Biological treatment of a mixture of gaseous sulphur reduced compounds: identification of the total bacterial community's structure

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Abstract

BACKGROUND: This study deals with the potential of biological processes combining biofiltration and a biotrickling filter to treat a mixture of sulphur reduced compounds (SRC) including dimethylsulfide (DMS), dimethyldisulfide (DMDS) and hydrogen sulphide (H_2S). The first step in this work is to evaluate the influence of pH on SRC biodegradation in microcosms seeded with planktonic biomass from activated sludge of a rendering facility. In a second step, for each tested pH, evaluation of the influence of pH on total bacterial community diversity and structure has been investigated using denaturing gradient gel electrophoresis (DGGE).

RESULTS: At pH 7 and 5, H_2S, DMS and DMDS were completely removed. In return, the abatement of DMS and DMDS is low, around 20%, for pH 3 and pH 1 microcosms. The selective pressure imposed in the microcosms (concentration and pH) is sufficient to influence strongly the community diversity and composition. The inoculum community seems to make a significant contribution to the 67-day community in the considered pH microcosms.

CONCLUSION: These results suggest that distinct communities from different inocula can achieve high and stable functionality. The results obtained provide relevant information for improving the treatment of different SRC using biotrickling filter/biofilter combination.

Keywords: malodorous gases; sulphur reduced compounds; biological processes; removal efficiency; pH; microbial communities

INTRODUCTION

Rendering facilities generate odorous gaseous emissions that are a serious concern as they may induce odour annoyance to the surrounding populations, generate complaints and lead to a lack of acceptance of the facility. 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.[1] Depending on weather and topographical conditions, odours may be carried away several kilometers but are generally only judged as significant within 500 m of the site.[2] Hence, legislation in developed countries and states is becoming more and more restrictive and sets minimum distances from the facility to the nearest housings (Germany: 300 m, France: 200 m)[1].

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. Among these molecules, the sulphur reduced compounds (SRC) are an important class of odorous compounds. Because of their very low odor thresholds (around 1 μg-N m^{-3}), these compounds deserve special attention.[3] Moreover, they a potentially corrosive effect on some materials. Generally the most frequently studied SRCs are hydrogen sulphide (H_2S), dimethylsulphide (DMS), dimethyldisulfide (DMDS) and methanethiol (MT).[4,5]

For these reasons an adequate waste gas management strategy has to be implemented by industrial facilities to control odor emissions.[6,7] Among the gas treatment processes, biotechniques, and more particularly biofiltration[8,9,10] and biotrickling,[11,12] 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. Nevertheless, as such gaseous emissions contain a mixture of chemical molecules (oxygenated, sulphur and nitrogenous compounds), the performance of these techniques has to be

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improved to reach removal efficiency levels sufficient to prevent these odour annoyances. Consequently, the complete elimination of sulphur reduced compounds has to be assessed as the perception threshold of these compounds is particularly low.

Sulphide oxidizing bacteria (SOB) allowing oxidation of sulfide encompass several genera such as *Thiobacillus, Acidithiobacillus, Achromatium, Beggiatoa, Thriothrix, Thioplaca*, and *Thermotrix*. *Thiobacillus* is one of the most widely studied.[13] According to literature,[14] it has been shown that these bacterial communities involved in the biodegradation of SRC are sensitive towards environmental stress conditions as pH.

This study deals with the potential of a combination of biofiltration and biotrickling filter for the treatment of a mixture of SRCs including DMS, DMDS and H_2S. Hence, the first step of this work was to evaluate the influence of pH on the SRC biodegradation activity under controlled operating conditions. The optimal pH range within which the SRC mixture is completely removed from the gas phase has been determined in microcosms seeded with a planktonic biomass.

In a second step the aim of this work was then to characterize the total bacterial community in terms of diversity and structure, using denaturing gradient gel electrophoresis (DGGE). An increasing number of studies propose statistical investigations of DGGE banding patterns, which undoubtedly leads to refined results. These advanced analyses are based on computer-assisted characterization of the banding patterns and subsequent treatment of the data using a statistic approach. Guidelines for the computer assisted analysis of fingerprinting profiles was proposed by Rademaker[15] using the GelCompar software package (Applied Maths, Kortijk, Belgium). Clustering techniques, such as the unweighted pair group method using arithmetic averages (UGPMA), are applied to DGGE profiling with the aim of identifying those samples which generate similar patterns.[16,17,18] One advantage of this presentation is that coherence of the fingerprinting patterns can be assessed rapidly.

