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### ► To cite this version:

Luc Malhautier, Alexandre Soupramanien, Sandrine Bayle, Janick Rocher, Jean-Louis Fanlo. Potentialities of coupling biological processes (biotrickler/biofilter) for the degradation of a mixture of sulphur compounds. *Applied Microbiology and Biotechnology*, 2015, 99 (1, SI), pp.89-96. 10.1007/s00253-014-5842-0 . hal-02914340

**HAL Id: hal-02914340**

**<https://hal.science/hal-02914340v1>**

Submitted on 26 May 2021

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# Potentialities of coupling biological processes (biotrickler/biofilter) for the degradation of a mixture of sulphur compounds

Luc Malhautier · Alexandre Soupramanien · Sandrine Bayle · Janick Rocher · Jean-Louis Fanlo

**Abstract** This study deals with the potential of biological processes combining a biotrickler and a biofilter to treat a mixture of sulphur-reduced compounds including dimethyl sulphide (DMS), dimethyl disulphide (DMDS) and hydrogen sulphide (H<sub>2</sub>S). As a reference, duplicated biofilters were implemented, and operating conditions were similar for all bioprocesses. The first step of this work was to determine the efficiency removal level achieved for each compound of the mixture and in a second step, to assess the longitudinal distribution of biodegradation activities and evaluate the total bacteria, *Hyphomicrobium* sp. and *Thiobacillus thioparus* densities along the bed height. A complete removal of hydrogen sulphide is reached at the start of the experiment within the first stage (biotrickler) of the coupling. This study highlighted that the coupling of a biotrickling filter and a biofilter is an interesting way to improve both removal efficiency levels (15–20 % more) and kinetics of recalcitrant sulphur compounds such as DMS and DMDS. The total cell densities remained similar (around  $1 \times 10^{10}$  16S recombinant DNA (rDNA) copies g dry packing material) for duplicated biofilters and the biofilter below the biotrickling filter. The relative abundances of *Hyphomicrobium* sp. and *T. thioparus* have been estimated to an average of  $10 \pm 7.0$  and  $0.23 \pm 0.07$  %, respectively, for all biofilters. Further investigation should allow achieving complete removal of DMS by starting the organic sulphur compound degradation within the first stage and surveying microbial community structure colonizing this complex system.

**Keywords** Volatile sulphur compounds · Biofiltration · Biotrickling filter · Coupling · Hyphomicrobium · Thiobacillus

## Introduction

Malodorous gases are emitted from industrial biowaste treatment facilities like rendering facilities and wastewater treatment plant. They may induce odour annoyance to the surrounding populations, generate complaints and lead to a lack of acceptance of these facilities. While odorous emissions are not considered to cause health problems directly, they may well be associated with negative health effects, which may cause defensive reactions of people due to psychological effects (Schlegelmilch et al. 2005).

Legislation controlling the emissions of air pollutants (VOC, toxics and odours) has proliferated and became more and more restricted and sets minimum distances from the facility to the nearest housings (Germany, 300 m; France, 200 m; Fanlo 2005; Schlegelmilch et al. 2005).

Field data analysis and literature survey reveal the emission of a large variety of volatile compounds such as oxygenated (volatile fatty acids, ketones, aldehydes and alcohols) nitrogen and sulphur compounds (Van Langenhove et al. 1985). Due to their obnoxious smell and their very low odour thresholds (around  $1 \mu\text{g Nm}^{-3}$ ), especially hydrogen sulphide (H<sub>2</sub>S) and organic sulphur compounds such as dimethyl disulphide (DMDS) and dimethyl sulphide (DMS) often cause odour annoyance and deserve special attention (Sercu et al. 2006).

For these reasons, an adequate waste gas management strategy has to be implemented by industrial facilities to control odour emissions (Both 2001; Mahin 2001). Among the gas treatment processes, biotechniques, more particularly biofiltration (Chung et al. 2010; Kennes et al. 2009; Rehman

