Appl Microbiol Biotechnol (2006) 72: 1090–1098
DOI 10.1007/s00253-006-0382-x

ENVIRONMENTAL BIOTECHNOLOGY

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H_2S degradation is reflected by both the activity and composition of the microbial community in a compost biofilter

Received: 25 January 2006 / Revised: 15 February 2006 / Accepted: 15 February 2006 / Published online: 31 March 2006

Abstract In this study, 16S rRNA- and rDNA-based denaturing gradient gel electrophoresis (DGGE) were used to study the temporal and spatial evolution of the microbial communities in a compost biofilter removing H_2S and in a control biofilter without H_2S loading. During the first 81 days of the experiment, the H_2S removal efficiencies always exceeded 93% at loading rates between 4.1 and 30 g m^{-3} h^{-1}. Afterwards, the H_2S removal efficiency decreased to values between 44 and 71%. RNA-based DGGE analysis showed that H_2S loading to the biofilter increased the stability of the active microbial community but decreased the activity-based diversity and evenness. The most intense band in both the RNA- and DNA-based DGGE patterns of the H_2S-degrading biofilter represented the sulfur oxidizing bacterium *Thiobacillusthioparus*. This suggested that *T. thioparus* constituted a major part of the bacterial community and was an important primary degrader in the H_2S-degrading biofilter. The decreasing H_2S removal efficiencies near the end of the experiment were not accompanied by a substantial change of the DGGE patterns. Therefore, the decreased H_2S removal was probably not caused by a failing microbiology but rather by a decrease of the mass transfer of substrates after agglutination of the compost particles.

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Introduction

Hydrogen sulfide and volatile organic sulfur compounds are emitted from wastewater treatment plants and composting facilities, rendering plants and food/feed production plants during heating and/or anaerobic decay of sulfur-containing amino acids (Smet and Van Langenhove 1998). Also, Kraft pulp mills are known to emit high concentrations of H_2S (Bordado and Gomes 2002). Biofilters are often used to treat H_2S-containing odorous emissions (Easter et al. 2005). Compost is frequently used as filter material in biofilters because it contains a high number and diversity of microorganisms, has a good water-holding capacity and air permeability, provides pH buffering, and contains a large amount of nutrients (Delhomenie and Heitz 2005).

In general, pilot- or full-scale biofilters show odor-removal efficiencies exceeding 90% (Easter et al. 2005; Goodwin et al. 2000; Luo 2001). However, lower removal efficiencies can occur depending on, e.g., medium age, pollutant loading rates, and filter bed moisture content. In practice it can be difficult to define the major causes of low odor-removal efficiencies and, thus, to propose a straightforward solution for the problem. It has been reported that laboratory- or pilot-scale investigations can overlook problems occurring in full-scale installations such as long-term accumulation of intermediates (Van Langenhove et al. 1989) or inhibition effects of other waste gas compounds (Liu et al. 2005; Smet et al. 1997). Next to physical–chemical parameters, microbiological parameters could have an added value when used as indicators for the functioning or stability of a biofilter. Microbial indicators based on community composition analysis could include microbial diversity, microbial community dynamics, or the occurrence of specific species or groups of microorganisms. Several H_2S-degrading bacteria have been isolated and quantified in biofilters. Degorce-Dumas et al. (1997) identified *Thiobacillus thioparus*, *Thiobacillus denitrificans*, and *Thiobacillus novellus* as the most numerous neutrophilic thiobacilli and *Acidithiobacillus thiooxidans* as an acidophilic thiobacillus in a H_2S-degrading biofilter.

In addition, the number of all thiobacilli increased during H_2S biofiltration except when acidification occurred (pH<5.5). In the latter case, the number of neutrophilic autotrophs decreased. Also, Cook et al. (1999) isolated mainly acidophilic chemolitotrophic microorganisms in biofilters at low pH, while at neutral pH values, more neutrophilic and heterotrophic microorganisms occurred. Although these studies indicated the importance of thiobacilli during H_2S degradation, they cannot provide information about the activity of these bacteria compared with the total bacterial community. It is known that culture-dependent studies can impose a bias toward microorganisms that are able to grow on the selected media (Wagner et al. 1993; Webster et al. 1997). Therefore, molecular techniques can provide a more representative picture of the general composition of the microbial community.

In this study, an H_2S-removing compost biofilter was set-up that was operated during 109 days. Based on the applied loading rates, the total biofiltration period was divided in four periods, with average loading rates between 5.6 and 25.3 g m^{-3} h^{-1}. The microbial composition of both the most active and abundant bacteria in the biofilter was studied with 16S rRNA- and rDNA-based denaturing gradient gel electrophoresis (DGGE) analysis, respectively, and compared with a control biofilter not removing any H_2S. The Shannon diversity, the evenness, and moving windows analysis (based on Pearson correlation) were used to study the stability of the active microbial populations and the relation between the active microbial populations and H_2S removal. Dominant ribotypes were sequenced to identify possible key players in the H_2S removal process.

