H₂S removal and bacterial structure along a full-scale biofilter bed packed with polyurethane foam in a landfill site

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HIGHLIGHTS

- Full-scale biofilter was used successfully for landfill site H₂S removal.
- Removal differed obviously between lower part and upper part of the biofilter.
- Different microorganisms took part in H₂S elimination in the biofilter bed.
- Odorants introduced shifted the distribution of the existing microbial populations.
- Reveal H₂S removal and bacterial community structure along the biofilter bed.

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ABSTRACT

Hydrogen sulfide accumulated under a cover film in a landfill site was treated for 7 months by a full-scale biofilter packed with polyurethane foam cubes. Sampling ports were set along the biofilter bed to investigate H₂S removal and microbial characteristics in the biofilter. The H₂S was removed effectively by the biofilter, and over 90% removal efficiency was achieved in steady state. Average elimination capacity of H₂S was 2.21 g m^{−3} h^{−1} in lower part (LPB) and 0.41 g m^{−3} h^{−1} in upper part (UPB) of the biofilter. Most H₂S was eliminated in LPB. H₂S concentration varied along the polyurethane foam packed bed, the structure of the bacterial communities showed spatial variation in the biofilter, and H₂S removal as well as products distribution changed accordingly. The introduction of odorants into the biofilter shifted the distribution of the existing microbial populations toward a specific culture that could metabolize the target odors.

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1. Introduction

Landfills are the most popular method of municipal solid waste (MSW) disposal and have been extensively applied worldwide. In China, about 200 million tons of MSW was disposed of via sanitary landfilling in 2010. The waste, especially the domestic waste, contains a considerable amount of organic compounds, which generate odors due to decomposition and anaerobic fermentation under low oxygen conditions. With continuing urbanization and demands for improved living environment quality, odor control at landfill sites has become increasingly important.

To decrease the impact of landfill odors, some closed or temporarily unused landfill areas in Beijing were covered by a layer of high density polyethylene (HDPE) film to not only control the release of gases, but also prevent rainwater and surface water from percolating into the site and influencing the generation of leachate.

However, odors accumulate under the films and still pose a threat to human health and the environment. The sulfur containing compounds, amines, hydrocarbons, phenols and other organic compounds presented in odors. Among them, H₂S was the predominant odor-causing substance.

Biological technologies such as the biofilters have been widely applied in the treatment of H₂S emitted from industrial processes, wastewater treatment, and waste disposal in landfills. They provide an alternative to conventional physical–chemical methods owing to their efficiency, cost-effectiveness, and environmental acceptability (Burgess et al., 2001; Mudliar et al., 2010). Within bioreactors, H₂S in odors are converted into odorless compounds, e.g. elemental sulfur or sulfate, carbon dioxide and water by microorganisms attached on packing material. Bacteria such as *Thiobacillus*, *Thiosphaera*, *Sulfolobus*, *Pseudomonas* and *Alcaligenes*, are principal microorganisms for H₂S biodegradation in previous reports (Chung et al., 1996; Honma and Akino, 1998; Omri et al., 2011; Ramírez et al., 2011). To optimize this process, previous studies have demonstrated the direct impacts of media moisture content, pH and temperature parameters on pollutant removal

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efficiency (Elias et al., 2002; Morgan-Sagastume and Noyola, 2006; Jover et al., 2012; Charnnok et al., 2013). The analysis of physico-chemical parameters should be associated with microbial biodegradation to both improve control and maintain maximum efficiency of the biofilters. Based on DNA extracted directly from natural samples, various analyzes have been developed to clarify microbial ecology (Theron and Cloete, 2000; Singh and Ward, 2005). Temporal and spatial changes in bacterial populations and microbial community diversity during biodegradation have been examined by sophisticated molecular methods (Widada et al., 2002; Omri et al., 2011; Ramírez et al., 2011). However, such investigations have not been regularly applied to characterize microbial populations associated with bioreactors treating air contaminants, especially those in landfill sites.