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et al. 2009) and biotrickling (Arellano-Garcia et al. 2009; Chung et al. 2005), are proving to be efficient, economic and environment-friendly technologies for the treatment of industrial airstreams characterized by high flow rates and low pollutant concentrations. These biotechniques are based on the ability of microorganisms to use volatile pollutants for growth and have been successfully applied for the treatment of gaseous emissions emitted from different industrial processes (Malhautier et al. 2005). Whereas there has been an explosion in the number of papers dealing with biofilters (Iranpour et al. 2005), there has not been nearly as many reports on waste air treatment using biotrickling filters (BTFs) even if the number of full-scale BTFs is rapidly increasing. In the past few 2–3 years, several successful conversions of full-scale chemical scrubbers to BTF have been demonstrated (Mudliar et al. 2010). Moreover, these bioreactors have been shown to be suitable in several instances when accurate control of the environmental conditions or the degradation of a mixture of complex chemical molecules (Estrada et al. 2011; Hassan and Sorial 2011; Paca et al. 2007; Sempere et al. 2010). However, these biotechnologies are claimed to support low removal efficiencies for the hydrophobic fraction of malodorous emissions, whose elimination is mandatory for efficient odour abatement (Lebrero et al. 2014).

Sulphide-oxidizing bacteria allowing oxidation of sulphide encompass several genera such as *Thiobacillus*, *Acidithiobacillus*, *Achromatium* and *Beggiatoa* (Ramirez et al. 2009; Tang et al. 2008). It also has been shown that the inoculation of *Hyphomicrobium* in a biofilter allowed increasing the DMS and DMDS elimination capacity (Smet et al. 1996).

Thus, this study deals with the potential of a combination of two traditional biotechniques (biofiltration and BTF) for the treatment of a mixture of sulphur compounds including DMS, DMDS and H<sub>2</sub>S. So, the aims of this work are, in the first step, to determine the efficiency removal level achieved for each compound of the mixture and, in a second step, to assess the longitudinal distribution of biodegradation activities along the bed height and, finally, to evaluate the total bacteria, *Hyphomicrobium* sp. and *Thiobacillus thioparus* densities by using real-time PCR. We focus on *Hyphomicrobium* and *T. thioparus* as both genera are well-documented aerobic DMS degraders that have both been identified in biofilters treating volatile organic sulphur compounds through isolation (Cho et al. 1991) and via molecular techniques (Sercu et al. 2006; Hayes et al. 2010).

## Material and methods

### Pilot unit

The system consists of two identical and independent biofiltration units (BFA and BFB) and a coupling of

biotrickler/biofilter (BTF/BF). Each biofiltration unit is consisted of PVC columns with an inner diameter of 30 cm and filled up to 1 m of bed height, with an alternating of sawmill chips/coconut fibre every 20 cm. The last 20-cm section was filled with pine barks as indicated on Fig. 1. Gas sampling ports are located at the inlet and outlet of the biofilter and along the height of each column every 20 cm from the inlet up to 100 cm, respectively (10, 30, 50, 70 and 90 cm). The biotrickler is consisted of a tank of 300 L and a PVC column with an inner diameter of 30 cm and filled with Hiflow rings up to 1 m of bed height (Fig. 1). Gas sampling ports are located at the inlet and outlet of the biotrickler (0 and 100 cm).

### Packing material

The physicochemical characteristics of the used packing materials are summarized in Table 1. The mean particle diameter (mm; Sauter diameter) was performed from a granulometric distribution. Each packing material was sifted by using height sieves: 5, 6.3, 8, 10, 12.5, 14, 16 and 20 mm. The maximal water-holding capacity is determined by measuring the weight difference between a known volume of dry and moist packing material previously kept in water for 8 h and drained. Material porosity is obtained by weighing a known volume of drained packing material, then by covering it with water and weighing again. Determination of pH was performed from the aqueous suspension after submerging the packing material in water for 1 h under controlled conditions of temperature (20 °C) and shaking (15 Hz; Vibro-Shaker MM200, Retsch, Haan, Germany).

### Inoculation

For each column, the packing material was seeded with supernatant of wastewater (10 L) collected at a rendering facility (ATEMAX and SOLEVAL Sud-Est, Viriat, France). The inoculum was recirculated (5 L h<sup>-1</sup>) for 24 h. During inoculation, biofilters were supplied only with air.