Materials and methods

Reactor set-up

Biofilter reactors consisted of three detachable parts made of Plexiglas with an internal diameter of 0.195 m and an overall height of 1 m (Fig. 1). The sections are indicated as 1 (inlet), 2 (middle), and 3 (outlet). Each biofilter section was filled with 20 cm of compost material mixed with dolomite (<2 mm) in a compost/dolomite ratio of 1.1 (w/w) (total filter bed volume, 20 l), supported by a perforated Plexiglas plate. The compost used was produced from source-separated municipal organic waste (the garden, fruit, and vegetable fraction) by the so-called double process, i.e., anaerobic digestion followed by aerobic treatment (Gellens et al. 1995). Gas sampling points were provided at the influent (port A) and effluent (port D), between Sections 1 and 2 (port B) and between Sections 2 and 3 (port C). Air was supplied by a diaphragm pump (KNF Neuberger, Freiburg, Germany) and was humidified in a scrubber (>98% relative humidity) before entering the biofilters in down flow mode. In one biofilter hydrogen sulfide was dosed in the humidified air stream with a rotameter (Gilmont Instruments, Barrington, IL) from a gas bottle (99% H_2S, Praxair). The other biofilter acted as a control reactor, and no pollutant was added to the humidified air stream. The air flow rate entering each biofilter was 33 l min^{-1}, corresponding to an empty bed residence time of 36 s. Compost samples for microbiological and physical/chemical analyses were taken by opening the biofilters during short interruptions of the air flow (maximum 30 min). During the first 82 days, samples were taken from the upper 10 cm of each biofilter section. Afterwards, the compost in the biofilter sections was mixed before sampling. The mineral medium that was added on day 93 consisted of 3 g l^{-1} K_2HPO_4, 3 g l^{-1} KH_2PO_4, 3 g l^{-1} NH_4Cl, 0.5 g l^{-1} $MgSO_4 \cdot 7H_2O$, and 0.01 g l^{-1} $FeSO_4 \cdot 7\ H_2O$ (pH 7).

Physical–chemical analyses

Hydrogen sulfide concentrations from 0 to 100 ppmv were measured with a Lifeline II Extractive Gas Detector (Zellweger Analytics, Zaventem, Belgium), having a detection limit of 1 ppmv. Concentrations exceeding 50 ppmv were measured with a Varian 3700 gas chromatograph equipped with a flame photometric detector. A 30-m DB-5 column (J&W Scientific, Folsom, CA) was used with an internal diameter of 0.53 mm and a film thickness of 1.5 μm. Using a Pressure-Lok precision analytical syringe (Alltech Ass., Deerfield, IL) 1-ml gas samples were injected. An external standard was prepared by diluting the pure gas.

Analysis of the moisture content and pH was performed as previously described by Sercu et al. (2005a). The pressure drop over the biofilter bed was measured with a Testo 452 electronic pressure probe (Testo, Ternat, Belgium). Total sulfur and extractable sulfate were

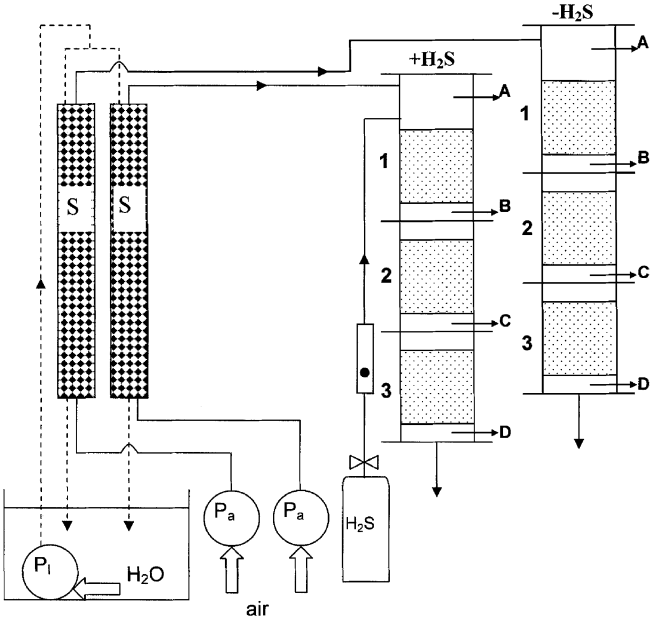

Fig. 1 Experimental set-up of the H_2S and control biofilter. The positions of the gas sampling points (**A**–**D**) and the biofilter Sections (1, 2, 3) are shown; P_l indicates liquid pump; P_a, air pump; *S*, humidification column

determined according to the method of Tack et al. (1997), using 1 g of wet compost per analysis. Sulfate amounts in the final solutions were determined with an IC 761 compact ion chromatograph (Metrohm, Herisau, Switzerland) with a metrosep A supp 5 column and a metrosep A 4/5 guard column.

