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Biofiltration of reduced sulphur compounds and community analysis of sulphur-oxidizing bacteria

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ABSTRACT

The present work aims to use a two-stage biotrickling filters for simultaneous treatment of hydrogen sulphide (H_2S), methyl mercaptan (MM), dimethyl sulphide (DMS) and dimethyl disulphide (DMDS). The first biofilter was inoculated with *Acidithiobacillus thiooxidans* (BAT) and the second one with *Thiobacillus thioparus* (BTT). For separate feeds of reduced sulphur compounds (RSC), the elimination capacity (EC) order was DMDS > DMS > MM. The EC values were 9.8 $g_{MM\text{-}S}/m^3/h$ (BTT; 78% removal efficiency (RE); empty bed residence time (EBRT) 58 s), 36 $g_{DMDS\text{-}S}/m^3/h$ (BTT; 94.4% RE; EBRT 76 s) and 57.5 $g_{H2S\text{-}S}/m^3/h$ (BAT; 92% RE; EBRT 59 s). For the simultaneous removal of RSC in BTT, an increase in the H_2S concentration from 23 to 293 ppmv (EBRT of 59 s) inhibited the RE of DMS (97–84% RE), DMDS (86–76% RE) and MM (83–67% RE). In the two-stage biofiltration, the RE did not decrease on increasing the H_2S concentration from 75 to 432 ppmv.

1. Introduction

The emission of volatile reduced sulphur compounds such as hydrogen sulphide (H_2S), methyl mercaptan (MM), dimethyl sulphide (DMS) or dimethyl disulphide (DMDS), a group of compounds known as reduced sulphur compounds (RSC), can be problematic due to their foul smell and low odour threshold. RSC are often emitted as mixtures from a variety of industries such as wastewater treatment, kraft pulping and animal rendering in concentrations that are well suited for treatment with biological technologies such as biofiltration (Andersson, 2006).

The biological removal of H_2S by biofiltration has been extensively studied (Mahmood et al., 2007) but the simultaneous removal of mixtures of RSC has not been addressed. The removal of RSC has been carried out by biofiltration in biofilters and biotrickling filters. It is known that the use of a two-stage biofiltration system can improve the removal efficiency (RE) of these compounds. The main advantage of these technologies is that different microorganisms, carriers and operational conditions can be used in each stage. The two-stage biofiltration has been investigated for the removal of H_2S in the presence of DMS (Sercu et al., 2005), MM (Pinjing et al., 2001), DMS/MM (Ruokojärvi et al., 2001) and DMS/DMDS (Wani et al., 1999), but little is known about the simultaneous removal of MM/DMS/DMDS in the presence of H_2S in two-stage biotrickling filters.

A variety of microorganisms have been used for RSC removal: *Thiobacillus thioparus* (Park et al., 1993; Tanji et al., 1989), *Hyphomicrobium* and *Xanthomonas* (Cho et al., 1992), *Methylophaga sulfidovorans* (De Zwart et al., 1997), and *Microbacterium* sp. and *Pseudomonas* (Ho et al., 2008; Shu and Chen, 2009). It has been suggested that more work on microbial ecology of biofilters is needed, in particular for monitoring microbial populations dynamics during these treatments. Recently, molecular techniques have recently been used to determine the composition of microbial communities in biofiltration processes and to monitor biofilters colonization by specific degradative populations (Chung et al., 2010).

Motivated by the need to establish an economical and environmentally friendly RSC control technology, the aim of this work was to study the simultaneous removal of a mixture of H_2S, MM, DMS and DMDS using two-stage biotrickling filters in series; the first filter was inoculated with *Acidithiobacillus thiooxidans* (BAT) for H_2S removal and the second was inoculated with *T. thioparus* (BTT) for MM, DMS and DMDS removal. The removal of a mixture of RSC and the removal of DMDS and MM separately was studied with BTT. The removal of H_2S with BAT was also evaluated. At the same time, in order to gain further insights into the bacterial diversity in the biofilters, the microbial community was observed by denaturing gradient gel electrophoresis (DGGE). The DGGE technique allows to obtain and compare the fingerprints of

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bacterial populations in complex samples. DGGE was used here to investigate the evolution of the bacterial communities in the two biotrickling filters (BTT and BAT) operated under different biofiltration conditions.

2. Methods

2.1. Microorganisms and media preparation

The original pure-culture strain of the *T. thioparus* ATCC 23645 was obtained from the American Type Culture Collection. *T. thioparus* is a chemolithotrophic neutrophilic sulphur-oxidizing bacterium that is able to utilize sulphide, sulphur, thiosulphate and tetrathionate as source of energy. Organic sulphur compounds like DMS, DMDS and MM can also be used as energy sources by *T. thioparus* (Kelly et al., 2005).