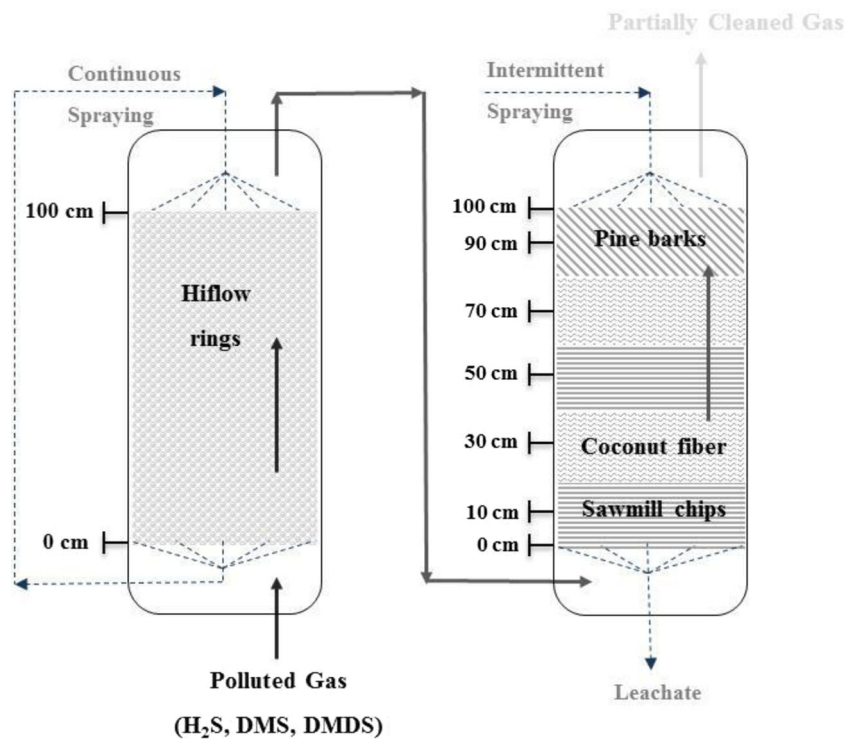
### Experimental set-up

The biofiltration units (BFA and BFB) and the coupling (BTF/BF) were operated in parallel, at ambient temperature, for 3 months (Fig. 1). Each system was supplied with a synthetic gaseous effluent at a velocity of 100 m h<sup>-1</sup> (EBRT of 36 s) in an upward flow mode.

The synthetic gaseous effluent is composed of H<sub>2</sub>S (Messer France SAS, Puteaux, France), DMS and DMDS (VWR, West Chester, PA) at concentrations of 30, 20 and 20 mg m<sup>-3</sup>, respectively.

Concerning the BTF, the recirculation of the aqueous phase is of 250 L h<sup>-1</sup> (water velocity of 3.5 m h<sup>-1</sup>), and pH of the

**Fig. 1** Schema diagram of the pilot unit (→ denotes air to be treated)



aqueous phase was adjusted at neutrality with a sodium hydroxide solution (5 N).

For biofilters, the humidity of packing material was kept by spraying ( $20 \text{ L h}^{-1} \text{ m}^{-3}$  packing material) with a nutrient solution (pH=7.5) containing  $10 \text{ mg L}^{-1}$  of total nitrogen (Optiplan fertilizer, N/P/K of 24/20/2, Duclos International Engrais, Lunel, France). An automated humidification system was carried out. No recirculation of water was operated. The water phase within the bed height was adjusted at neutrality with a sodium hydroxide solution (5 N) on the basis of daily pH values of percolate waters.

Daily, gaseous volatile compounds were detected and quantified online by a gas chromatograph (Varian CP-3800, Varian, Palo Alto, CA) equipped with a pulsed flame photometric detector and a 30-m Varian CP-Sil 5 CB for sulphur capillary column (0.32-mm internal diameter, 0.4- $\mu\text{m}$  film thickness). The volatile compound removal efficiency (RE) is calculated to evaluate biodegradation activity of the microbial community.

Percolate waters were collected at the bottom of the column once a week to determine total nitrogen (Nitrogen total reagent set, HACH LANGE, Duesseldorf, Germany), sulphate anion (sulphate reagent SulfaVer<sup>®</sup> 4, HACH LANGE, Duesseldorf, Germany) and pH.