Microbiological analysis

Prior to nucleic acids extraction, a mixed sample of 4×0.5 g compost was taken from the biofilter sections. Nucleic acids were extracted using the slightly modified protocol of Boon et al. (2003) to allow the larger sample size. For this purpose, all reagents were added in four times the required quantities. The crude DNA extract was purified using the Wizard DNA clean-up system (Promega, Madison, WI), while the crude RNA was purified using the RNeasy kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's instructions.

DNA and RNA were amplified using the primers P338F (with GC clamp) and P518r (Ovreas et al. 1997). Due to the short amplicon, the selected primer pair does not result in the most robust phylogenetic information. However, it has been reported that this primer combination allowed superior resolution on a DGGE gel in addition to the higher sensitivity when compared to other "universal" polymerase chain reaction (PCR)-DGGE primers, e.g., 968F-GC/1401r (Pruden et al. 2001; Chang et al. 2000). Shorter target sequences are also less likely to result in the formation of undesirable chimeras (Wang and Wang 1996). PCR was performed as described by Boon et al. (2002). Reverse-transcriptase (RT)-PCR was performed using the OneStep RT-PCR kit (Qiagen) according to manufacturer's instructions. The final concentrations of the different compounds in the RT-PCR mastermix were 0.6 μM of each primer, 400 μM of each deoxynucleoside triphosphate (dNTP), 1× Qiagen OneStep RT-PCR buffer, 1/25 μl of Qiagen OneStep RT-PCR enzyme mix and 1/25 μl diluted template in DNase- and RNase-free filter-sterilized water (Sigma). The RT-PCR temperature program was the following: 30 min at 50°C, 15 min at 95°C followed by 30 cycles of 1 min at 94°C, 1 min at 53°C, and 2 min at 72°C. At the end a final elongation step for 12 min at 72°C was added. To find the optimal dilutions of the nucleic acid extracts for optimal amplification, the dilutions 0, 1:5, 1:20, and 1:100 were tested for PCR, and 0, 1:2, 1:5, 1:10, and 1:100 for RT-PCR. Based on the signal intensity on the agarose gels after amplification, the DNA and RNA extracts were diluted 1:5 and 1:10, respectively, prior to amplification.

DGGE was performed using the Bio-Rad D gene system (Bio-Rad, Hercules, CA) based on the protocol of Muyzer et al. (1993) as described previously by Boon et al. (2002). A denaturing gradient ranging from 45 to 55% was used. The 16S rRNA-based DGGE patterns reflect the activity of the community members and are potentially more related with the H_2S degradation activity of the biofilter compared with the 16S rDNA-based DGGE patterns. Therefore, next to the visual observations, additional analyses were performed on the former gel patterns. The gels were analyzed using the Bionumerics Software 2.0 (Applied Maths, Kortrijk, Belgium). The Shannon Diversity Index (Shannon and Weaver 1963) was calculated based on the DGGE band intensities (Sercu et al. 2005b). The evenness was calculated by dividing the diversity index by log S, with S being the number of bands. Both indices, when based on RNA analysis, should not be regarded as structural diversity parameters sensu stricto related to the abundance of the bacteria. Instead, they represent the diversity (A_{Sh}) and evenness (A_E) of the activity of bacteria. The stability of the microbial communities in time was determined using moving windows analysis (Wittebolle et al. 2005) and expressed by the Pearson correlation coefficients ($R_{i,i+1}$) between two banding patterns from consecutive sampling dates (i and i+1). Gel patterns were also compared by calculating the Pearson correlation coefficient (R) between different biofilter sections or reactors. Correlation coefficients were always determined between samples on a same gel to avoid negatively biased correlations due to between-gel variations.

For sequence analysis purposes, the desired DGGE band fragments were cut-out and cloned with the pCR 2.1-TOPO cloning kit (Invitrogen, Carlsbad, CA) according to the manual instructions. DNA sequencing was carried out by IIT Biotech-Bioservice (Bielefeld, Germany). DNA sequence analysis was performed using the Sequence Match software and the Ribosomal Database Project database (Cole et al. 2005).

The GenBank accession numbers of the sequences obtained from the DGGE bands were DQ398578–DQ398584.

Results

Biofiltration of H_2S

The experiment was divided into four periods according to the influent loading rate: period I (days 1–46, 5.6±0.8 g m^{-3} h^{-1}), period II (days 47–64, 12.5±2.3 g m^{-3} h^{-1}), period III (days 65–81, 25.3±3.3 g m^{-3} h^{-1}), and period IV (days 82–108, 13.5±3.3 g m^{-3} h^{-1}; the period without pollutant loading is not included in the mean value) (see Fig. 2). During periods I and II, H_2S was generally removed efficiently, with effluent concentrations below the detection limit (1 ppmv). After increasing the influent concentration in period III, H_2S was always detected at the biofilter outlet, and the removal efficiency decreased to 97±2%. For the total biofilter a maximal elimination capacity (EC) of 28.5 g m^{-3} h^{-1} was obtained with 95% removal efficiency. Between days 1 and 81, the H_2S removal efficiencies were always higher than 93%. Between days 82 and 98, however, values below 71% were measured. Due to the increased compost moisture content, the operation of the biofilter was interrupted between days 99 and 102 to dry the compost. Thereafter, slightly increased removal efficiencies were obtained, although still lower than during periods I–III.