MATERIALS AND METHODS

Inoculum and mineral medium

The inoculum was obtained from a rendering facility (ATEMAX and SOLEVAL Sud Est, Viriat, France) and comprises an aqueous suspension sampled from the retention tank where waste water is collected after primary treatment and deposit of solid sulfur. The composition of the mineral medium used for microbial growth is as follows: KH_2PO_4: 1.36 g L^{-1}; Na_2PO_4: 1.42 g L^{-1}; KNO_3: 0.51 g L^{-1}; $(NH_4)_2SO_4$: 2.38 g L^{-1}; $MgSO_4{}^*7H_2O$: 0.05 g L^{-1}; $CaCl_2{}^*2H_2O$: 15 mg L^{-1}; $Fe(NH_4)_2(SO_4)_2{}^*6H_2O$: 3.53 mg L^{-1}; HCl conc. (38%): 19 mg L^{-1}; H_3BO_3: 2.86 mg L^{-1}; $MnSO_4{}^*4H_2O$: 1.54 mg L^{-1}: $CuSO_4$: 2.5 µg L^{-1}; $ZnCl_2$: 2.1 µg L^{-1}; $CoCl_2{}^*6H_2O$: 4.1 µg L^{-1}; $Na_2MoO_4{}^*2H_2O$: 2.5 µg L^{-1}.

Experimental setup and operation

Polluted gas generation and microcosms

The dimethylsulfide (DMS) and dimethyldisulfide (DMDS) liquid mixture is continuously injected and vaporized into an air stream regulated at 5 L min^{-1} (humidity content 5%), via a syringe-pump (Precidor, Infors AG, Basel, Switzerland). Gaseous hydrogen sulphide is directly injected in the compressed airstream using a flow rate regulator (5850TR, Brooks Instrument, Hatfield, PA, USA) controlled at 0.35 mL.min^{-1} (Fig. 1). The concentration of each compound in the synthetic polluted gas is 100 mg m^{-3}.

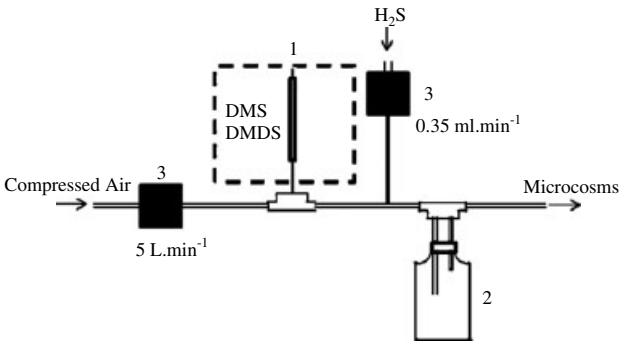

Figure 1. Gaseous effluent generation system: (1) syringe pump; (2) homogeneous flask; (3) flow rate regulator.

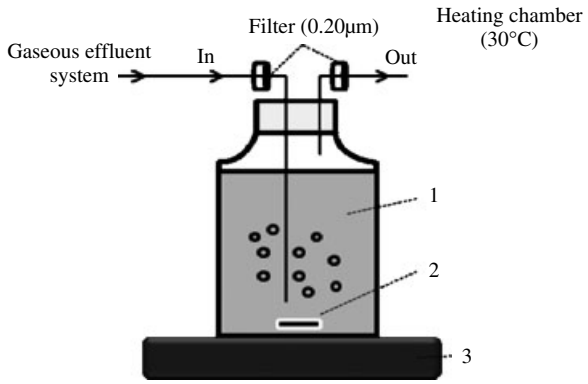

Figure 2. Microcosm culture: (1) mineral medium; (2) magnetic stirring bar; (3) magnetic agitator.

This synthetic gaseous effluent passes continuously through homogeneous flasks. The flow rate of the airstream is 100 mL min^{-1} and the concentration of each compound of the mixture is 100 mg m^{-3} (Fig. 2). Tests were performed in 1 L sterile flasks containing 1 L of mineral salt solution and sealed with a Teflon-coated cap (Interchrom, Montluçon, France). The gaseous effluent inlet and outlet are equipped with a 0.22 µm filter in order to avoid microbial contamination. Each flask except the negative control is seeded with 25 mL of aqueous suspension and 11 mL of solid deposit sulphur suspended in the mineral medium. Incubation was performed at 30 °C at an agitation speed of 150 rpm for 2 months.

Two experiments (C1 and C2) were realized with duplicates (C1.1 and C1.2; C2.1 and C2.2). At the beginning, pH of the mineral medium of each flask was adjusted to neutrality. Then, the pH value of the microcosms was maintained constant at specified levels (7, 5, 3, 1) using 5N NaOH solution. Two controls are also realized, one containing mineral medium and inoculum and fed by compressed air and the other containing just mineral medium and supplied with the polluted gas. The pH of these two controls was maintained at neutrality.