#### Bacterial community analysis

#### DNA extraction

The procedure for cell dispersion and DNA extraction from biofilter packing material was optimized on the basis of the systemic guideline provided by Cabrol et al. (2010). For all biofilters, at steady state, samples of 5 g of each packing material were collected, suspended in 15 mL of sterile physiological saline solution ( $\text{NaCl } 9 \text{ g L}^{-1}$ ) and shaken by using a Vibro-Shaker MM200 (Retsch, Haan, Germany) at rotating frequency of 10 Hz for 2 min. After dispersion, each packing material was discarded, and liquid phase containing the

**Table 1** Physicochemical characteristics of the used packing materials

Packing material	Sawmill chips	Coconut fibre	Pine barks	Hiflow rings
Mean diameter (mm)	11.4	ND	10.7	21.8
Porosity (%)	0.60±0.02	0.80±0.03	0.60±0.02	0.88±0.01
Water-holding capacity ( $\text{m}^3 \text{ H}_2\text{O m}^{-3}$ material)	0.27±0.03	0.09±0.04	0.14±0.04	0.03±0.01
pH	5.5±0.4	6.8±0.2	4.4±0.2	7.5

ND not determined

suspended microorganisms was centrifuged at 10,000 rpm for 15 min. The cell pellet was recovered and DNA was extracted using FastDNA<sup>®</sup> SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA). Extracted DNA was visualized by gel electrophoresis on 1 % (w/w) agarose gel. DNA quantification was determined by absorbance at 260 nm using a multiscan spectrum spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

#### qPCR analysis

Investigation of the total bacterial *Hyphomicrobium* sp. and *T. thioparus* population densities was performed by quantitative PCR (qPCR) targeting the *rrs* gene. The qPCR reactions were performed according to the protocol described by (i) Gadal-Mawart et al. (2012) using primers V3F (5'-ACTCCC TAGCGGGAGGCAGCAG-3') and V3R (5'-ATTACCGC GGCTGCTGGCAC-3') and (ii) Hayes et al. (2010) using primers hypho F (5'-GGCTCAACCTCGGACT-3') and hypho R (5'-CGAATTTACCTCTACTAGGAT-3'), thio F (5'-CCTCACGTTATTTCGAGCGG-3') and thio R (5'-ACGCAC TCTAGACTGCCA-3'), respectively. The qPCR is realized with a Bio-Rad SsoFast<sup>™</sup> EvaGreen Supermix kit (Bio-Rad, Hercules, CA) and achieved by a thermocycler Rotor-Gene<sup>™</sup> 6000 (Corbett, Mortlake, NSW). In addition to environmental samples, a standard range and a negative control were included. All samples, standard range and negative control were replicat-

ed. Efficacy of standards was higher than 90 %, and an error rate of less than 2 % was observed for all samples.

## Results

### Efficiency levels

The removal profiles are similar for both biofilters as indicated on Fig. 2a, whatever the pollutant, revealing that duplicated biofilters exhibit a highly reproducible functioning. For coupling, complete removal of hydrogen sulphide is reached at the start of the experiment, while, for duplicated biofilters, similar performance is reached after only 8 days of operation. High and stable efficiency level of DMS (70 %) and DMDS (around 80 %) was observed for duplicated biofilters. DMS and DMDS efficiency levels are higher for coupling. DMDS was completely removed from day 60, and DMS efficiency level was close to 90 % from day 80 (Fig. 2b). The presence of sulphate in liquid phases highlighted the biological oxidation of volatile sulphur compounds. The conversion rate of gaseous sulphur compounds into sulphate (SO<sub>4</sub>) has been assessed at steady state (from day 65 to day 83) for biofilters BFA and BFB and for coupling BTF/BF between gas and liquid phases as follows:

$$\sum (S-H_2S + S-DMS + S-DMDS)g_i = \sum (S-H_2S + S-DMS + S-DMDS)g_f + \sum (S-SO_4)l_f$$

where  $g$  is gas,  $l$  is liquid,  $i$  is the initial day (day 65) and  $f$  is the final day (day 83).