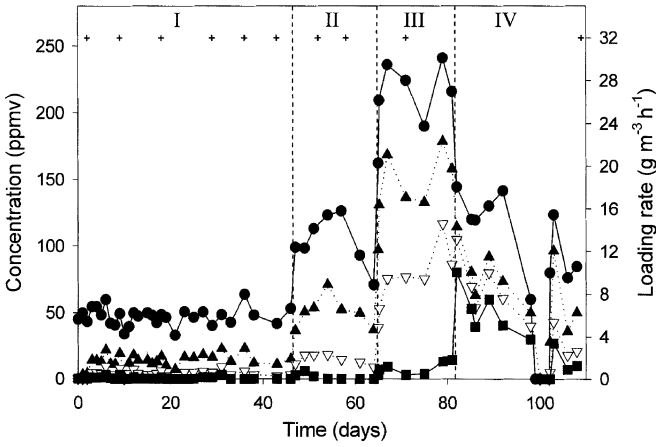

Fig. 2 Evolution of the H_2S concentrations (*left axis*) in time at the gas sampling points A (●), B (▲), C (▽), and D (■). The equivalent volumetric loading scale is shown in the *right axis*. Sampling dates for analyzing the microbiological community composition of the compost are indicated with "+." Periods I–IV are separated by *dashed lines*

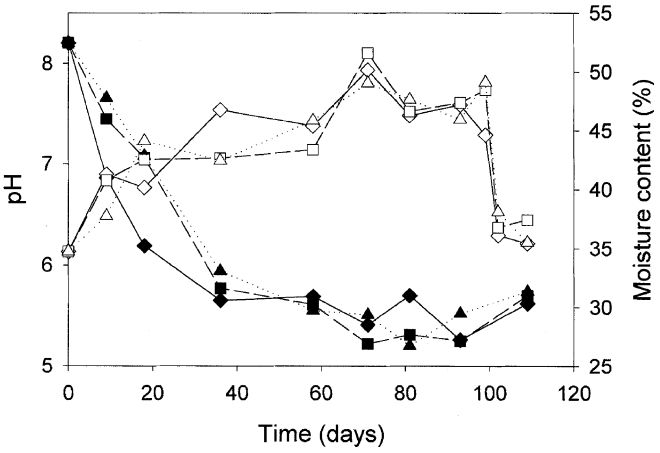

Fig. 3 Compost pH (*black symbols*) and moisture content (*white symbols*) for Sections 1 (◆, ◊), 2 (■, □), and 3 (▲, △) of the H_2S-degrading biofilter

Evolution of the compost parameters

From day 35, the pH of the compost in the three sections of the H_2S-degrading biofilter remained almost constant after an initial change (5.5±0.2 for all sections of the biofilter, see Fig. 3). A slightly faster pH decrease was observed in the inlet side of the biofilter. The moisture content of the compost/dolomite mixture reached a mean value of 44±2% between days 18 and 58, and 48±2% between days 71 and 99. Between days 99 and 102 the filter material was dried by bypassing the scrubbers, resulting in a moisture content of 37±1% (days 102–109). The extractable sulfate and total sulfur concentrations gradually increased in the three biofilter sections (Table 1). Only from day 82 were the biofilter sections mixed before sampling, leading to a redistribution of the accumulated species. The total theoretical sulfur content in the biofilter material, calculated based on the amount of H_2S that was oxidized in each section, agreed well with the experimental results. The experimental values [minus the average background of 2.2 mg S $(g\ DW)^{-1}$ in the control biofilter] showed deviations of −30, −13, and +0.5% from the calculated values on day 109 for Sections 1, 2, 3, respectively.

In the control biofilter, the moisture content increased slightly from 47% on day 0 to 53±3.9% from day 9 in all three biofilter sections. The pH decreased slightly from 8.2 on day 0 to 7.5±0.3 from day 9. The total sulfur and sulfate contents of the compost material in the control biofilter remained low [2.2±0.6 mg S $(g\ DW)^{-1}$ and 0.4±0.3 mg $SO_4^{2-}S$ $(g\ DW)^{-1}$].

Table 1 Concentrations of total sulfur and extractable sulfate in different sections of the control and H_2S-degrading biofilters [mg S $(g\ DW)^{-1}$]

Day	36	58	81	99	109	109 (th)
Sulfate						
Control						
Section 1	0.3	0.7	0.2	ND	ND	
H_2S						
Section 1	7.7	11.1	8.3	11.9	15.7	
Section 2	4.1	10.1	10.4	13.9	17.5	
Section 3	4.2	8.9	11.2	10.5	15.5	
Total S						
Control						
Section 1	2.5	1.6	2.6	ND	ND	ND
H_2S						
Section 1	23.2	31.6	23.2	21.3	35.2	47.1
Section 2	11.0	13.6	15.2	19.3	25.7	26.9
Section 3	6.9	10.1	10.6	15.0	23.3	21.0