Analytical methods

The biodegradation was monitored by gas phase measurement at inlet and outlet sampling ports. Hydrogen sulphide concentration was measured using a gas chromatograph equipped with a photoionization detector (PID) (HNU Systems Model 311, Newton, MA, USA). The minimum detectable level for hydrogen sulphide is estimated at 1 mg m^{-3}. DMS and DMDS concentrations were

measured using a gas chromatograph equipped with a flame ionization detector (FID) (Trace, Thermo Fisher Scientific Inc., Waltham, MA). A fused silica capillary column was used (Equity-1, Sigma Aldrich, St Louis, MO, USA): length 30 m, internal diameter 0.32 mm, film thickness 0.25 μm and quantification limit 1 mg m^{-3}. The detector and injection temperature are 220 °C and 100 °C, respectively, gas vector flows are 350 mL min^{-1} for air and 35 mL min^{-1} for H_2.

Removal efficiency

The removal efficiency (RE) defined the functional performance in the different cultures. It is based on the SRC concentration (C) inlet and outlet at the different microcosms and was calculated according to:

$$RE = \frac{C_{inlet} - C_{outlet}}{C_{inlet}} \quad (1)$$

DNA isolation and 16S RNA gene amplification

DNA was extracted from the aqueous suspension present in each microcosm using a FastDNA® SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA). Replicates were realized for each DNA extraction. For that, 200 mL of aqueous suspension were taken and centrifuged at 10 000 rpm for 15 min. The amount of cell pellet used for extraction and the elution volume for final DNA recovery were standardized for each microcosm (200–350 mg and 50 μL, respectively). Extracted DNA (5–7 μL) was visualized by gel electrophoresis on 1% agarose gel. The V3 region of the eubacterial 16S rRNA gene was PCR amplified using primers V3F (5′-ACTCCTACGGGAGGCAGCAG-3′) and V3R (5′-ATTACCGCGGCTGCTGGCAC-3′), both slightly modified from Muyzer.[19] A 40-base GC clamp was attached to the 5′ end of the forward primer. Amplification was performed on ∼100 ng DNA template (approx. 1–2 μL) in a final volume of 50 μL containing 0.6 μmol L^{-1} of each primer (Sigma Aldrich, St Louis, MO, USA), 10 mmol L^{-1} of dNTP mix (200 μmol L^{-1} each dNTP, Abcys, Paris, France), 5 μL of 10× ThermoTaq Reaction Buffer (Thermo Fisher Scientific Inc., Waltham, MA, USA), and 0.75 U of Thermo Taq High fidelity DNA Polymerase (Thermo Fisher Scientific Inc., Waltham, MA, USA). PCR was achieved by Tpersonal thermocycler (Biometra, Goettingen, Germany) using the following program: initial denaturation at 98 °C for 4 min, 30 cycles consisting of 30 s denaturation at 98 °C, 30 s annealing at 68 °C and 30 s extension at 72 °C, and final extension at 72 °C for 10 min. Negative controls were included to verify the absence of contamination. PCR products were visualized by electrophoresis on 2% agarose gel.

Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis (DGGE) was performed according to the protocol of Muyzer,[19] using 8% polyacrylamide gels prepared with denaturing gradient ranging from 30% to 60% (where 100% denaturant contained 7 mol L^{-1} urea and 40% (v/v) formamide). 500 ng of PCR samples product were loaded on the gel, along with reference standards for further pattern alignment. Electrophoresis was run at 60 °C and 100 V in 1× TAE buffer, for 16 h. Gels were stained with Sybr green I (Sigma-Aldrich), washed, and photographed with a camera (Vilber, Quantum ST4-1100/26M. X-Press, Eberhardzell, Deutschland). Gel images were analyzed with Gel Compar II (Applied Maths, Saint-Martens-Latem, Belgium).

Sequencing and cloning of DGGE fragments

DNA fragments to be sequenced were excised from the gel, placed into sterile Eppendorf tubes containing 25 μL of sterilized water, and incubated overnight at 4 °C. A 4 μL volume of DNA diffused in water served as template for PCR amplification. The amplified products were subjected to a new DGGE step to confirm their electrophoretic mobility. The PCR product was cloned by using a TOPO TA Cloning kit with plasmid vector pCR4-TOPO (Introvigen, Carlsbad, CA). The sequences obtained were investigated on the RDP-II database.

Statistical analysis

The diversity of the total bacterial community is evaluated by calculating the Shannon index (I), which took into account both the number of bands (obtained by DGGE) and their relative intensity according to Equation (2) where p_i is the relative abundance of the *i*th band of the profile:

$$I = -\sum_{i}^{n} p_i \log(p)_i \quad (2)$$

The uhted pair group method using arithmetic averages (UGPMA), are applied to the DGGE profiling to identify those samples which generate similar patterns. In effect, the initial data matrix (relative intensities according to position) was reduced and transformed in order to downweight the influence of more abundant species. The pair-wise similarity index $S_{i,j}$ between a profile *i* and *j* was calculated by Bray–Curtis coefficient, on the basis of both band positions and their relative abundance:

$$S_{i,j} = 1 - \sum_{k=1}^{s} \frac{|p_{i,k} - p_{j,k}|}{p_{i,k} + p_{j,k}} \quad (3)$$

where $p_{i,k}$ is the relative abundance of the *k*th band in the profile *i*, and $p_{j,k}$ is the relative abundance of the *k*th band in the profile *j*.