A. thiooxidans (DSM11478) was obtained from Minas Gerais (Brazil) from the Universidade Estadual Paulista (UNESP). *A. thiooxidans* is a chemoautotrophic acidophilic sulphur-oxidizing bacterium that grows in liquid medium using elemental sulphur, thiosulphate or tetrathionate as source of energy (Kelly and Wood, 2005).

Table 1
Composition of basic media.

Medium	ATCC290:S6		0 K medium	
Composition (g/L)	$Na_2S_2O_3$[a]	10.0	$(NH_4)_2SO_4$	3.0
	KH_2PO_4	1.8	K_2HPO_4	0.5
	Na_2HPO_4	1.2	$MgSO_4 \cdot 7H_2O$	0.5
	$MgSO_4 \cdot 7H_2O$	0.1	KCl	0.1
	$(NH_4)_2SO_4$[a]	0.1	$Ca(NO_3)_2$	0.01
	$MnSO_4$	0.02		
	$CaCl_2$	0.03		
	$FeCl_3 \cdot 6H_2O$[b]	0.033		
pH		7.0		2.5
Temperature (°C)		30		30

[a] For recirculation medium in biofiltration experiments (referred to as TTN+): the $Na_2S_2O_3$ (energy source) was removed and the $(NH_4)_2SO_4$ concentration was increased to 1 g/L.

[b] $FeCl_3 \cdot 6H_2O$ was prepared as a separate solution and sterilized by filtration (0.22 μm pore size membrane).

Two identical biotrickling filters were used. One biotrickling filter was inoculated with *T. thioparus* ATCC 23645 (BTT) and the other with *A. thiooxidans* DSM11478 (BAT). The compositions of the basic media are shown in Table 1. The ATCC290:S6 medium was used in the propagation and immobilization of *T. thioparus*. The TTN+ and 0 K media were used as the trickling solutions in the biotrickling filters, TTN+ for BTT and 0 K for BAT.

2.2. Carrier material

Small cubes of polyurethane foam (PUF) (1 cm^3 in size) were used as the carrier material. PUF is an inert material with a very low commercial cost. The principal properties are density (20 kg/m^3) and porosity (96%).

2.3. Experimental set-up

A scheme of the experimental set-up for the laboratory-scale biotrickling filters and gaseous generating system are shown in Fig. 1.

The biotrickling filters consisted of a transparent PVC columns (i.d. 105 mm, height 1500 mm) with two stages (module height 250 mm, total packed volume 4.33 L) joined with a screwed clamp. Five gas sampling ports were distributed along the packed biofilters at heights of 120, 200, 370, 450 and 500 mm. The trickling solution was kept at 30 °C using a tubing coil submerged in a temperature-controlled water bath. The pH was maintained at 7.0 in the BTT and 2.0 in the BAT by adding sterile NaOH (2 N) using two pH controllers (Biocontroller ADI 1030, APPLIKON and PH28, CRISON) and an electrode with a sleeve diaphragm (CRISON, 5221). The medium was recirculated at a constant velocity of 7.85 m/h with a centrifugal pump (ECOCIRC, D5-8/810 N).

The compressed air was passed through three consecutive filters, which were filled with silica gel, active carbon and glass wool, respectively; the filtered air was then sterilized using a Millipore filter SLG05010 (0.45 μm) and humidified by passing through a column filled with distilled water. The synthetic waste gas stream was supplied to the biotrickling filters from two compressed gas cylinders: H_2S (0.5 vol%, balance N_2) and MM (0.1 vol%, balance N_2).