The species present, their population diversities, metabolic transformations, and interactions with the environment and each other are fundamental to biofilter operation. Studies on both laboratory and full-scale odor treating biofilters indicate that highly diverse microbial communities exist even in bioreactors fed with only one or a few air contaminants and they are distributed unevenly in packing material beds. Despite their key role in biofilter function, no information is currently available on genetic structure and microbial diversity along packing material beds because such material has been regarded as a whole reactor system in previous reports. It is important to characterize differences in the community from the inlet to the outlet through the depth of the biofilter, since the concentration of oxygen and contaminants will decline in the biofilter at increasing distances from the gas entry point.

This study focused on a full-scale biofilter treating H_2S in odors accumulated under HDPE film in a landfill site. Sampling ports were set along the biofilter bed packed with polyurethane foam cubes (PUFCs) to investigate H_2S removal and microbial characteristics in the biofilter. Distribution of H_2S and O_2 along the PUFCs packed bed was determined periodically. The cloning/sequencing method was applied to characterize the community structure of the bacteria in the biofilter regardless of cell culturability. The products of H_2S biodegradation on the PUFCs were observed and analyzed by scanning electron microscopy together with energy dispersive X-ray spectroscopy and inductively coupled plasma. The objectives of the present study were to characterize H_2S removal and bacterial structure along a full-scale biofilter bed and explore their correlation in the process of biodegradation.

2. Methods

2.1. Landfill site and odor control system

The present study was carried out in a sanitary landfill site established in Beijing in 2006. Average quantity of waste presently received at the landfill site is in the order of 1000 tons per working day. A temporarily unused area (8000 m^2) was covered by a layer of HDPE film to prevent odor emission. Odors accumulated under the HDPE film were treated by a full-scale biofilter. As H_2S was the predominant odor-causing substance, the present study focused on H_2S removal.

A biofilter with an effective volume of 1.0 m^3 was installed in a thermal insulation building to maintain a suitable temperature in winter and prevent damage from rain and the sun in summer. Gases collected by pipes were introduced into the biofilter by a blower and discharged after purification (Fig. 1). Previous investigation indicated that oxygen content under the HDPE film was usually below 10%. Two blowers supplied the airflow. A stream of air was mixed with the odor stream to provide enough oxygen for H_2S oxidation and reduce the inlet load of H_2S for the biofilter. The flow rate ratio of the two streams was 1:1. Total flow rate and empty bed residence time were 60 $\text{m}^3 \text{h}^{-1}$ and 1.0 min, respectively. The float flow meters (LZB-50 and LZB-100, China) were used to meter gas flow to the biofilter. Polyurethane foam cubes 1.0 cm^3 in size, with a density of 19 kg/m^3 and porosity of 97%, were used as packing material in the biofilter. They have high porosity, low density, high water absorption and low pressure drop across the biofilter. The total height of the PUFC packed bed was 1.2 m. The section from 0 m to 0.6 m was assigned to lower part of the biofilter (LPB) and 0.6–1.2 m was belong to upper part of the biofilter (UPB). Five sampling ports were located in the biofilter to monitor changes in H_2S concentration, humidity, pH, and temperature. In addition to the inlet (sampling port 1) and outlet (sampling port 5), sampling port 2, sampling port 3 and sampling port 4 were located 0.30 m, 0.6 m and 0.90 m from the bottom of the packing material bed, and were used to analyze the microbial and chemical characteristics on the PUFC packed in the UPB and LPB of the biofilter, respectively.

2.2. Biofilter start up

Initially, the packing material bed was seeded with aerobic microbial cultures of H_2S -oxidizing bacteria obtained from an odor treatment bioreactor in the laboratory for a fast start up. The cultures on the bioreactor packing material served as an inoculum (INOCM). Approximately 20 g of packing material were cut into pieces and soaked in 20 L of nutrient solution containing 4.0 g glucose, 10.0 g K_2HPO_4 , 100 g KH_2PO_4 , 2.0 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 40.0 g NH_4Cl , 0.2 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 40.0 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ (pH 6.5–7.0). The mixture was shaken for 30 min in an ultrasonic oscillator (KQ-250B, Kunshan, China). The liquid with suspended cells was then cultured in 10 L nutrient solution at 36 °C (Jiang et al., 2011; Li and Liu, 2013) and 160 rpm for 3 days, and then transferred into the same nutrient solution three times for enrichment of microorganisms capable of degrading H_2S . The microbial cultures were harvested by refrigerated centrifuge at 3000 rpm for 20 min (Bio-fuge Stratos, Heraeus, Germany). The concentrated cells were resuspended in 20 L mineral nutrient solution and inoculated onto the biofilter packing material. Mineral nutrients containing K_2HPO_4 0.5 g L^{-1} , KH_2PO_4 5.0 g L^{-1} , NH_4Cl 2.0 g L^{-1} , and NaCl 2.0 g L^{-1} were sprayed into the biofilter at periodical intervals to maintain an adequate supply of nutrients and moisture for microorganism growth. H_2S was regularly collected from each sampling