The error mass balance ( $e$ ) is defined as follows:

$$e(\%) = \left( \sum S \text{ inlet} - \sum S \text{ outlet} \right) / \left( \sum S \text{ inlet} \right) \times 100$$

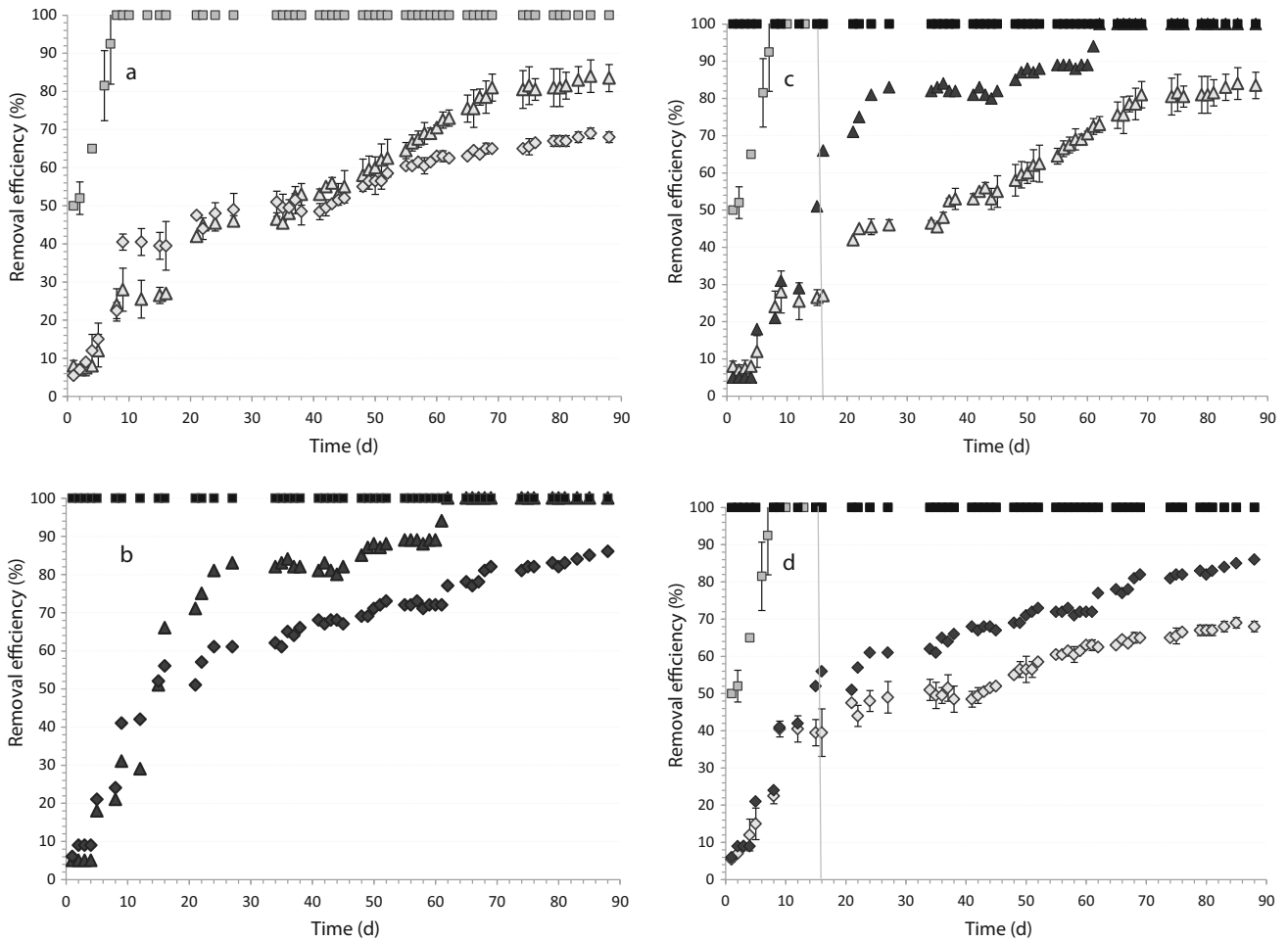
The error mass balance is low and corresponds to 1, 2.3 and 4.7/7.3 % for BFA, BFB and BTF/BF, respectively. These results seem to reveal that the sulphur compounds are mainly oxidized all the way to sulphate which is the end-product of sulphur compound oxidation (Sercu et al. 2006). For DMDS and DMS, the delay needed to reach steady state is of around 60 and 80 days respectively, irrespective of the bioreactor. While the acclimatization phase delay remains important for both duplicated biofilters and coupling, DMS and DMDS removal efficiency kinetics are higher for coupling than those for duplicated biofilters (Fig. 2c, d) from day 16.