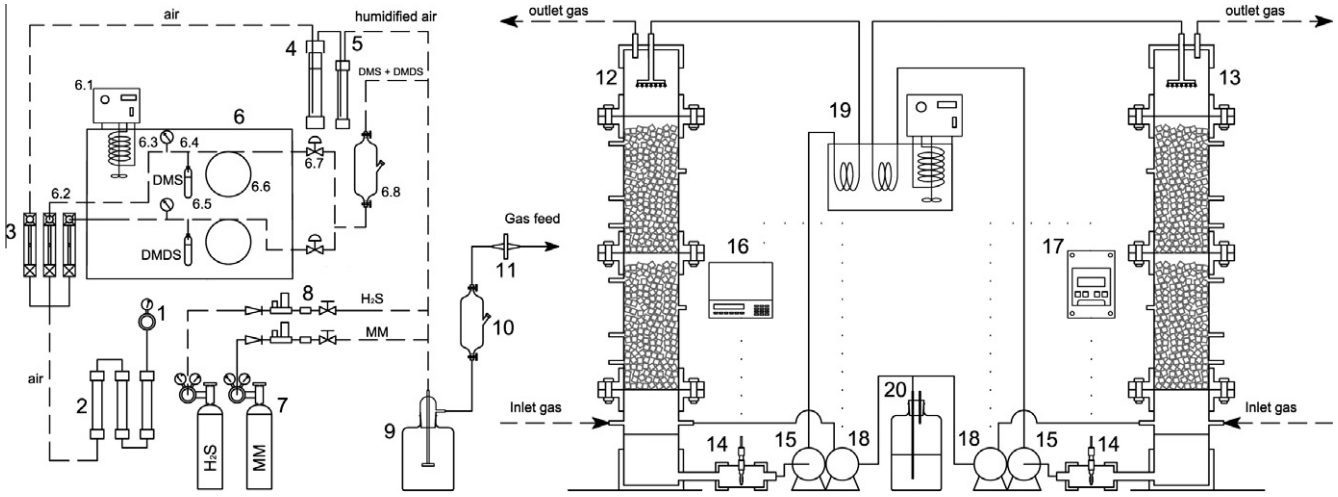

Fig. 1. Schematic diagram of the laboratory-scale biotrickling filter system and gaseous generating system. (1) Pressure regulator, (2) air filters, (3) flow meter, (4) humidifier, (5) water trap, (6) gaseous DMS and DMDS generation system, (6.1) heater pump recirculator, (6.2) flow meters, (6.3) pressure gauge, (6.4) T joint, (6.5) steel vessels, (6.6) steel-tubing coils, (6.7) needle valves and (6.8) sampling glass bulb, (7) compressed gas cylinders (H_2S and MM), (8) mass flow controllers, (9) expansion tank, (10) sampling glass bulb, (11) membrane filter, (12) BTT, (13) BAT, (14) pH probe, (15) recirculating pumps, (16) pH controller (Applikon), (17) pH controller (Crison), (18) NaOH pumps, (19) thermostatic bath, (20) NaOH container.

The flow rates of the H_2S and MM streams were regulated by mass flow rate controllers (Bronkhorst F-201C). DMS and DMDS streams with constant concentrations were produced using the system described by Smet (1996). Capillary steel tubing (O.D. 1/16″, I.D. 0.53 mm, AISI 316) was used to carry gaseous DMS and DMDS, by diffusion, from a steel vessel (Hoke, USA, vol. 75 mL) filled with the liquid; >98.0% pure DMDS and > 95.0% pure DMS were obtained from Fluka and Sigma–Aldrich, respectively. Needle valves and 3 m long steel tubing spirals were used to apply an overpressure (~0.27 bar) on the diffusion tube to reduce variations in the final concentrations of DMS and DMDS. The stream was then combined with humidified air. An expansion tank with a capacity of 2.5 L was used to homogenize the input stream. The final concentration was sampled from a glass bulb.

2.4. Immobilization of microorganisms and initial conditions

The immobilization of *T. thioparus* in the BTT was carried out in 16 Erlenmeyer flasks (vol. 1 L) containing 0.5 L of ATCC290:S6 medium, 0.1 L of inoculum and 5.4 g of PUF. The flasks were inoculated with a culture growing in the exponential phase and incubated in a rotary shaker (30 °C, 150 rpm). The thiosulphate concentration was monitored and the mineral medium was replaced with fresh ATTC290:S6 medium after this substrate was depleted. This operation was repeated three times to obtain enough biomass attached to the PUF. The BTT was packed with the colonized PUF and 1.5 L of ATCC290:S6 medium, without thiosulphate, was added as a mobile phase and recirculated. After the immobilization the study of DMS removal was carried out (89 day) as described by Arellano-García et al. (2009).

The immobilization of *A. thiooxidans* in the BAT was carried out with the PUF colonized from a biotrickling filter (10 g, total working volume of 1.278 L) previously used by Ramírez et al. (2009b). The BAT was packed with 10 g of PUF colonized (old) and 40 g of new PUF (25 g in each stage). The biotrickling system was fed with an air flow of 0.26 m^3/h and a concentration of H_2S in the range 30–50 ppmv. Fresh medium (500 ml) was added whenever the sulphate concentration reached 30 g/L. This biofilter was continuously operated for a period of 405 days.

2.5. Biofiltration experiments

The operational conditions are summarized in Table 2. The removal of MM and DMDS by the biotrickling filter was evaluated by carrying out BTT Experiments 1–6. The main parameters studied were the empty bed residence time (EBRT), inlet load (L) and sulphate concentration. The performance of the BAT was analyzed in Experiment 7 by increasing the H_2S concentration. Mixtures of H_2S, DMS, DMDS and MM were subsequently tested in the BTT (Experiment 8) and the H_2S concentration was increased in order to assess its effect on the RE of the rest of the RSC. Finally, the simultaneous removal of RSC was studied by the two biotrickling filters in series, with the first filter BAT and the second one BTT (Experiment 9).

The EBRT (s), RE (%), L (gS/m^3/h), and elimination capacity, EC (gS/m^3/h), were determined using the relationships between the inlet concentration, C_{in} (gS/m^3), outlet concentration, C_{out} (gS/m^3), the gas flow rate, Q (m^3/h), and the filter bed volume, V (m^3), as follows: EBRT = (V/Q); RE = $[(C_{in} - C_{out})/C_{in}] \times 100$; $L = C_{in}/V \times Q$; EC = $(C_{in} - C_{out})/V \times Q$.