RESULTS AND DISCUSSION

SRC removal efficiency

The synthesis of all results of this study is presented on Fig. 3(a), (b) and (c).

The removal efficiency of H_2S (Fig. 3(a)) is very high (approximately 90–95%) from the fourth day of functioning. After the 10 days of functioning, H_2S is completely removed, whatever the pH of the microbial suspension.

The efficiency of removal of DMS and DMDS (Fig. 3(b), (c)) follows a similar temporal evolution. Nevertheless, two groups can be distinguished: pH 7 and 5 microcosms on the one hand and pH 3 and 1 microcosms on the other. After 10 days of functioning, the efficiency of removal of these compounds increases, whatever the microcosms considered (pH 7, pH 5, pH 3 and pH 1), reaching removal efficiency values close to 60% for DMS and 40% for DMDS. For pH 1 and 3 microcosms, a decrease in the efficiency of removal of these compounds is then exhibited. The abatement values remain stable around 10 and 15% for DMS and DMDS, respectively. In return, for pH 7 and 5 microcosms, complete elimination of both compounds is obtained after 20 days of functioning.

These results can be explained by several phenomena. First, for pH 1 and pH 3 microcosms, it is probable that there is competition between substrates; the biomass would first metabolize the most

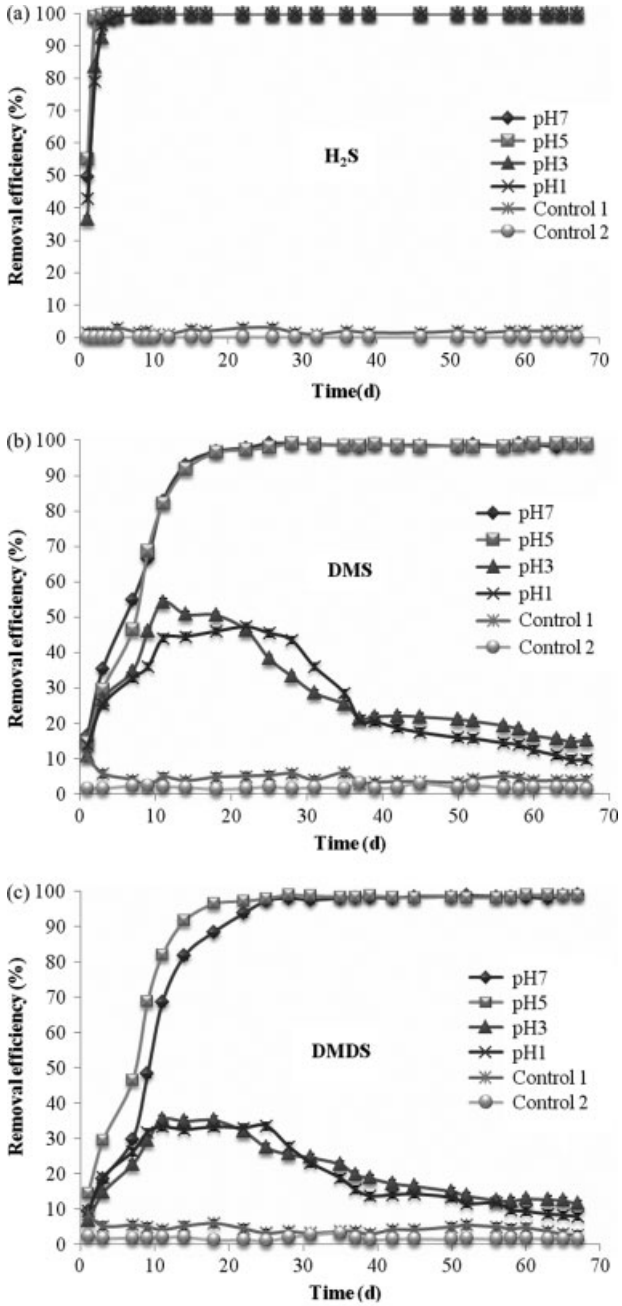

Figure 3. SRC removal efficiency versus time for different tested pH: (a) H_2S; (b) DMS; (c) DMDS.