Fig. 1. (a) Schematic diagram of the biofilter. 1. Domestic waste; 2. odor; 3. HDPE film; 4. blower; 5. flow meter; 6. sampling port 1; 7. sampling port 2; 8. sampling port 3; 9. sampling port 4; 10. sampling port 5; 11. sprayer; 12. outlet; 13. Temp/RH meter; 14. mineral nutrients tank; 15. pump; 16. packing material; 17. biofilter; "→": Gas flow direction.

port to monitor the performance of the biofilter. The PUFCs were sampled from the biofilter for microbial and chemical analysis.

2.3. Chemical analysis

Gas phase H_2S was collected from the biofilter using 1 L Tedlar bags and was analyzed by gas chromatography (GC, Agilent 6890 N, USA) with a flame photometric detector (FPD) and DB-1701 capillary column (30 m $\times$ 0.32 mm $\times$ 0.25 μ m, Hewlett Packard, USA). The temperatures of the oven, injection, and detector were 100 °C, 50 °C, and 200 °C, respectively. Nitrogen was used as the carrier gas. The pH values were measured by a pH meter (pH-3C, Shanghai, China). Relative humidity (RH) and temperature were detected by a Dewpoint Thermohygrometer (WD-35612, OAKTON, Germany). The products of H_2S biooxidation were determined by analysis total sulfur containing compounds on the PUFCs. Inductively coupled plasma (ICP) (ULTIMA, HORIBA Jobin Yvon S.A.S., France) analysis was performed to evaluate the contents of total sulfur containing compounds. The samples were cut into pieces and dissolved in 2 ml nitric acid/1 ml hydrogen peroxide. They were then heated in an oven for 4 h at 160 °C. Finally, the solution was diluted to 10 ml with pure water. Observation of sulfur containing compounds on the packing material surface was carried out with Scanning Electron Microscopy & Energy dispersive X-ray apparatus (SEM/EDX) (HITACHI S-3000N /EDAX Inc., Japan).

2.4. Microbiological analysis

The PUFC as biofilter packing material was used to support the microorganisms and provide access to the airflow contaminants. As its nature influences not only removal performance but also operational costs, packing material is considered the core of a biofilter. The PUFCs from LPB and UPB were collected for microbial analysis after 220 days of biofilter operation. Conventional detection methods combined with molecular biology techniques were applied to detect the microbial population formed in the PUFC packed biofilter bed. Microbial population of the INOCM was also investigated.

For microbial enumeration, 1.0 g of moist PUFC was taken from sampling port 2 and 4, respectively, after 7 months of steady biofilter operation. They were cut into pieces, soaked in 100 mL of sterile distilled water, and then homogenized for 15 min using a magnetic stirrer. Total culturable bacteria were incubated in nutrient agar (BR, Aoboxing Biotech Co., China) at 30 °C for 48 h (Jiang et al., 2011; Li and Liu, 2013). Composition of the mineral medium for H_2S oxidizing bacteria enumeration was (in grams per liter): 0.5 g K_2HPO_4 , 5.0 g KH_2PO_4 , 0.1 g $MgSO_4 \cdot 7H_2O$, 2.0 g NH_4Cl , 0.01 g $FeSO_4 \cdot 7H_2O$, 2.0 g $Na_2S_2O_3 \cdot 5H_2O$, and 15.0 g agar. All chemicals used were provided by Beijing Aoboxing Biotech Co, Ltd. Results were expressed as colony forming units per gram dry PUFC ($CFU\ g^{-1}$).