2.6. Molecular biology techniques

For the PUF sampling all of the cubes were removed randomly from the biotrickling filters from the bottom (first stage) and top levels (second stage). The identifications numbers of samples for molecular analysis genomic are showed in Table 2. In the BAT, samples from the old (PUF used as inoculum) and the new PUF were taken separately. The PUF cubes were sonicated for 15 min in TNN+ medium (25 mL) using an Ultrasons-H sonicator operating at 40 kHz in order to allow the total desorption of immobilized microorganisms. The PUF cubes were then removed and the TNN+ medium (with the microorganisms in suspension) was centrifuged at 10000*g* for 15 min. The resulting pellet was 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 of the bacterial 16S rRNA

Table 2
Operating conditions and identifications of samples for molecular analysis genomic.

Experiment	Compounds	EBRT (s)	Inlet load (gS/m^3/h)	C_{in} (ppmv)**	$[SO_4^{=}]$ (g/L)	Biotrickling filter	Number of sample for molecular analysis genomic			
							Top	Foam	Bottom	Foam
0*	DMS	54 ± 1	8.2 ± 0.5	94 ± 6	<10	BTT	15	new	14	new
1	MM	58 ± 1	1.2 to 12.1	16 to 144	<10	BTT	17	new	18	new
2	MM	31 to 208	5.1 ± 0.04	34 to 230	<10	BTT				
3	MM	58 ± 1	5.0 ± 0.2	62 ± 2	3 to 23	BTT				
4	DMDS	78 ± 0.6	3.0 to 37.3	25 to 312	<10	BTT	11	new	12	new
5	DMDS	78 to 15	27.7 ± 3.9	42 to 205	<10	BTT				
6	DMDS	78 ± 1	24.6 ± 0.4	206 ± 3	5 to 15	BTT				
7	H_2S	59 ± 1	4.2 to 102.0	54 to 1320	<30	BAT	2***	new	1***	new
							4***	old	3***	old
							10	new	9	new
8	H_2S	59 ± 1	1.8 to 29.5	23 to 376	<10	BTT	20	new	19	new
	MM		2.4 ± 0.2	30 ± 2						
	DMS		1.8 ± 0.3	23 ± 3						
	DMDS		7.2 ± 0.7	46 ± 5						
9	H_2S	59 ± 1	5.9 to 33.9	75 to 432	<10	BAT + BTT(in series)	8 (BAT)	old	7 (BAT)	new
	MM		2.7 ± 0.2	34 ± 3					6 (BAT)	old
	DMS		2.3 ± 0.2	29 ± 2					16 (BTT)	new
	DMDS		5.7 ± 0.8	36 ± 5						

* Feed of DMS to the BTT for study of the bacterial population. The operational performance was study previously by Arellano-García et al. (2009).
** At 30 °C.
*** PUF cubes taken after the experiments were completed.

gene was PCR- amplified using the forward primer GC-338F (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) described by Muyzer et al. (1993) and the reverse primer 907R (5′-CCG TCA ATT CCT TTG AGT TT-3′) described by Yu and Morrison (2004). The amplified fragments were 586 bps in length. The 25 μL reaction mixtures contained: 2.5 μL of 10X AccuBufer® (Bioline), 0.25 μL of 50 μM each primer prepared at a concentration of 50 μM, 0.5 μL of 10 mM dNTP mix, 1 μL of total DNA, 0.25 μL of 50 mM $MgCl_2$, 1 μL of 10 mg/mL of bovine serum albumin (Promega®) and 0.5 μL of 2.5 U/μl ACCUZYME™ DNA polymerase® (Bioline) in 18.75 μL of DNase and RNase-free sterilized water (Promega®).

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 products were analyzed by electrophoresis on 1.5% agarose gels in 1X TBE buffer (100 mM Tris, 90 mM Boric Acid, 0.001 mM EDTA) stained with PlusOne™ DNA Silver Staining Kit (GE Healthcare®) to confirm the product size and estimate the DNA concentration. The PCR samples were stored at −20 °C.

The bacterial populations present in the biofilters during the different biofiltration experiments were evaluated by DGGE using 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 35% to 55% (where 100% denaturant contained 7 M urea and 40% formamide). Electrophoresis was performed at 60 °C and 75 V for 15 h. After electrophoresis, the gel was stained with DNA Silver Staining Kit (GE Healthcare®) according to the manufacturer's instructions. The Gel Documentation Systems Imagen-Quant 100 GE Healthcare® was used for documenting the gels.

