, 43–51, 51–78 and 121–132. It can be observed that the nitrate consumption rate is markedly lower than that of the sulphide consumption. Without oxygen for complete denitrification, the nitrate/sulphide ratio is 0.4 for partial H_2S oxidation and 1.6 for complete H_2S oxidation [27], meaning that H_2S was mainly oxidized using oxygen rather than nitrate. However, between days 97 and 99 the airflow was shutdown and the nitrate consumption rate rose to 0.153 mmol N-NO_3^- h^{-1} as a result of the oxygen depletion in the trickling medium. Thus, facultatively anaerobic bacteria such as *T. denitrificans* can be useful in biotrickling filters in the case of gas shutdown or poor oxygen mass transfer.

3.7. Bacterial population

Several characteristic bands were observed in the DGGE analysis (Fig. 5): 3 bands in the biofilm (1A, 2A and 3A), 2 bands in the trickling medium (2A and 2B), and one band for *T. denitrificans* pure culture (3A). Band 3A can be said to provide evidence that *T. denitrificans* is part of the population of the biofilm.

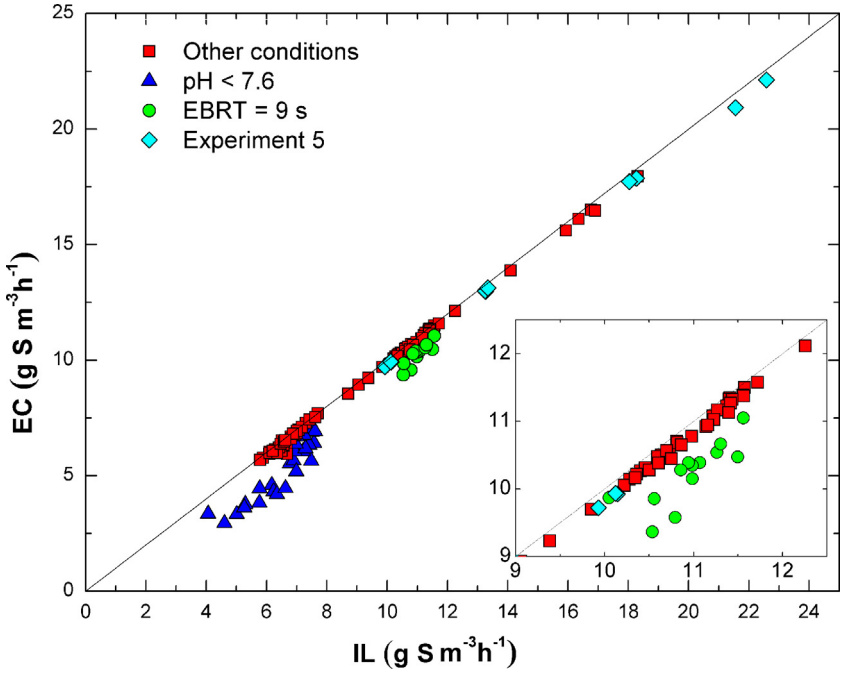

Fig. 4. EC vs. H_2S IL.

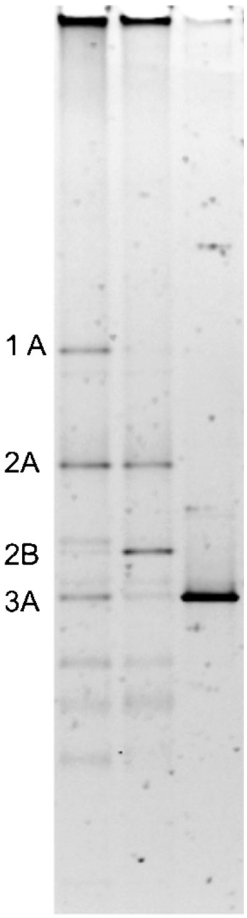

Fig. 5. DGGE fingerprints of the 16S rRNA (V3–V5). Lane 1: sample 1 (biofilm), lane 2: sample 2 (trickling medium), lane 3: sample 3 (pure culture).