Microbial distribution in the PUFCs was observed with microscope objectives using scanning electron microscopy (HITACHI S-3000N/EDAX Inc., Japan). Microbial populations on the packing media were assayed with molecular tools as described below.

2.4.1. Clone library construction and phylogenetic analysis of sequences

Microbial suspensions taken for preparation of total DNA extracts were harvested by vibration in an ultrasonic oscillations instrument, centrifugation (4 °C, 10 min, 10,000 rpm), and then suspended in a 1000 μ l (0.01 M) potassium phosphate buffer (pH 7.2). Isolation of total DNA was accomplished with a Magnetic System-16 (TanBead, Taiwan). Primers 27F and 1492R were used to amplify the segment of eubacterial 16S rRNA as described in previous report (Han et al., 2012). The PCR products generated from

each sample were purified by an Agarose-Gel Extraction Kit (OMEGA, USA). After purification, the products were ligated to the 1.0 μ l pMD19-T vector (Takara, Japan), and transformed into *Escherichia coli* DH5 α competent cells. Clones were cultured on LB medium with X-gal, IPTG and Amp. A total of 60 clones were selected for PCR detection, and their positive clones were submitted for sequencing using the ABI 3730DXL DNA sequencer (AB, USA).

All sequences obtained from the clone libraries were manually checked and trimmed to exclude vector sequences, then checked for chimeras using Bellerophon on the Greengenes website (<http://greengenes.lbl.gov/>). These sequences were aligned using DNAMAN software. Aligned sequences were grouped into operational taxonomic units (OTUs) at a threshold of 97% minimum similarity. They were named as “sampling port name-number” in the present study. Homology of representative sequence searches of each OTU was conducted using the GenBank server of the National Centre for Bio-technology Information (NCBI) and the BLAST algorithm. The representative OTU sequences obtained in this study were deposited in the NCBI database under accession numbers (Table 1). To identify the phylogenetic affiliation of all OTUs, a phylogenetic tree including representative sequences of each OTU and related sequences from the NCBI database was constructed using the neighbor joining algorithm in MEGA version 4.1.

2.4.2. Diversity and richness estimation of clone libraries

To determine whether the clone library number was large enough to represent the diversity of an original community, the coverage of each clone library was calculated according to the equation $C = 1 - (n/N)$, where n is the number of unique clones and N is the total number of sequences examined (Good, 1953). The Shannon Wiener index (H) was used to estimate the diversity of each clone library (Ding et al., 2008).

3. Results and discussion

3.1. H_2S removal in the biofilter bed

The performance of the PUFC packed biofilter was observed over 7 months. Inlet and outlet concentrations of H_2S were monitored periodically. The changes of H_2S concentration and removal efficiencies in the biofilter are shown in Fig. 2 as a function of operating time. After inoculation, the biofilter took a few days to adapt to the concentration and reached a steady state relatively quickly. The removal efficiency of H_2S increased gradually from 32% to 85% within 2 weeks. After the adaptation stage, the inlet concentrations of H_2S were 6.70–38.30 $mg\ m^{-3}$ and the outlet concentrations were 0–4.90 $mg\ m^{-3}$. Thus, over 90% removal efficiency was achieved and the H_2S accumulated under the HDPE film was removed effectively by the biofilter.

The biofilter itself had a H_2S elimination capacity of 2.10 $g\ m^{-3} \cdot h^{-1}$, which was obtained at an inlet concentration of 38.30 $mg\ m^{-3}$ and at a superficial gas velocity of 60 $m^3\ h^{-1}$. Results showed that H_2S removal in the LPB was quite different from that in the UPB.

Table 1
Accession numbers of OTUs from all samples in GenBank database.

Sampling port	Names of OTUs in Fig. 4	Accession numbers
INOCM	INOCM-1, INOCM-4, INOCM-5	JX855303 to JX855305
LPB	LPB-1 to LPB-8 LPB-13	JX534169 to JX534176 JX855292
UPB	UPB-1 to UPB-11 UPB-12	JX534186 to JX534196 JX855299

INOCM: inoculum, LPB: lower part of biofilter, UPB: upper part of biofilter.