Theoretical calculated total sulfur concentrations on day 109 are indicated in column 109 (th)
ND, Not determined

Microbiological analyses

DGGE analysis of the 16S rRNA showed gradual changes in the active microbial communities during H_2S loading (Fig. 4b). Considering the first 81 days of biofiltration, when the H_2S was most efficiently removed, 7 from a total of 24 initial ribotypes remained active throughout this period of H_2S biofiltration. On day 81, 7 new ribotypes were active (out of 24) that were not or hardly active on day 9 of biofiltration. Hence, a significant change in activity of the bacteria occurred during H_2S biofiltration. In Fig. 4a it is shown for selected sampling dates that the DNA-based DGGE patterns for Section 1 were very similar to the RNA-based patterns. As indicated by the numbered arrows, the most intense bands were common for both DGGE patterns. The Pearson correlation coefficient (RNA-based) between days 9 and 81 ($R_{9,81}$) was only 0%. The composition and evolution of the active microbial populations in Section 3 were highly similar to those in Section 1 (results not shown). The appearance of the most intense bands occurred later in time (about 10–30 days) in Section 3. From day 29, the microbial active populations in Section 1 of the H_2S-degrading biofilter were highly stable ($R_{i,i+1}$>78%) (Fig. 5a). Also, the DNA-based microbial community composition was stable from day 36, as shown by the highly similar patterns between days 36 and 81 in Fig. 4a. The A_{Sh} in Section 1 varied during H_2S biofiltration between a maximum of 3.3 (after an initial increase) and a minimum of 2.5. The decreasing trend in A_{Sh} did not occur until the end of the experiment, however. The A_E showed a very similar evolution as the A_{Sh} (Fig. 5b,c).

The evolution of the 16S rRNA-based DGGE patterns from the control biofilter was somewhat different than from the H_2S-degrading biofilter. Although the patterns were highly similar between samples of both biofilters on day 2 (R=90% for Section 1), a decrease of the correlation coefficients with time was observed for equal sample dates. For instance, on days 36 and 71, the correlation coefficients were only 40 and 24%, respectively, between samples of Section 1 of the H_2S-degrading and control biofilters. The gradual and persistent changes of the activity of the microbial communities that were visually observed in the gel pattern of the H_2S-degrading biofilter were not visible in the control. Instead, a more random change was observed (Fig. 4c). The latter is reflected by the generally