2.7. Analytical techniques

Gaseous DMS and DMDS concentrations were determined using an FID-GC (Agilent Technologies 6890) with a Porapak Q packed column (HP, 5′ × 1/8″, 80/100 mesh) and using nitrogen (40 mL/min) as the carrier gas. Temperatures of 190, 200 and 200 °C were used, respectively, for the oven, injector and detector. The injected sample volume was 200 μL in all cases. A specific sensor (Gasbadge Pro, ISC-Oldham) with a constant flow hand pump (ISC-Oldham) was employed to analyze the H_2S concentration in the gas phase for H_2S concentrations below 500 ppmv (0–500 ppmv in 0.1 ppmv increments). For concentrations greater than 500 ppmv the H_2S concentration was measured by iodometric titration (iodine, 5×10^{-3} N; starch indicator, 2 g/L) with a Tutweiler burette. MM gas concentrations were measured by gas detector tubes (Gastec 810-71, SKC USA). The detection ranges of the MM gas tube were 0.25–140 ppmv. A Tedlar bag (10 L single PP fitting 232-08, SKC USA) was used for the sampling gas. Sulphate concentration was analyzed by a turbidimetric method according the Standard Methods (APHA et al., 1998). The thiosulphate concentration was measured by iodometric titration according to Rodier (1998).

3. Results and discussion

3.1. Effect of the inlet load of MM and DMDS on the RE

In Experiments 1 and 4, the effect of the inlet load was studied at a constant EBRT of 58 ± 1 and 78 ± 0.6 s for MM and DMDS, respectively. The minimum time between each inlet concentration value was 6 h. Ramírez et al. (2009a) reported that the steady-state on working with *T. thioparus* for H_2S removal was reached after 40 times the EBRT. The total durations of the Experiments were 5 and 8 days for MM and DMDS, respectively.

For DMDS, the critical elimination capacity was 31.27 $g_{DMDS\text{-}S}/m^3/h$ (RE 99.6%) and for MM the RE maximum was only 94% for 1.14 $g_{MM\text{-}S}/m^3/h$ (Fig. 2). Cho et al. (1991b) reported that the specific uptake rates for these compounds using *T. thioparus* DW44 were H_2S > MM ⩾ DMDS > DMS. In this study, a greater degradation rate of DMDS was observed.

3.2. Effect of the EBRT on MM and DMS removal

In Experiments 2 and 5, the effect of the EBRT was studied at two constant loads of 5.10 ± 0.04 $g_{MM\text{-}S}/m^3/h$ and 27.7 ± 3.9 $g_{DMDS\text{-}S}/m^3/h$ for MM and DMDS, respectively. The minimum time between each EBRT was 6 h. For MM removal, the initial EBRT was 59 s (RE 95%). A decrease in the EBRT from 59 to 31 s affected the RE of MM by 24%. For DMDS, when the EBRT was decreased from 77.5 to 27.2 $g_{DMDS\text{-}S}/m^3/h$, the decay in the RE was only from 98% to 94% even with a DMDS load 5.4 times higher. For a constant EBRT over 40 s, the EC values were 33.56 $g_{DMDS\text{-}S}/m^3/h$ (RE 95%), 3.56–4.58 $g_{MM\text{-}S}/m^3/h$ (RE 71–91%) and 11.85 $g_{DMS\text{-}S}/m^3/h$ (RE 98%) for DMDS, MM and DMS (Arellano-García et al., 2009), respectively. Therefore, for a separate RSC feed the EC order was DMDS > DMS > MM.

MM is an intermediate oxidation product in the metabolic pathway of *Thiobacillus* sp. (Kelly and Smith, 1990; Visscher and Taylor, 1993) and the degradation rate of MM should therefore be greater. These pathways are not consistent with the results, but the existence of another pathway is unlikely. It is possible that other microorganisms were present in the biotrickling filter. After long operation times, the colonization of biofilters by other microorganisms is possible. Sercu et al. (2005) analyzed the microbial community present after 60 days of operation of a biotrickling filter at pH 7, the initially inoculated bacteria (*Hyphomicrobium* VS) was not the dominant microorganism. The EC of each compound has been reported to depend on the microbial community present in biofilters. Cho et al. (1991a) obtained an EC of H_2S greater than of that of MM in a biofilter initially inoculated with *Thiobacillus* sp. HA43 and heterotrophic bacteria. These authors suggest the presence of other H_2S-degrading microorganisms or an enhancement of the ability to remove H_2S of strain HA43 in the presence of other microorganisms.