Fig. 2. The performance of the biofilter in H₂S removal.

Fig. 3. The concentrations and removal efficiencies of H₂S along the PUFC packed bed in the biofilter.

Average elimination capacity of H₂S was $2.21 \text{ g m}^{-3} \text{ h}^{-1}$ in LPB and $0.41 \text{ g m}^{-3} \text{ h}^{-1}$ in UPB. Thus, most H₂S accumulated under the HDPE film was eliminated in LPB.

In a biofilter, removal efficiency of pollutants from exhaust is concentration-dependent (Dehghanzadeh et al., 2005). Samples from different height (0 m, 0.3 m, 0.6 m and 1.2 m) of PUFC packed biofilter bed were analyzed periodically to determine the vertical distribution of H₂S. Variations in H₂S concentration and removal efficiency along the bed are shown in Fig. 3. The H₂S concentration decreased gradually and removal efficiency was obviously enhanced with increasing height of the packing material bed. At the same time, oxygen content in the biofilter decreased slightly, from 15.7% to 14.2% on average. The proportion of oxygen in the inlet stream was much higher compared to H₂S. There was enough oxygen present to provide for microorganisms in H₂S biodegradation. The drop in H₂S concentration along the biofilter bed was due to H₂S biodegradation by sulfur oxidizing bacteria. Gas stream in the biofilter was in up-flow mode. H₂S in odorous stream first entered the LPB through the inlet and then passed through the UPB. As the airflow rate was maintained at $60 \text{ m}^3 \text{ h}^{-1}$, the H₂S loading rate depended on its inlet concentration. Therefore, the biofilter received a high H₂S load in the LPB and less in the UPB. The difference in elimination capacity between LPB and UPB was probably due to the different loads along the biofilter bed. High elimination capacity was achieved under large substrate load. Similar results have been obtained in other research on odor removal (Rene et al., 2010; Zilli et al., 2001). In addition, higher concentrations of substrates will be treated more effectively under many conditions because it will drive the contaminant into the biofilm more rapidly

and speed biodegradation, especially in a case of diffusion limitation. Therefore, the elimination capacity of H₂S in LPB was much higher than that of UPB.

3.2. Microbial characteristics in the biofilter bed

3.2.1. Microbial morphology observation

Microphotographs provided detailed images of the PUFC structure and the microbial distribution along the biofilter bed (Fig. S1 in the Supplementary materials available on-line). The PUFCs without cells had porous surfaces with honeycomb-like structures (Fig. S1a in the Supplementary materials available on-line). Microorganisms more readily colonize on rough, porous, and hydrophilic materials. As packing material for microorganism support, PUFCs have an adequate pore size and relatively homogeneous aeration, which provide a suitable environment for inoculation and microorganism growth (Marín-Cervantes et al., 2008). The INOCM was primarily composed of bacillus (Fig. S1b in the Supplementary materials available on-line). Compared with the INOCM, multiplicity of microorganisms emerged in the biofilter bed after seven months of operation (Fig. S1c–f in the Supplementary materials available on-line). The microphotographs revealed that large amounts of biomass were accumulated on the surface, forming a dense biofilm, which was mixed with coccus, bacillus and fungi mycelium (Fig. S1c and d in the Supplementary materials available on-line). In microbial ecosystems, cells commonly organize themselves into films and attach onto packing material surfaces to avoid being carried away by airflow. The number of microorganisms decreased with depth of the foam cubes. Only a few bacillus and coccus appeared at the center of the PUFCs (Fig. S1e and f in the Supplementary materials available on-line).

3.2.2. Distribution of biomass in the biofilter bed

During inoculation, inoculation fluid was sprayed by nozzles from the top of the packing material bed. Thus, total number of bacteria on the UPB was greater than that on the LPB after inoculation (Table 2). The ratios of sulfur oxidizing bacteria to total bacteria were maintained in the range of 50.09–54.03% from the surface to the center of the PUFCs, indicating that sulfur oxidizing bacteria dominated in the biofilter. Obvious variation was observed in the microbial population after 7 months of biofilter operation. Total bacteria on the PUFCs sampled from LPB were greater than that attached on the PUFCs collected from UPB. Compared to those colonized in the center of the PUFCs, most bacteria grew on the surface, which was in agreement with results obtained from the SEM observation. The ratio of sulfur oxidizing bacteria was

Table 2
Counts of microbial population in the PUFC.