available or biodegradable compounds. Second, competition between species can be assumed as SRC oxidizing bacteria are diverse and hence different specialized groups can grow. Furthermore, this result could be explained by the accumulation of sulfuric acid, which is a by-product of the aerobic oxidation of sulfide by SOB. It seems that sulfuric acid induces a decrease in DMS and DMDS removal efficiency by inhibiting the growth of the bacteria concerned.[20,21,22] For pure DMDS-degrading strains isolated from various environments, it has been shown that optimum growth was observed for pH values between 6 and 8.[23,24] Moreover, the outcome of competition for common substrate in the mixed cultures seems to be dictated mainly by pH value of the microbial suspension. What is the impact of this

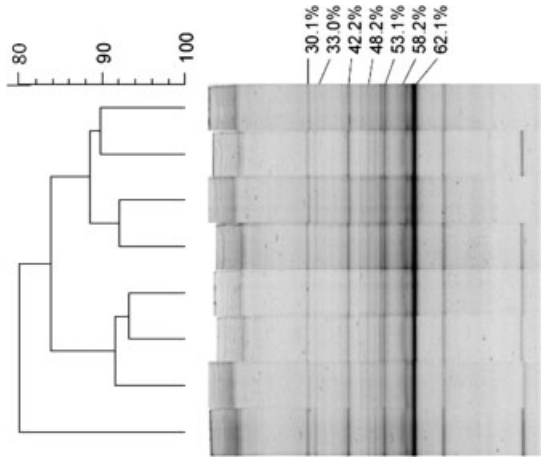

Figure 4. Test of reproducibility based on Bray–Curtis index.

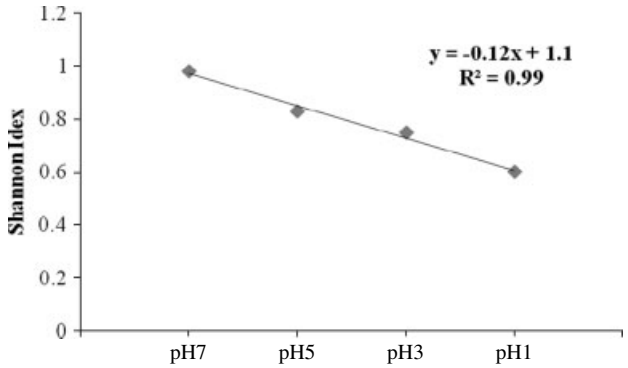

Figure 5. Influence of diversity by calculating the Shannon index for different tested pH.

operating parameter (pH) on total bacterial community diversity and structure?

Bacterial community analysis

Influence of pH on total bacterial community

The reproducibility of the whole experiment from sampling until DGGE is represented on Fig. 4. The similarity index within the eight-replicate pool was 88%. This reproducibility was the same range as previous studies where duplicate profiles were characterized by high similarity (≥85% according to the Jaccard coefficient[25]).

The diversity of the total bacterial community is evaluated by calculating the Shannon index, which took into account both the number of bands (obtained by DGGE) and their relative intensity (Fig. 5).

As indicated on Fig. 5, a diminution of the diversity of the total bacterial community is observed when the pH of the aqueous solution becomes acid (1 ± 0.09 to 0.6 ± 0.02). This diminution of diversity has been linked with the removal efficiency of SRC. At steady state, for pH 3 and pH 1 microcosms, the abatement value of DMS and DMDS is low (around 20%). It can be assumed that the acidification of the aqueous phase inhibited the growth of specific populations having the ability to degrade DMS and DMDS. In return, around neutrality (5–7), higher bacterial community diversity seems to induce an enhanced activity biodegradation level as SRC are completely removed. These first results indicate that the bacterial community involved

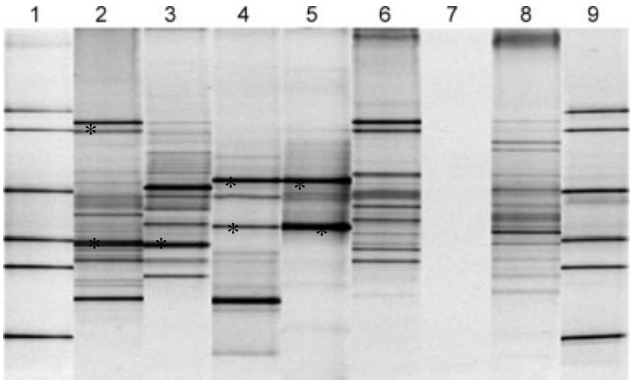

Figure 6. Total bacterial community structure for each microcosms: (1, 9) marker; (2) pH 7; (3) pH 5; (4) pH 3; (5) pH 1; (6) control 1; (7) control 2; (8) Inoculum.