3.3. H_2S removal by the BAT

Four gas sampling ports at fixed bed heights of 120, 200, 370 and 500 cm (the end of reactor) were used, in order to define 4

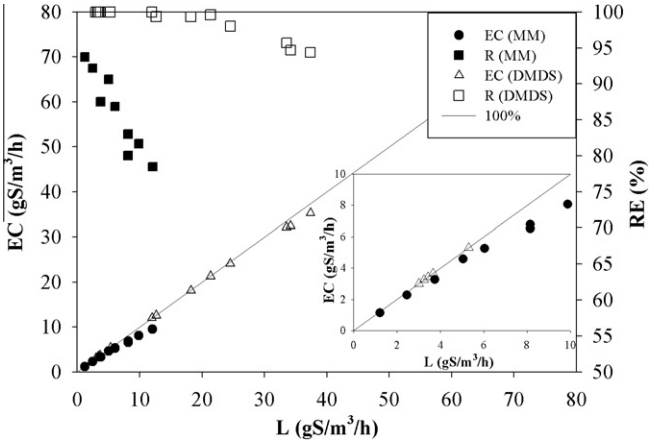

Fig. 2. Elimination capacity (EC) and removal efficiency (RE) versus inlet load for MM and DMDS removal.

reactor sections. The consecutive combined sections studied were: Section 1 (from 0 to 120 mm bed heights), followed by combined Sections 1–2 (from 0 to 200 mm), 1–3 (from 0 to 370 mm), and finally, the whole reactor. Our previous results for H_2S removal were obtained on a biotrickling filter of 1.28 L (Ramírez et al., 2009b). Therefore, in order to consider two identical biofilters it was necessary to study the removal of H_2S from a greater volume. The critical EC obtained by Ramírez et al. (2009b) was 58.7 $g_{H2S\text{-}S}/m^3/h$ (EBRT 24.4 s, RE 98%). In the BAT, for an EBRT of 23.6 s (Fig. 3, Experiment 7, Section 1–2) the EC was 59.6 $g_{H2S\text{-}S}/m^3/h$ (RE 88%). For an inlet load of 48.1 $g_{H2S\text{-}S}/m^3/h$ (Section 1–2) the RE increased to 94%. The minimum time between each inlet concentration value was 6 h for a period of 8 days. The results were very similar to those obtained previously; therefore, there is a very good possibility that the system could be successfully scaled-up. The maximum EC value was 70.6 $g_{H2S\text{-}S}/m^3/h$ with a RE of 68% (whole reactor, 59 s). Fig. 3 shows that when EBRT decreases the values of ED are separated from the line marking 100% of removal efficiency. This could be explained by a decrease in mass transfer between gas and liquid phase. An increased of the EBRT allows the solubility of H_2S in the liquid–biofilm phase and the H_2S degradation for the biomass immobilized.

For *T. thioparus*, an EC of 14.9 $g_{H2S\text{-}S}/m^3/h$ was obtained in a similar biotrickling filter (Ramírez et al., 2009a). A similar performance was observed by Aroca et al. (2007) using polyethylene rings as the carrier.

3.4. H_2S, MM, DMS and DMDS removal by the BTT

A mixture stream with the four compounds was supplied to the BTT (Experiment 8). For a constant concentration of MM, DMS and DMDS, the inlet concentration of H_2S was increased from 23 to 376 ppmv (Fig. 4). The minimum time between each inlet concentration value was of 6 h for a period of 6 days. When the H_2S concentration was increased, the RE decreased: from 97% to 67% (EC 1.75–1.20 $g_{DMS\text{-}S}/m^3/h$), 86% to 71% (EC 6.19–5.11 $g_{DMDS\text{-}S}/m^3/h$) and 83% to 33% (EC 1.96–0.79 $g_{MM\text{-}S}/m^3/h$) for DMS, DMDS and MM, respectively. Furthermore, when the H_2S concentration increased the RE of RSC decreased by 30%, 15% and 50% for DMS, DMDS and MM, respectively. Therefore, the presence of H_2S inhibited the capacity of *T. thioparus* to degrade MM, DMS and DMDS.

Wani et al. (1999) studied the elimination of H_2S in the presence of DMDS and DMS. They did not find any significant changes in the maximum removal rate of H_2S for three different biofilters packed with mixtures of compost/perlite (4:1), hog fuel/perlite (4:1) and compost/hog fuel/perlite (2:2:1), respectively. Nevertheless, the RE of DMS was <30% and the RE of DMDS between 30% and 35%. RE values of 100% for H_2S, MM, DMS and DMDS have been achieved in a biotrickling filter with *P. putida* treating gas at low concentrations for odour abatement (H_2S load: 0.03–0.48 $gH_2S/$

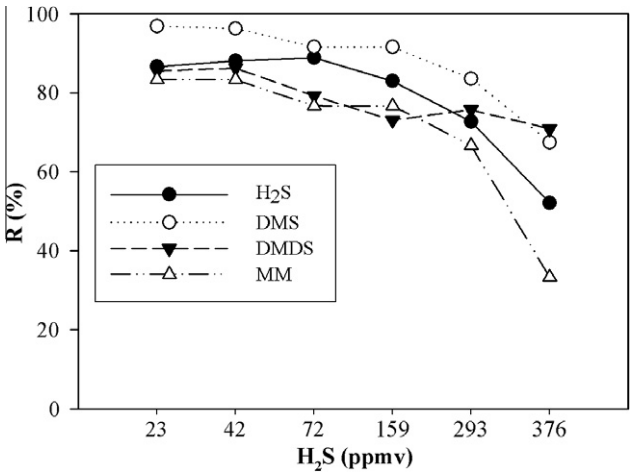

Fig. 4. Removal efficiency of H_2S/DMS/DMDS/MM versus inlet H_2S concentration. Biofilter BTT.