### Degradation profiles

For duplicated biofilters, the profiles at steady state (Fig. 3) enable to distinguish two groups of contaminants. On the one hand, hydrogen sulphide is very efficiently eliminated: Its complete removal required less than 10 % of bed height (i.e. 10 cm). On the second hand, organic sulphur compounds present slower degradation kinetics. The total column height is required to eliminate 65 and 80 % of inlet concentration of DMS and DMDS, respectively.

For coupling, the biofilter is fed with DMS and DMDS only, as H<sub>2</sub>S is completely removed within the BTF, i.e. 0.85 m (Fig. 3e). The global behaviour for both organic sulphur compounds remains unchanged. Nevertheless, the DMS and DMDS profiles exhibit a trend shift with an increasing rate of compound degradation. Moreover, DMDS is completely removed and its elimination required 85 % of the bed height. The difference of DMDS and DMS degradation kinetics between duplicated biofilters and the biofilter below the BTF is observed after just over 13 days of operation.





**Fig. 2** Removal efficiency levels of H<sub>2</sub>S (□), DMS (◇) and DMDS (Δ) for both duplicated biofilters (*grey symbols*, **a**) and for biotrickling/biofilter coupling (*dark symbols*; **b**). Comparison of removal efficiency levels of DMDS (**c**) and DMS (**d**) for both duplicated biofilters and for

biotrickling/biofilter coupling. Removal efficiency levels of H<sub>2</sub>S for duplicated biofilters are also mentioned. The *vertical line* (day 16) indicates the shift of removal efficiency values for BTF/BF

### Bacterial densities

The molecular evaluation of cell densities by qPCR, along the bed height of all biofilters, has been realized from packing material sampled at steady state (day 88). The total cell densities ranged between  $8 \times 10^9$  and  $1.5 \times 10^{10}$  16S recombinant DNA (rDNA) copies g dry packing material for duplicated biofilters and the biofilter below the BTF. No statistical difference was observed for duplicated biofilters (Student's *t* test  $P \gg 0.05$ ) and between duplicated biofilters and the biofilter below the BTF (Student's *t* test  $P \gg 0.05$ ).

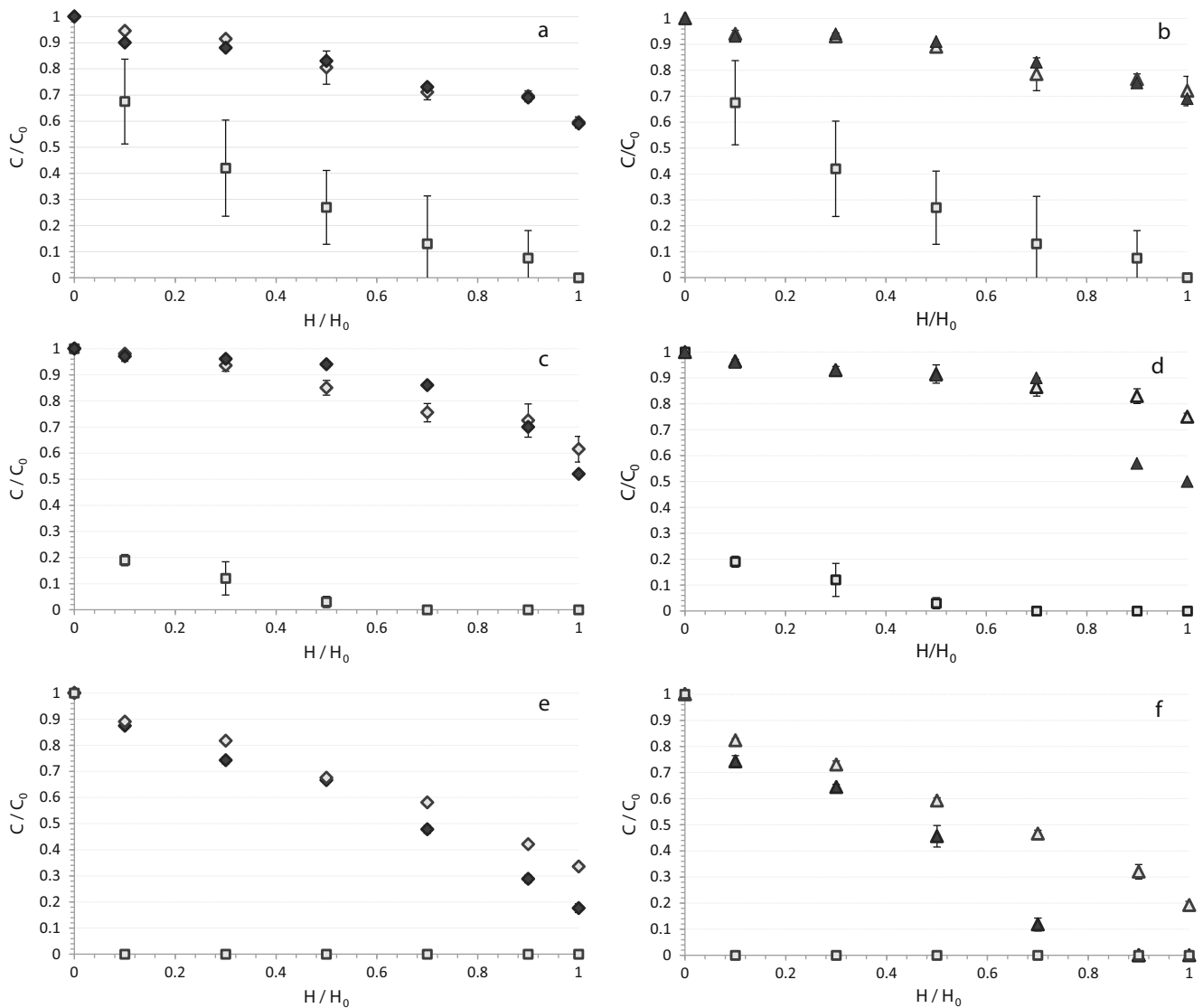
Cell densities of bacterial populations enabled to degrade DMS and DMDS are also investigated: *Hyphomicrobium* sp. and *T. thioparus*. For duplicated biofilters, the cell densities are similar (Student *t* test  $P \gg 0.05$ ) around  $2.1\text{--}2.8 \times 10^8$  and  $1.5\text{--}2.1 \times 10^7$  16S rDNA copies g dry packing material, respectively. The same trend is also observed between both biofilters and the biofilter below the BTF ( $3.4 \times 10^8$  and  $3.2 \times 10^7$  16S rDNA copies g dry packing material, respectively)