Table 1. Similarity percentages of excised DGGE bands as compared with the RDP database

Microcosms	Microorganisms	Similarity(%)	Class
pH 7	*Thioalkalibacter*	43	Gammaproteobacteria
pH 5	*Dyella*	73	Gammaproteobacteria
pH 3	*Acidithiobacillus*	99	Gammaproteobacteria
pH 1	*Acidithiobacillus*	98	Gammaproteobacteria

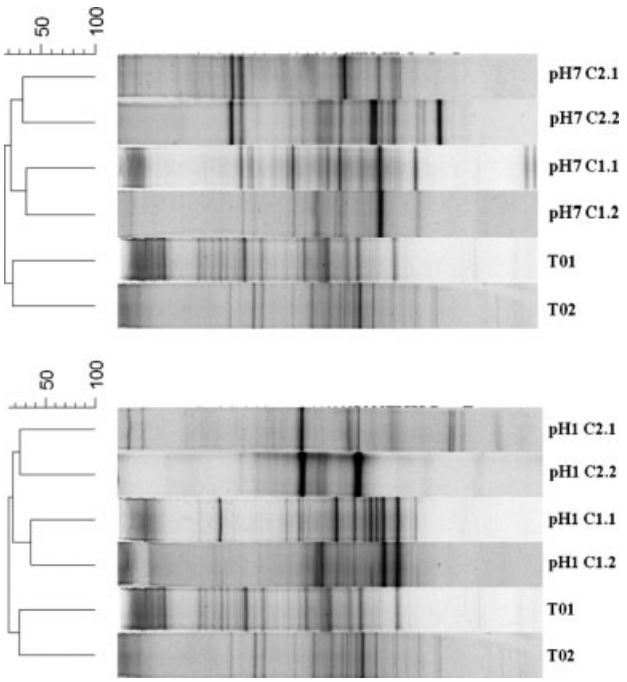

Figure 7. UPGMA clustering of DGGE patterns based on Bray–Curtis similarities for pH 7 and pH 1 microcosms and for both tests (C1 and C2).

in the biodegradation of these compounds is sensitive towards environmental conditions.

Figure 6 presents the DGGE profiles of the total bacterial community for each tested pH at steady-state (day 67) for C2.2. After 67 days of functioning, the total bacterial community structure differs from the *inoculum* community whatever the tested pH. This result could be due to the adaptation of bacterial communities to the environmental conditions. According to pH, the bacterial community structure differs. As indicated in DGGE profiles, the bacterial community of pH 1 microcosm reveals two main bands, while the DGGE profiles of pH 7 microcosm exhibits various prevailing populations. A similar evolution is observed for C1 and C2.1 (data not shown). Hence, the selective pressure imposed in the microcosms (contaminant concentration and pH) is sufficient to influence strongly the community diversity and composition. These results have to be confirmed by statistical analysis. It has been reported[26] that despite similar reactor performance with respect to chemical parameters (pH and temperature), the underlying community structures are different.

The analysis of DGGE profiles of pH 5 and pH 7 microcosms suggested that a stable function could be maintained, notwithstanding different bacterial community structure. It can be suggested that some populations seem to be favored with specific environmental conditions as some main bands are only visualized in pH 5 microcosms DGGE profile for example. For acidic environments, the low DMS and DMDS removal efficiency levels could be explained by the inhibition of specific bacterial populations. As indicated on Fig. 6, some dominant bands for pH 7 and pH 5 microcosms are not detected by the analytical technique.

The major bands from the DNA-based DGGE pattern of pH-microcosms were excised (Fig. 6) and sequenced (Table 1).

The identified bacteria belonged to the same bacterial class: Gammaproteobacteria. Similarities less than 80% to classified bacterial species were found except for pH 7 and pH 5 microcosms. Moreover, different studies have shown that these microorganisms are involved in the biodegradation of SRC and H_2S.

It has been reported that alkaliphilic sulfur oxidizing bacteria (ASB) can oxidize reduced sulfur compounds such as sulfides and *Thioalkalibacter* are used in H_2S removal studies.[27] These chemolithoautotrophic microorganisms can also grow in saline environments.

Microorganisms like *Dyella, Burkholderia, Alcaligenes*, are able to grow chemolithotrophically with a medium containing dimethylsulfide and dimethyldisulfide and exhibit growth coupled with their oxidation. It has been reported that optimal pH range is between 5 and 7.

In an acid environment, the chemolitrophic biooxidation of sulphide (H_2S) has been widely investigated using a number of organisms including *thiobacillus* and acidithiobacillus.[14,28]

Influence of the inoculum

Figure 7 shows UPGMA clustering of DGGE patterns based on Bray–Curtis similarities for pH 7 and pH 1 microcosms and for both experiments.