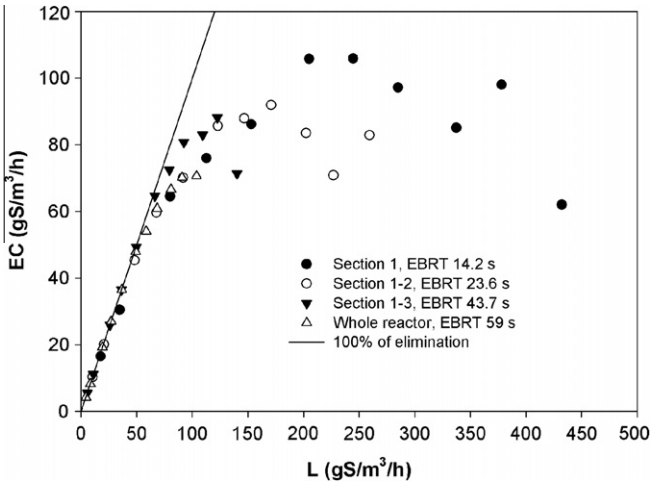

Fig. 3. Elimination capacity (EC) versus inlet load (L) at different sections of the biofilter (BAT).

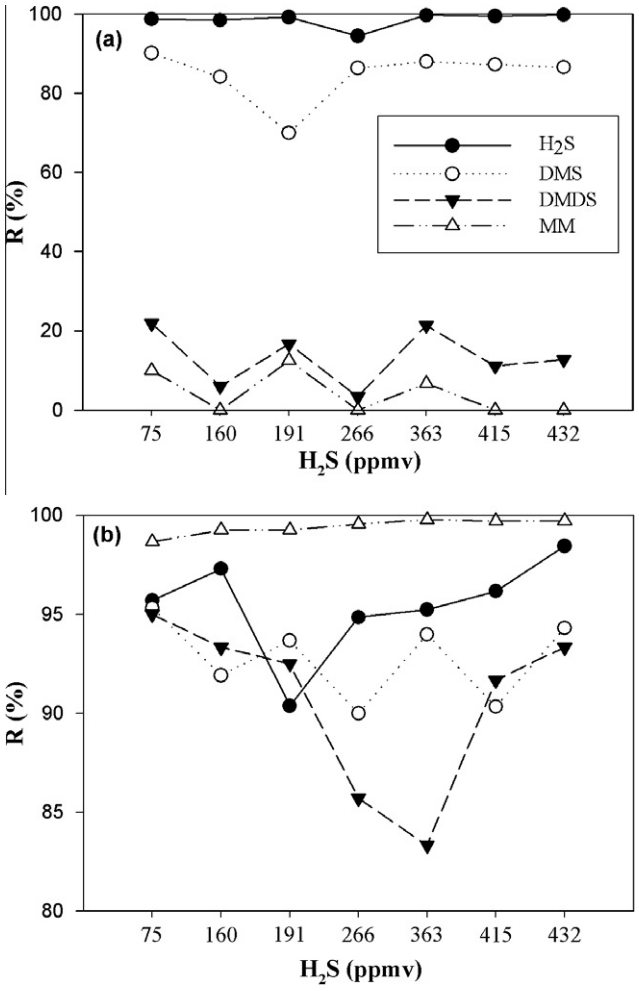

Fig. 5. Removal efficiency of H_2S/DMS/DMDS/MM versus inlet H_2S concentration. Biofilter BAT (a); BAT and BTT in series (b).