with no statistical difference (Student's *t* test  $P \gg 0.05$ ). The relative abundances of *Hyphomicrobium* sp. and *T. thioparus* have been estimated to an average of  $10 \pm 7.0$  and  $0.11 \pm 0.03$  %, respectively, for all biofilters. These values have been computed by assuming that two copies of the 16S recombinant RNA (rRNA) gene are located in regions of the genome of *T. thioparus* and that *Hyphomicrobium* spp. have one 16S rRNA gene copy (Fukushima et al. 2013; Hayes et al. 2010; Beller et al. 2006).

### Discussion

#### Removal efficiency of sulphur compounds

The results reveal that, for gases containing mixed sulphur compounds, the preference of sulphur-oxidizing bacteria to use hydrogen sulphide as an energy source over tested organic



**Fig 3** Degradation profiles of  $H_2S$  ( $\square$ ), DMS ( $\diamond$ ) and DMDS ( $\Delta$ ) for both duplicated biofilters (grey symbols) and for the biofilter combined to biotrickling (dark symbols) at day 9 (a, b), day 13 (c, d) and steady state (from day 80 to day 88; e, f).  $C$  is the outlet concentration ( $mg\ m^{-3}$ ) at the

height  $H$  of the biofilter (m),  $C_0$  the inlet concentration ( $mg\ m^{-3}$ ), and  $H_0$  is the total height of the column (m). This biofilter is fed with DMS and DMDS only, as  $H_2S$  is completely removed within the biotrickling filter. Error bars are indicated

sulphur compounds promotes low DMS and DMDS consumption (Arellano-Garcia et al. 2009; Caceres et al. 2012; Myung Cha et al. 1999; Ramirez et al. 2011). This result also confirms the higher substrate consumption rate of DMDS compared to that of DMS and seems to highlight that DMS is more recalcitrant than DMDS.