As illustrated by clustering (Fig. 7), three distinct groups are separated on the basis of species composition and relative abundances: sludge *inocula*, pH 7 or pH 1 microcosms seeded with inoculum1 (T01), pH 7 or pH 1 microcosms seeded with inoculum2 (T02). The sludge inocula constitute the most distinct group, with only 16 and 13% similarity with pH 7 or pH 1 microcosms, respectively. Moreover, even in the presence of contaminants, the inoculum community (T0) seems to make a significant contribution to the 67-day community in pH 7 or pH 1 microcosms. After 67 days, the pH 7 microcosm community does not result in the same structure as the pH 7 C1 microcosm community, and is only 22% similar to the pH 7 C2 microcosm community. The same trend is observed for the communities of the other microcosms (pH 3 and

5 microcosms). Nevertheless, for a given pH microcosm, the SRC removal efficiency is equivalent, whatever the chosen *inoculum*. These results indicate that distinct communities from different inocula can achieve high and stable functionality.

The long-term impact of inoculation has been discussed. In bioreactors under constant operating conditions, achieving the same functionality but differently seeded, distinct communities can develop at steady-state (in terms of density, diversity and structure), indicating the emergence of different fitted species due to the *inoculum* origin.[14,29] These interesting data must be cautiously interpreted as the extent of microbial dynamics has not been surveyed during the steady-state period. To conclude, it seems that inoculation brings enough diversity to allow the emergence of adapted species.

CONCLUSIONS

The present study assesses the influence of pH of the environment (aqueous phase) on SRC biodegradation and community structure in bioreactors seeded with planktonic biomass and under constant operating conditions.

The results obtained show that the pH value of the aqueous phase has a great impact on the abatement of SRC. Complete elimination of SRC is observed when pH level decreases from 7 to 5. In return, for acidic pH (3 and 1), the efficiency of removal of DMS and DMDS is low at around 20%. The abatement of hydrogen sulfide is complete, whatever the pH level of the considered microcosm.

Moreover, the selective pressure imposed in the microcosms (contaminant concentration and pH) is sufficient to influence strongly the community diversity and composition. The inoculum community (T0) makes a significant contribution to the 67-day community in the pH microcosms investigated. These results indicate that distinct communities from different inocula can achieve high and stable functionality. Sulphur oxidizing bacteria (SOB) represent a wide-ranging and highly diversified group of microorganisms such as *Thiobacillus*,[30] *Hyphomicrobium*,[14] *Pseudomonas*,[31] and *Arthrobacter*.[32] Competition between species can be assumed as SRC oxidizing bacteria are diverse and hence different specialized groups can emerge. At the different pH levels it will be of interest to identify, from DGGE profiles, those emergent populations that contribute to SRC biodegradation.

These results provide interesting data in order to improve the abatement of SRC by using a biotrickling filter/biofilter combination. It is worth noticing that the adjustment of pH level of the aqueous phase is easier to control in a biotrickling filter than in biofilters. Moreover, in these bioprocesses, microorganisms are grown on an organic or inorganic support and form a biofilm. Hence, as the growth conditions change, the impact of pH level on fixed microbial communities can be slightly different, as has been observed in microcosms seeded with planktonic biomass.