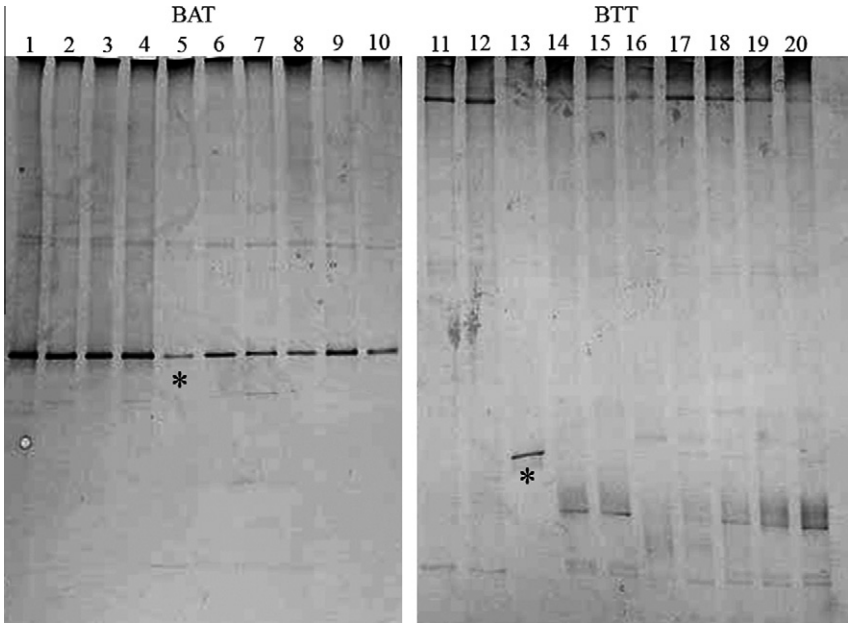

Fig. 6. DGGE banding patterns for BAT and BTT. Bands marked with an asterisk correspond to the pure strain (samples 5 and 13).

m^3/h) (Ho et al., 2008). However, when the H_2S concentration was increased the RE of RSC decreased. In an experiment with a peat biofilter inoculated with *Hyphomicrobium* sp. I55, the removal of DMS was inhibited by the presence of H_2S and MM but the removal of H_2S was not affected by the presence of DMS. In biotrickling filters inoculated with activated sludge the inhibition of DMS removal was observed when the H_2S concentration exceeded 200 $mg_{H2S\text{-}S}/m^3$ (Ruokojärvi et al., 2000).

3.5. H_2S, MM, DMS and DMDS removal in the BAT and BTT in series

A mixture stream containing the four RSC was fed into the system with two biotrickling filters in series (Experiment 9). In the first biotrickling filter (BAT), the RE was approximately constant at 98.5 ± 1.4%, 84.6 ± 5.0%, 13.3 ± 5.3% and 4.2 ± 4.1% for H_2S, DMDS, DMS and MM, respectively (Fig. 5(a)). When the RE was measured with both biotrickling filters in series the total RE was 99.4 ± 0.3%, 92.8 ± 1.5%, 95.4 ± 1.9% and 90.7 ± 3.3% for H_2S, DMDS, DMS and MM, respectively (Fig. 5(b)). Therefore, the BAT efficiently removed the H_2S and a high percentage of the DMDS. The global system increased the total RE. The BTT removed almost all of the MM and DMS and as well as the rest of the DMDS. The EC values in the global system were 33.7 ± 0.1%, 5.38 ± 0.1%, 2.2 ± 0.05%, 2.4 ± 0.05% gS/m^3/h for H_2S, DMDS, DMS and MM, respectively.

Ruokojärvi et al. (2001) obtained EC values of 47.9, 36.6 and 2.75 gS/m^3/h for H_2S, DMS and MM, respectively, in two-stage biotrickling filters inoculated with a consortium of microorganisms enriched from the sludge water of a Finnish refinery (some microbes with budding typical of *Hyphomicrobium*). The first biotrickling filter was operated at low pH and removed approximately half of the DMS and almost all of the H_2S, and the rest of the MM and DMS was oxidized in the second biotrickling filter at neutral pH.

Comparison between Fig. 5(b) and 4 shows that the biotrickling filters in series were more efficient. When the H_2S levels increased, the RE of RSC did not decrease.

3.6. Bacterial populations monitoring

Fig. 6 shows the DGGE profiles of the bacterial populations present in the biofilters. The fingerprint obtained for samples of the PUF (old and new) at two sampling points (top and bottom) with different RSC are shown.

A single band similar to the *A. thiooxidans* band (lane 5), was observed in the BAT, suggesting that the inoculated population of this microorganism, remained at different operation stages of the biofilter, regardless of the sample point and type of RSC that feed this biofilter. This fact can be explained because the low pH (2.0) that does not allow the colonization of this biofilter by other microorganisms.

In contrast, the bacterial populations present in the BTT changed after inoculation. Patterns for lanes 11 and 12 are the same; so this behaviour indicates that only one species was present in the biofilter that were responsible for DMDS degradation.

For lanes 14–20, other visible bands appeared, showing that other microorganisms colonized the biofilter. These results may explain the changes in EC order for MM, DMS and DMDS obtained in this work and by other authors (Cho et al., 1991b).

4. Conclusion

The results of this stage showed the feasibility of RSC removal by a two-stage biotrickling filter in series – the first one inoculated with *A. thiooxidans* (BAT) and the second one with *T. thioparus* (BTT).

For an inlet load of H_2S up to 33.7 $g_{H2S\text{-}S}/m^3$/h, the RE for MM, DMS and DMDS did not decrease in the two-stage biotrickling filter (BAT + BTT), and the RE was higher than in the single stage biofilter (BTT). The analysis of the bacterial populations present under different biofiltration conditions indicated that the bacterial populations varied depending on the operation conditions in the BTT biofilter.

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