Population (CFU g ⁻¹ PUFC)	LPB		UPB	
	Surface	Center	Surface	Center
<i>O-PUFC</i>				
SOB	2.68×10^9	5.63×10^7	5.36×10^9	2.16×10^9
TB	4.96×10^9	1.12×10^8	1.07×10^{10}	4.07×10^9
R (%) = $P_{\text{SOB}}/P_{\text{TB}} \times 100$	54.03	50.27	50.09	53.07
<i>U-PUFC</i>				
SOB	9.81×10^8	1.30×10^7	9.10×10^6	1.42×10^5
TB	3.19×10^9	5.93×10^7	3.53×10^7	1.72×10^6
R (%) = $P_{\text{SOB}}/P_{\text{TB}} \times 100$	30.75	21.92	25.78	8.26

PUFC: polyurethane foam cubes; O-PUFC: polyurethane foam cubes from the biofilter after inoculation; U-PUFC: polyurethane foam cubes from the biofilter operated over 7 months; LPB: lower part of biofilter; UPB: upper part of biofilter; SOB: sulfur oxidizing bacteria; TB: total bacteria; R: percentage of sulfur oxidizing bacteria in total bacteria; P_{SOB} : population of sulfur oxidizing bacteria; P_{TB} : population of total bacteria.

uneven along the PUFCs packed bed. Most sulfur oxidizing bacteria emerged at LPB, especially on the surface of the PUFCs. Biomass distribution in the PUFCs packed bed exhibited spatial variation.

There was a community of microorganisms present in the biofilter, which should grow and reproduce vigorously when food or substrate is abundant. The LPB received higher substrate load compared to UPB. Large amounts of H_2S may serve as an energy source or building material. Therefore, a dense biomass growth attached to the PUFCs at LPB, while fewer microorganisms were immobilized on UPB. In addition, the performance in H_2S removal relies on developing better microbial communities. The appearance of large amounts of sulfur oxidizing bacteria in LPB led to high H_2S elimination at this part of biofilter.

3.2.3. Microbial structure in the biofilter bed

Diversity and coverage of the 16S rDNA-based phylotypes were determined by analysis of clones from each of the clone libraries. A total of 108 clones were compared. There were 3 different OTUs among the 37 screened clones from the INOCM clone library, with 9 out of 33 and 12 out of 38 identified from the LPB and UPB clone libraries, respectively. Coverage analysis indicated that the bacterial libraries represented approximately 84.21–97.30% of the total number of clones examined, providing a dependable inventory of the bacterial 16S rRNA gene sequences present in the biofilter. Sequences were assigned to a bacterial phylum according to their position in the phylogenetic tree (Fig. 4). Bacteria that most closely

Fig. 5. Relative phylotype frequencies of clones isolated from examined samples.

represented the OTU microorganisms were those isolated from water and soil ecosystems.

Three sequences identified from the INOCM clone library were assigned to *Aquamicrobium defluvii* (JX855303), *Stenotrophomonas sp.* (JX855304) and *Ochrobactrum anthropi* (JX855305). Their percentages were 56.76%, 40.54% and 2.70%, respectively (Fig. 5). *O. anthropi* and *Aquamicrobium defluvii* were the major bacteria in the INOCM clone library, which were both affiliated with α -Proteobacteria. For LPB, the most represented bacterial groups were *O. anthropi* (36.36%), *Aquamicrobium defluvii* (15.15%), and *Rhizobium sp.* (15.15%), with *Delftia sp.*, *Brevundimonas sp.*, and *Brucella canis* forming minor groups. The other OTUs were either related to uncultured environmental bacteria or not closely related to any known sequence present in the databases. *O. anthropi* (23.68%) and *Aquamicrobium defluvii* (15.79%) also formed the majority of sequences in UPB. Other identified groups in UPB were *Rhodococcus fascians*, *Rhizobium sp.*, *Pseudomonas fluorescens*, *Pusillimonas sp.*, uncultured *Aquamicrobium sp.*, and uncultured alpha proteobacterium (Fig. 5).