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REFERENCES

1 Schlegelmilch M, Stresse J, Biedermann W, Herold T and Stegmann R, Odour control at biowaste composting facilities. *Waste Manage* **25**:917–927 (2005).
2 Héroux M, Pagé T, Gélinas C and Guy C, Evaluating odour impacts from a landfilling and composting site: involving citizens in the monitoring. *Water Sci Technol* **50**:131–137 (2004).
3 Langenhove HV, Roelstraete K, Schamp N and Houtmeyers J, GC-MS identification of odorous volatiles in wastewater. *Water Res* **19**:597–603 (1985).
4 Kim KY, Ko HJ, Kim HT, Kim YS, Roh YM, Lee CM, *et al*, Sulfuric odorous compounds emitted from pig-feedings operations. *Atmos Environ* **41**:4811–4818 (2007).
5 Panetta DM, Powers WJ and Lorimor JC, Management strategy impacts on ammonia volatilization from swine manure. *J Environ Qual* **34**:1119–1130 (2005).
6 Both R, Directive on Odour in Ambient Air: an established system of odour measurement and odour regulation in Germany. *Water Sci Technol* **44**:119–126 (2001).
7 Mahin TD, Comparison of different approaches used regulates odours around the world. *Water Sci Technol* **44**:87–102 (2001).
8 Chung YC, Cheng CY, Chen TY, Hsu JS and Kui CC, structure of the bacterial community in a biofilter during dimethyl sulfide (DMS) removal process. *Bioresource Technol* **100**:7176–7179 (2010).
9 Kennes C, Rene ER and Veiga MC, Bioprocess for air pollution control. *J Chem Technol Biotechnol* **84**:1419–1436 (2009).
10 Rehman ZU, Farooqi IH and Ayub S, Perfomance of biofilter for the removal of hydrogen sulphide odour. *Int J Environ Res Public Health* **3**:537–544 (2009).
11 Arellano-Garcia L, Revah S, Ramirez M, Gomez JM and Cantero D, Dimethyl sulphide degradation using immobilized *Thiobacillus thioparus* in a biotrickling filter. *Environ Technol* **30**:1273–1279 (2010).
12 Chung YC, Lin YY and Tseng CP, Removal of high concentration of NH_3 and coexistent H_2S by biological activated carbon (BAC) biotrickling filter. *Bioresource Technol* **96**:1812–1820 (2005).
13 Tang K, Baskaran V and Nemati M, Bacteria of the sulphur cycle: an overview of microbiology, biokinetics and their role in petroleum and mining industries. *Biochem Eng J* **44**:73–94 (2008).
14 Sercu B, Nunez D, Aroca G, Boon N, Verstraete W and Van Langenhove H, Inoculation and start-up of a biotrickling filter removing dimethyl sulfide. *Chem Eng J* **113**:127–134 (2005).
15 Rademaker JLW, Louws FJ, Rossbach U, Vinuesa P and De Bruijn F, Computer-assisted analysis of molecular fingerprints and database construction. *Molecular Microbial Ecology Manual*, Dordrecht, Kluwer Academic Publisher, chapter 7.1.3. (1999).
16 Boon N, De Windt W, Verstraete W and Top EM, Microbial community dynamics during production of the mexican fermented maize dough pozol. *Appl Microbiol Biotechnol* **66**:3664–3673 (2002).
17 Ibewke AM, Papiernik SK, Gan J, Yates SR, Yang CH and Crowley DE, Impact of fumigants on soil microbial communities. *Appl Environ Microbiol* **67**:3245–3257 (2001).
18 Yang CH, Crowley DE and Menge JA, 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and Phytopthora infected avocado roots. *FEMS Microbiol Ecol* **35**:129–136 (2001).
19 Muyzer G De, Waal HC and Uitierlinden AG, Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**:695–700 (1993).
20 Adib F, Bagreev A and Bandosz TJ, Effect of pH and surface chemistry on the mechanism of H_2S removal by activated carbons. *J Colloid Interface Sci* **216**:360–369 (1999).
21 Lee EY, Lee NY, Cho KS and Ryu HW, Removal of hydrogen sulfide by sulfate-resistant *Acidithiobacillus thiooxidans AZ11*. *J Biosci Bioeng* **101**:309–314 (2005).
22 Shinabe K, Oketani S, Ochi T and Matsumura M, Characteristics of hydrogen sulfide removal by *Thiobacillus thiooxidans KS1* isolated from a carrier-packed biological deodorization system. *J Ferment Bioeng* **80**:592–598 (1995).
23 Ito T, Miyaji T, Nekagawa T and Tomizuka N, Degradation of dimethyl sulfide by *Pseudomonas fluorescens* strain 76. *Biosci Biotechnol Biochem* **71**:366–370 (2007).
24 Smith NA and Kelly DP, Mechanism of oxidation of DMDS by *Thiobacillus thioparus strain 56*. *J Gen Microbiol* **134**:3031–3039 (1998).
25 Cabrol L, Malhautier L, Poly F, Lepeuple AS and Fanlo JL, Assessing the bias linked to DNA recovery from biofiltration woodchips for microbial community investigation by fingerprinting. *Appl Microbiol Biotechnol* **85**:779–790 (2010).

26 Egli K, Langer C, Siegrist HR, Zehnder AJB, Wagner M and Van der Meer JR, Community analysis of ammonia and nitrite oxidizers during a start-up of nitrification reactors. *Appl Environ Microbiol* **69**:3213–3222 (2003).
27 Olguín-Lora P, Le Borgne S, Castorena-Cortés G, Roldán-Carrillo T, Zapata-Peñasco I, Reyes-Avila J, *et al*, Evaluation of haloalkaliphilic sulfur-oxidizing microorganisms with potential application in the effluent treatment of the petroleum industry. *Biodegradation* **22**:83–90 (2010).
28 Rappert S and Müller R, Odor compounds in waste gas emissions from agricultural operations and food industries. *Waste Manage* **25**:887–907 (2005).
29 Khammar N, Malhautier L, Degrange V, Lensi R and Fanlo JL, Link between spatial structure of bacterial communities and degradation of a complex mixture of volatile organic compounds in peat biofilters. *J Appl Microbiol* **98**:476–490 (2004).
30 Malhautier L, Gracian C and Roux JC, Biological treatment process of air loaded with ammonia and hydrogen sulphide mixture. *Chemopshere* **50**:145–153 (2003).
31 Chung YC, Huang C and Tseng CP, Biological elimination of H_2S and NH_3 from waste gases by biofilter packed with immobilized heterotrophic bacteria. *Chemosphere* **43**:1043–1050 (2001).
32 Lee EY, Cho KS and Ryu HW, Simultaneous removal of H_2S and NH_3 in biofilter inoculated with *Acidithiobacillus thiooxidans TAS*. *J Biosci Bioeng* **99**:611–615 (2005).