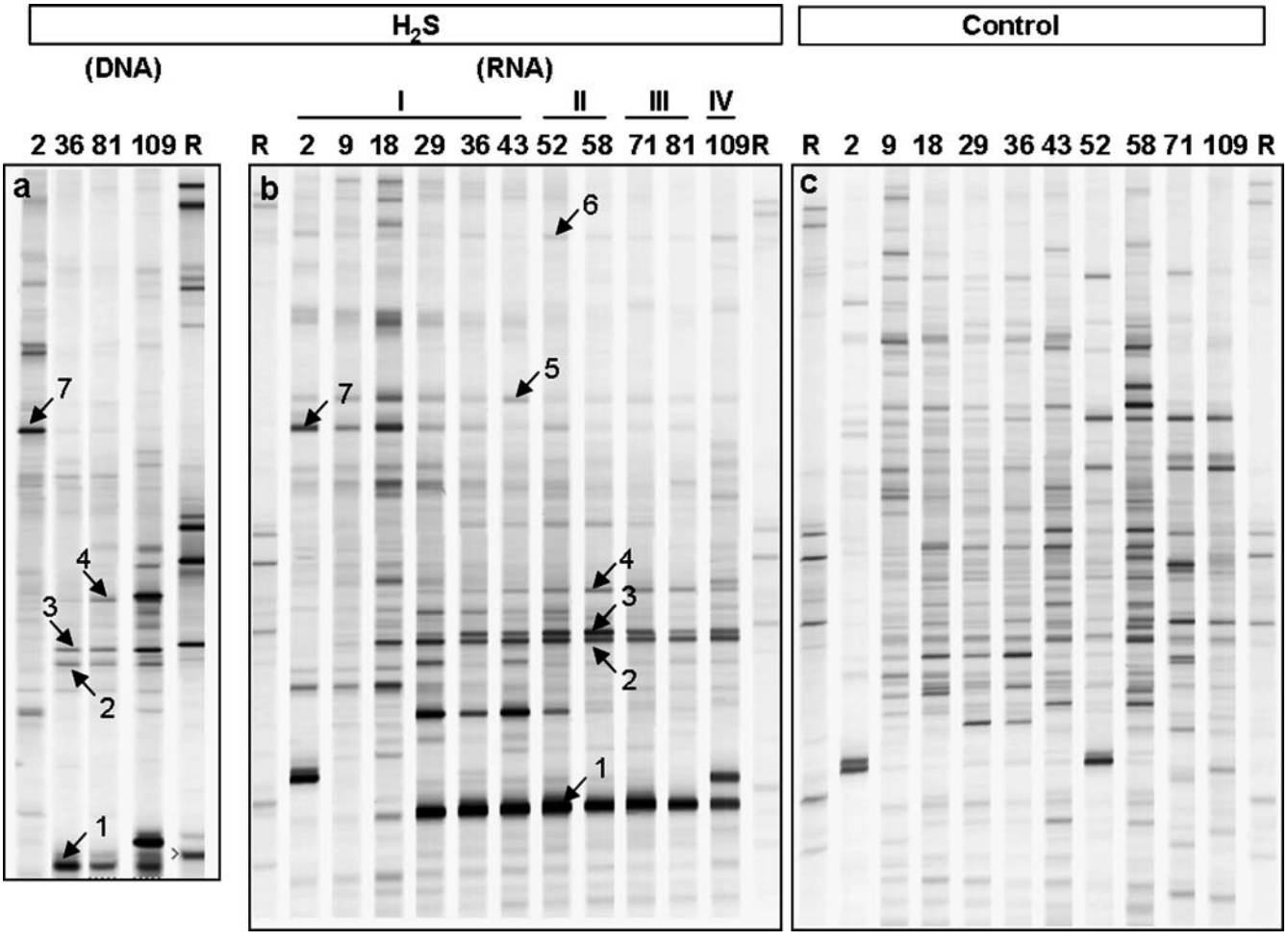

Fig. 4 DGGE patterns of the compost samples from Section 1 at different times during biofiltration. **a** H_2S-degrading biofilter (16S rDNA); **b** H_2S-degrading biofilter (16S rRNA); **c** control biofilter (16S rRNA). The *numbers* above the picture indicate the number of days after biofilter start-up and biofilter periods I–IV; *R*, reference lane. *Numbered arrows* indicate bands that were sequenced (*B1–B7*)

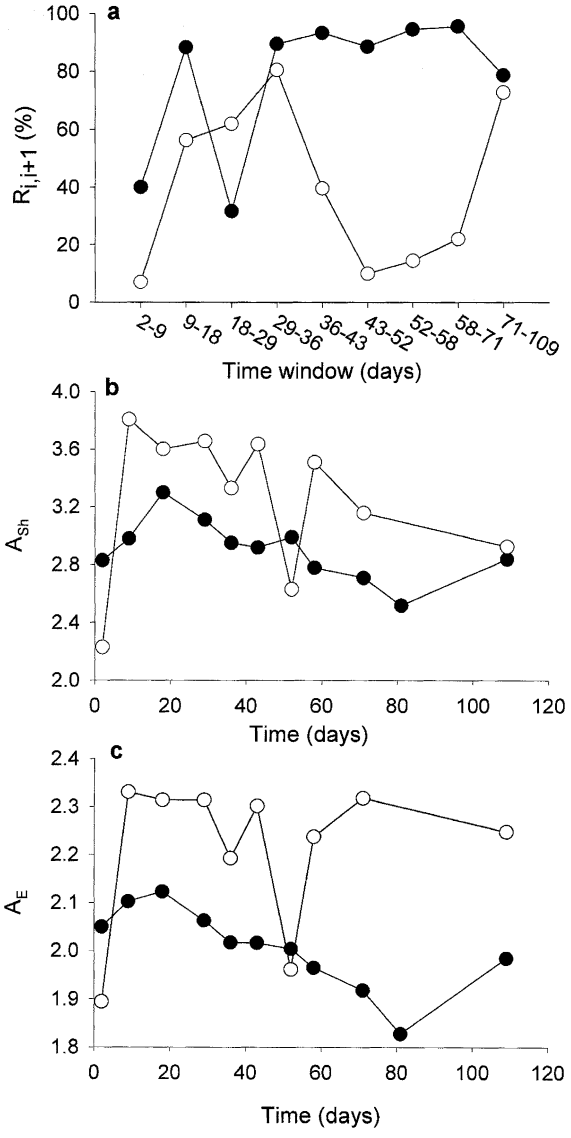

Fig. 5 **a** Pearson correlation ($R_{i,i+1}$) between consecutive samples; **b** Shannon Diversity Index (A_{Sh}); and **c** evenness (A_E) of the microbial communities in Section 1 of the H_2S-degrading (●) and control (○) biofilters based on 16S rRNA analysis

lower $R_{i,i+1}$ values in Section 1 of the control biofilter compared with those of the H_2S-degrading biofilter (Fig. 5a). The A_{Sh} in the control biofilter decreased but is generally 9–28% higher than in the H_2S-degrading biofilter, except on days 2, 52, and 109. The A_E in the control biofilter also remained higher than in the H_2S-degrading biofilter from day 9 onwards, except on day 52.

The major bands from the RNA-based DGGE pattern of Section 1 of the H_2S-degrading biofilter were excised and sequenced (Table 2). The sequence matches of the second-closest match were never in contradiction with the closest match. For instance, for band 1, the second-closest match (90.8%) belonged to an uncultured *Thiobacillus* sp. For band 6, members of the classes Flavobacteria and Bacteroidetes were also found (54.4–55.0% similarity). However, the sequence similarities were very low for this band. Only two of the seven identified active bacteria belonged to the same bacterial class (Gammaproteobacteria). All other ribotypes were assigned to a variety of Gram-negative and Gram-positive classes. Similarities less than 95% to classified bacterial species were found, except for band 4.