The other bands confirm that other bacterial populations had been established in the biotrickling filter during the 197 days of operation. One of these bands (2A) was present in the biofilm as well as in the trickling medium. Once the system was not kept completely sterile during the course of experiment, the presence of a consortium – predominantly composed of *T. denitrificans* – was observed. Sercu et al. [28] reported that the population in the biofilm was very stable in comparison to the trickling medium though the stability of the biofilm population is strongly influenced by the pH conditions, as pointed out by Ramírez et al. [19]. The bacteria population in a biotrickling filter at low pH (2.0) was found to be very stable; whereas at neutral pH the population was likely to change depending on the operational conditions even with a pure culture inoculation. This change is due to the possibility that the establishment of other bacteria is greater, as observed in the present study when working with a pH close to 7.

4. Conclusions

The results of the present investigation showed that a sulphur-oxidizing microbial culture containing *T. denitrificans* can be used successfully for the removal of H_2S in biotrickling filters. The sulphur oxidation capacity in the biotrickling filter was found to be satisfactory even in the presence of other bacteria, with a maximum elimination capacity of 22 g S m^{-3} h^{-1} (RE 98%). The optimal conditions determined for the process were as follows: sulphate concentration below 16.8 g L^{-1} (RE > 97%) and EBRT higher than 16 s (RE 99%). The optimum pH range was 7.6–8.5 (RE 97–98%).

Acknowledgements

This work was financially supported by the Ministerio de Ciencia e Innovación of Spain through the Project CTM2009-14338-C03-02, the Brazilian National Council for Scientific and Technological Development (CNPq), and the Alfa BIOPROAM project (contract no. AML/190901/06/18414/II-0548-FC-FA) to which the authors greatly express their heartiest gratitude and indebtedness.