For DMDS and DMS, the delay needed to reach steady state is important, around 60 and 80 days, respectively. This lag phase duration can be attributed to biological factors: substrate availability (poorly soluble compounds) (Chen et al. 2008), biodegradability limitation and/or slow degradation kinetic (Cabrol et al. 2012). Moreover, competitive exclusion phenomena within the bacterial community colonizing the packing material could delay the emergence of functional populations. Moreover, for all bioreactors and at steady

state, as almost all (or all) the bed heights (90–100 cm) are available for degrading organic sulphur compounds only, it is possible that both substrate availability and slow degradation kinetic mainly affect the removal efficiency of these compounds.

#### Performances of BTF/BF

Higher removal efficiencies of DMS and DMDS (15–20 %) are observed by combining BTF and BF. This result can be explained by the spatial distribution of degradation activities: Hydrogen sulphide is completely removed within the first stage (biotrickling) while DMS and DMDS are eliminated within the biofilter only. This spatial distribution could

prevent inhibition effect of H<sub>2</sub>S on the bio-oxidation rate of DMS and DMDS.

Moreover, in the case of the biological treatment of a complex mixture of sulphur compounds containing H<sub>2</sub>S and organic sulphur compounds by using one-stage bioprocess, it is possible that competition between bacterial taxa, through the selection of fitted H<sub>2</sub>S-degrading populations, slows down the development of DMS and DMDS-degrading populations, thus inducing negative effect on performances as it has been revealed in this work (Fig. 3).

Some studies dealing with the use of biological processes in series are scarce. Nevertheless, it has been reported (Ramirez et al. 2011; Ruokojärvi et al. 2001) that the removal efficiency of such compounds is enhanced when two BTFs fed with a mixture stream containing H<sub>2</sub>S, methyl mercaptan, DMS and/or DMDS are combined.

### Bacterial densities

At steady state, the bacterial growth is homogeneous along the bed height, whatever the targeted populations (total bacteria, *Hyphomicrobium* sp. and *T. thioparus*). Concerning total bacteria densities, it has been reported that, on a long term, the density of bacterial colonization tends to homogenize along the biofilter height (Grove et al. 2004; Juteau et al. 1999).

The presence of both *Hyphomicrobium* and *Thiobacillus* genera is not surprising as both genera have the capacities to metabolize organic sulphur compounds such as DMS and DMDS (Hayes et al. 2010). Nevertheless, the density profiles of both genera exhibited a surprising longitudinal homogeneity in gas biofilters. Almost all (or all) the bed heights (90–100 cm) is effectively needed to remove both organic sulphur compounds. The absence of correlation between the physico-chemical and microbial spatial distributions can be explained by the environmental standardization along the bed height, arising from (a) the supply of carbon and energy source by the packing material (Friedrich et al. 2003) or/and (b) the percolation of water and nutrients through the packing material in biofilters (Cabrol and Malhautier 2011); as in our study, the flow rate of the nutritive solution is high (20 L h<sup>-1</sup> m<sup>-3</sup> packing material). It also can be due to the growth of other active populations involved in DMS biodegradation and unidentified in this study.

The results revealed that the bacterial densities of *Hyphomicrobium* genus are higher (×50) than those of *T. thioparus*. Both genera have been also detected in biological processes treating volatile organic sulphur compounds. Nevertheless, the relative abundances can differ as they have been reported by different authors. Within a BTF-removing DMS (removal efficiency close to 100 %), the number of copies of 16S rDNA from *T. thioparus* was several orders of magnitude more abundant than that of 16S rDNA from

*Hyphomicrobium* sp. (Sercu et al. 2006). In return, other works highlighted that 16S rDNA copies of *Hyphomicrobium* sp. exceeded those of *Thiobacillus* sp. at the section where the bulk of biodegradation was occurring in biofilters used for the treatment of DMS and methanol (Hayes et al. 2010; Zhang et al. 2006).

To conclude, this study highlighted that the coupling of a BTF and a biofilter is an interesting way to improve both removal efficiency levels and kinetics of recalcitrant sulphur compounds such as DMS and DMDS. The bacterial growth is homogeneous along the bed height, whatever the targeted populations (total bacteria, *Hyphomicrobium* sp. and *T. thioparus*). Further investigation should allow (i) achieving complete removal of DMS by starting the organic sulphur compound degradation within the first stage (BFT) and (ii) surveying microbial community structure colonizing this complex system.

**Acknowledgments** The authors are very thankful to the Orientations Stratégiques des Ecoles des Mines (OSEM) for its financial support.

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