Discussion

The biofilter had good H_2S removal efficiencies (>93%) at loading rates up to 30.1 g m^{-3} h^{-1}. At the lowest loading rates applied (periods I and II), H_2S was usually removed to concentrations below 1 ppmv, but in period III, at loading rates between 20 and 30 g m^{-3} h^{-1}, effluent concentrations of 3–14 ppmv were measured. The maximal EC obtained in this study was 28.5 g m^{-3} h^{-1} for the total biofilter, while in Section 3, a maximal EC of 39 g m^{-3} h^{-1} was measured during the end of period III (day 79; η=94%). This corresponds well with results from Elias et al. (2000), who obtained a removal efficiency lower then 90% at inlet loading rates exceeding 40 g m^{-3} h^{-1} (empty bed residence time, 27 s). In this study, it was not attempted to determine the absolute EC_{max} of the biofilter. In other studies, a wide range of EC_{max} values have been reported, ranging between 55 and 300 g m^{-3} h^{-1} in organic biofilters (Cook et al. 1999; Degorce-Dumas et al. 1997; Oyarzun et al. 2003; Yang and Allen 1994).

No other studies were found that investigated the temporal changes (stability) of the active microbial

Table 2 Similarity percentages of excised DGGE bands as compared with the RDP database

Band	Accession nos.	Closest match(es), accession no(s).	Similarity (%)	Class
1	DQ398582	*Thiobacillus thioparus* ATCC 8158, M79426	94.5	Betaproteobacteria
2	DQ398578	>20 sequences belonging to the rhizobiales (e.g., type strain *Aquamicrobium defluvii* DSM 11603, Y15403)	93.0	Alphaproteobacteria
3	DQ398579	*Conexibacter woesei* DSM 14684, AJ440237	88.3	Actinobacteria
4	DQ398580	Uncultured xanthomonadaceae, DQ125745, DQ125697	100	Gammaproteobacteria
5	DQ398581	Uncultured *Pseudomonas* sp., AJ306778, AY972868	85.1	Gammaproteobacteria
6	DQ398584	*Flexibacter sancti*, M62795 Uncultured *Chitinophagae*, AJ318121, AB078068	57.0	Sphingobacteria
7	DQ398583	Uncultured Flavobacteriaceae bacterium, AJ853590	85.1	Flavobacteria

GenBank accession numbers are given for the sequences corresponding with each band and for the closest matches

communities in organic biofilters. In this study, we showed the occurrence of an adaptation period, after which, a stable active microbial community was present. This was clearly visualized in the 16S rRNA-based DGGE pattern and was reflected by the stable $R_{i,i+1}$ values (>75%) from day 29 in Section 1 of the H_2S-degrading biofilter, while stable and high values were not obtained in the control biofilter. Similar changes as in Section 1 occurred in Section 3 of the H_2S-degrading biofilter, although usually later in time. This was probably caused by the loading rates in the latter section, which were on average 12, 7, and 3 times lower than in Section 1 during periods I, II, and III, respectively. The increasing H_2S loading rates did not affect the stability of the active microbial community. Also, the structural diversity (DNA-based) was stable after start-up. Assuming that rRNA and rDNA quantities reflect the metabolic activity and size of bacterial populations (Ka et al. 2001; Teske et al. 1996), the DGGE patterns suggested that the latter were closely related in the biofilter. The diversity indicators (A_{Sh} and A_E) were used to derive quantifiable data from the DGGE gel patterns, in addition to the visual observations that were made. The A_{Sh} and A_E values in Section 1 of both the control and the H_2S-degrading biofilter showed a decreasing trend after an initial increase. Both values were usually higher in the control biofilter than in the H_2S-degrading biofilter. While the differences in A_{Sh} values remained relatively constant during the experiment, the differences in A_E values increased during the course of the experiment. Therefore, H_2S loading to the biofilter decreased the activity-based evenness and diversity of the microbial populations.