References

[1] D. Gabriel, M. Deshusses, Retrofitting existing chemical scrubbers to biotrickling filters for H_2S emission control, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 6308–6312.
[2] M. Ramírez, J.M. Gómez, G. Aroca, D. Cantero, Removal of hydrogen sulfide by immobilized *Thiobacillus thioparus* in a biotrickling filter packed with polyurethane foam, Bioresour. Technol. 100 (2009) 4989–4995.
[3] R. Lebrero, L. Bouchy, R. Stuetz, R. Muñoz, Odor assessment and management in wastewater treatment plants: a review, Crit. Rev. Environ. Sci. Technol. 41 (2011) 915–950.
[4] G. Cabrera, M. Ramírez, D. Cantero, Bioreactors – design biofilters, in: M.-Y. Murray (Ed.), Comprehensive Biotechnology, vol. 2, 2nd ed., Elsevier B.V, 2011, pp. 303–318.
[5] C.G. Friedrich, D. Rother, F. Bardischewsky, A. Quentmeier, J. Fischer, Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism? Appl. Environ. Microbiol. 67 (2001) 2873–2882.
[6] G. Aroca, H. Urrutia, D. Núñez, P. Oyarzún, A. Arancibia, K. Guerrero, Comparison on the removal of hydrogen sulfide in biotrickling filters inoculated with *Thiobacillus thioparus* and *Acidithiobacillus thiooxidans*, Electron. J. Biotechnol. 10 (2007) 514–520.
[7] K. Tang, V. Baskaran, M. Nemati, Bacteria of the sulphur cycle: an overview of microbiology, biokinetics and their role in petroleum and mining industries, Biochem. Eng. J. 44 (2009) 73–94.
[8] Y.C. Chung, C.P. Huang, C.P. Tseng, Biological elimination of H_2S and NH_3 from wastegases by biofilter packed with immobilized heterotrophic bacteria, Chemosphere 43 (2001) 1043–1050.
[9] Y. Jin, M.C. Veiga, C. Kennes, Autotrophic deodorization of hydrogen sulfide in a biotrickling filter, J. Chem. Technol. Biotechnol. 80 (2005) 998–1004.
[10] D. Kelly, A. Wood, E. Stackebrandt, *Thiobacillus* Beijerinck 1904 b, 597^{AL}, in: D.J. Brenner, N.R. Krieg, G.M. Garrity, J.T. Staley, D.R. Boone, P. Vos, M. Goodfellow, F.A. Rainey, K.-H. Schleifer (Eds.), Bergey's Manual® of Systematic Bacteriology, Springer, New York, USA, 2005, pp. 764–769.
[11] K.L. Sublette, Microbial treatment of sour gases for the removal and oxidation of hydrogen sulphide, Gas Sep. Purif. 4 (1990) 91–96.
[12] K.L. Sublette, R.P. Hesketh, S. Hasan, Microbial oxidation of hydrogen sulfide in a pilot-scale bubble column, Biotechnol. Prog. 10 (1994) 611–614.
[13] Y. Ma, B. Yang, J. Zhao, Removal of H_2S by *Thiobacillus denitrificans* immobilized on different matrices, Bioresour. Technol. 97 (2006) 2041–2046.
[14] M. Ramírez, M. Fernández, C. Granada, S. Le Borgne, J.M. Gómez, D. Cantero, Biofiltration of reduced sulphur compounds and community analysis of sulphur-oxidizing bacteria, Bioresour. Technol. 102 (2011) 4047–4053.
[15] L.S. Clesceri, A.E. Greenberg, A.D. Eaton, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, 1999.
[16] J. Rodier, Análisis de las aguas. Aguas naturales, aguas residuales, agua de mar, Omega, Barcelona, 1998.
[17] J.M. Gomez, D. Cantero, C. Webb, Immobilisation of *Thiobacillus ferrooxidans* cells on nickel alloy fibre for ferrous sulfate oxidation, Appl. Microbiol. Biotechnol. 54 (2000) 335–340.
[18] D. Gabriel, H.H.J. Cox, M.A. Deshusses, Conversion of full-scale wet scrubbers to biotrickling filters for H_2S control at publicly owned treatment works, J. Environ. Eng. 130 (2004) 1110–1117.
[19] M. Ramírez, J.M. Gómez, D. Cantero, J. Paca, M. Halecky, E.I. Kozliak, M. Sobotka, Hydrogen sulfide removal from air by *Acidithiobacillus thiooxidans* in a trickle bed reactor, Folia Microbiol. 54 (2009) 409–414.
[20] J. Garcia-de-Lomas, A. Corzo, M. Carmen Portillo, J.M. Gonzalez, J.A. Andrades, C. Saiz-Jimenez, E. Garcia-Robledo, Nitrate stimulation of indigenous nitrate-reducing, sulfide-oxidising bacterial community in wastewater anaerobic biofilms, Water Res. 41 (2007) 3121–3131.
[21] R.B. Cardoso, R. Sierra-Alvarez, P. Rowlette, E.R. Flores, J. Gómez, J.A. Field, Sulfide oxidation under chemolithoautotrophic denitrifying conditions, Biotechnol. Bioeng. 95 (2006) 1148–1157.
[22] C. Ongcharit, K.L. Sublette, Y.T. Shah, Oxidation of hydrogen sulfide by flocculated *Thiobacillus denitrificans* in a continuous culture, Biotechnol. Bioeng. 37 (1991) 497–504.
[23] S. Kim, M. Deshusses, Understanding the limits of H_2S degrading biotrickling filters using a differential biotrickling filter, Chem. Eng. J. 113 (2005) 119–126.
[24] J. Chen, Removal of hydrogen sulfide in a biotrickling filter under extremely acidic conditions, in: International Conference on Digital Manufacturing & Automation, 2010, pp. 158–161.
[25] A.J.H. Janssen, R. Sleyster, C. van der Kaa, A. Jochemsen, J. Bontsema, G. Lettinga, Biological sulphide oxidation in a fed-batch reactor, Biotechnol. Bioeng. 47 (1995) 327–333.

[26] G.C. Stefess, R.A.M. Torremans, R. Schrijver, L.A. Robertson, J.G. Kuenen, Quantitative measurement of sulphur formation by steady-state and transient-state continuous cultures of autotrophic *Thiobacillus species*, Appl. Microbiol. Biotechnol. 45 (1996) 169–175.
[27] G. Soreanu, M. Béland, P. Falletta, K. Edmonson, P. Seto, Investigation on the use of nitrified wastewater for the steady-state operation of a biotrickling filter for the removal of hydrogen sulphide in biogas, J. Environ. Eng. Sci. 7 (2008) 543–552.
[28] B. Sercu, D. Núñez, H. Van Langenhove, G. Aroca, W. Verstraete, Operational and microbiological aspects of a bioaugmented two-stage biotrickling filter removing hydrogen sulfide and dimethyl sulfide, Biotechnol. Bioeng. 90 (2005) 259–269.