The ribotypes in Section 1 of the H_2S-degrading biofilter that remained active during the whole period or that gradually became more active were sequenced. Because these changes did not occur or were not that extensive in the control biofilter, the activity of these ribotypes is potentially stimulated by H_2S dosing. The major finding from the sequencing data is the dominant activity of a sulfur-oxidizing bacterium (band 1) in the H_2S-degrading biofilter. In addition, DNA-based DGGE analysis suggested that this is also one of the most abundant ribotypes. The ribotype showed the highest sequence similarity with *T. thioparus* ATCC 8158 (Lane et al. 1992). This provides indirect evidence for the importance of *T. thioparus* for H_2S removal in the biofilter, both with respect to the activity and abundance of the species. The ribotype in the RNA-based DGGE was very faint on days 2 and 9, but from day 29, it was the most dominant band in the pattern. Thereafter, its intensity remained rather constant, suggesting that the activity of the sulfur-oxidizing bacterium did not vary significantly anymore. The *T. thioparus*-related band also appeared in Section 3 of the biofilter. The high activity of *T. thioparus* in this study suggests that strategies to optimize H_2S biofiltration in a compost biofilter should provide optimal conditions for this species. The other active ribotypes identified in this study belonged to different phyla. The identity of ribotypes 2–7 was in accordance with previous culture-independent studies in organic biofilters, which showed the presence of rhizobiales, actinobacteria, xanthomonads, pseudomonads and Bacteroidetes (Friedrich et al. 1999, 2002, 2003; Khammar et al. 2005; von Keitz et al. 1999). Ribotypes 2 and 3 became increasingly active in this study, which corresponds particularly well with results from Friedrich et al. (1999, 2002), who showed the dominance of rhizobiales and actinobacteria in industrial biofilters. *T. thioparus* (ribotype 1) was not described in the abovementioned studies investigating the natural microbial population in biofilters, where no or low H_2S concentrations were present in the influent. This adds to the evidence that this genus is responsible for the H_2S degradation in this study. Ribotypes 4 and 5 could also be involved in H_2S oxidation because *Xanthomonas* sp. and *Pseudomonas* sp. have been described to oxidize H_2S (Cho et al. 1992; Chung et al. 1996). However, we expect that these heterotrophs are less important H_2S degraders, based on the decreasing or faint corresponding band intensities.

Previous studies, using culturing techniques, have shown spatial and temporal microbial community shifts between acidophilic and neutrophilic autotrophs and heterotrophs during H_2S biofiltration (Cook et al. 1999; Degorce-Dumas et al. 1997). Microorganisms, like *T. thioparus*, *T. denitrificans*, *Thiobacillus novellus*, *A. thiooxidans*, and also *Pseudomonas* spp., *Bacillus* spp., and *Penicillium* spp. were identified. The results from the current study confirm the importance of *T. thioparus* for H_2S oxidation as was observed in these culture-dependent studies. However, no shift to more acidophilic species was observed, which was probably related with the mild pH decrease that occurred in Section 1 of the H_2S-degrading biofilter (pH always higher than 5.2).

From day 82, the performance of the biofilter decreased somewhat. Comparing the ECs of the three biofilter sections showed that this was mainly related with low ECs in Section 2 and 3. Several explanations for this decreased performance were considered. The moisture content itself was probably not limiting because this value was almost equal during periods III and IV (before drying the compost). Also, nutrient limitation is unlikely because the addition of 600 ml mineral medium to the biofilter on day 93 did not improve reactor operation. Both sulfur and sulfate accumulation occurred in the biofilter. Jones et al. (2005) did not observe sulfate toxicity in a H_2S-degrading biofilter even at concentrations high enough to saturate the liquid phase, and they suggested sulfur precipitation as a possible cause of long-term biofilter inactivation. Kim et al. (2002) showed that accumulated elemental sulfur or $(NH_4)_2SO_4$ cause diminution of the active site and augmentation of the hydrophobic area on the surface of the packing materials, reducing biofilter performance. In this experimental biofilter, agglutination of the packing material was observed in Sections 2 and 3 from day 82 (after manual mixing), but not in Section 1. On day 98 this was also observed in Section 1. The start of the agglutination of particles corresponded very well with the decreased H_2S removal efficiencies in the different sections, and could be caused by the presence of $CaSO_4$ (gypsum) and water in the compost. After drying (days 99–102), the agglutination was

not observed anymore, and the H_2S removal efficiency improved somewhat. Morgan-Sagastume et al. (2003) also observed agglutination of compost particles during H_2S biofiltration, although in their case, it was related with excessive moisture content and not with sulfate formation. We believe that the major causes of the decreased H_2S removal efficiencies were agglutination and sulfur accumulation, and not microbial inhibition, e.g., by low pH, nutrient limitation, or inadequate moisture content.

Although small increases of A_{Sh} and A_E were observed in Section 1 on day 109, this was less the case in Section 3, where the H_2S removal decreased the most. According to the DGGE pattern, the activity of *T. thioparus* also did not change significantly between days 71 and 109 in both sections of the H_2S-degrading biofilter. The fact that no relation was found between the determined microbiological parameters and the H_2S degradation supports the above-mentioned idea that the major causes of the decreased H_2S removal efficiencies were agglutination and sulfur accumulation rather than microbial inhibition. However, further investigations are needed to confirm whether other species besides *T. thioparus* are degrading H_2S in biofilters and if 16S rRNA-based DGGE analysis is sensitive enough to detect significant changes of the activity of target bacteria. Such studies could ultimately provide a diagnostic tool to determine whether biofilter inactivation is caused by a failing microbiology or by alternative causes (e.g., channelling and low mass transfer).

Acknowledgements This work was supported by a scholarship from the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen) (IWT/SB/11314/Sercu). The authors acknowledge Peter Machilse at Intercommunale Grondbeleid en Expansie Antrwerpen (IGEAN) for providing the compost material, and Han Vervaeren and Robin Temmerman for critically reviewing the manuscript.

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