O. anthropi and *Aquamicrobium defluvii* originally in INOCM clustered along the packing material bed, indicating they thrived during the 7 months of operation. *Stenotrophomonas sp.* however, was not detected either from LPB or UPB. Microorganisms may require a period of acclimation before they biodegrade vigorously when exposed to substrates in the inlet stream. Fierce competition occurs between species consuming the same substrate and the less capable species may die out (Devinny et al., 1999). Therefore, the disappearance of *Stenotrophomonas sp.* from the biofilter was probably due to its low degradation capacity of H_2S and the unsuitable environment for survival in the biofilter.

Bacterial species diversities were 0.64, 2.37, and 2.68 for the INOCM, LPB, and UPB clone libraries, respectively. Both LPB and UPB clone libraries exhibited higher bacterial diversities than that of INOCM, indicating obvious variance in bacterial structure after 7 months of biofilter operation. This was consistent with the microbial morphology observation by SEM. Bacterial diversity distribution within the PUFC packed bed was uneven and exhibited spatial variation. Populations in UPB showed higher species richness compared to those in LPB. Bacterial diversity increased obviously along the PUFC packed bed.

3.3. Microbial populations and substrates introduced

The amount, species and distribution characteristics of the microorganisms depended on the properties of the compounds being treated. The microbial population in the biofilter, their closest relatives and substrates of the closest relatives is shown in

Fig. 4. Phylogenetic tree showing the relationship of representative sequences of OTUs in all samples and reference sequences in GenBank.

Table 3

The microbial population in the biofilter, closest relatives and substrates of the closest relatives.

Name of OTU			Closest relatives	Substrates of the closest relatives	References
INOCM	LPB	UPB			
INOCM-5	LPB-4	UPB-5	<i>Ochrobactrum anthropi</i>	H ₂ S or sulfide	Mahmood et al. (2009), Jiang et al. (2011) and Li and Liu (2013)
INOCM-1	LPB-2	UPB-4	<i>Aquamicrobium defluvii</i>	H ₂ S or sulfide	Liu et al. (2013)
	LPB-1	UPB-12	<i>Rhizobium sp.</i>	H ₂ S	Jiang et al. (2011)
	LPB-3		<i>Delftia sp.</i>	Phenolic compounds	Juarez Jimenez et al. (2012)
	LPB-13		<i>Brevundimonas sp.</i>	Nitrobenzene	Huang et al. (2012)
		UPB-6	<i>Pseudomonas fluorescens</i>	H ₂ S	Chung et al. (1996) and Honma and Akino (1998)
		UPB-10	<i>Rhodococcus sp.</i>	Dibenzothiophene; dimethylsulfoxide	Wang and Krawiec (1996), Yan et al. (2000), Gou et al. (2003) and Davoodi-Dehaghani et al. (2010)
		UPB-11	<i>Pusillimonas sp.</i>	Phenols; thiocyanate	Felföldi et al. (2010)

Table 3 with the aim to find the correlation between substrates introduced and microbial community in individual part of the biofilter.

Both *O. anthropi* and *Aquamicrobium defluvii* can produce either elemental sulfur or sulfate as a product of sulfide oxidation (Mahmood et al., 2009; Liu et al., 2013). In addition to *O. anthropi* and *Aquamicrobium defluvii*, *Rhizobium sp.* appeared in both LPB and UPB in the present study. Previous research has reported that the consortium of *O. anthropi* and *Rhizobium sp.* exhibited high elimination capacity in the treatment of H₂S from off-gas (Jiang et al., 2011). The combined percentages of *O. anthropi* and *Rhizobium sp.* were 51.51% in LPB and 36.84% in UPB. H₂S biooxidizing bacteria dominated along the PUFC packed bed due to H₂S being the major substrate in the odor stream. In addition, OTU of UPB-6(JX534191) in the UPB clone library had 99% similarity to *Pseudomonas fluorescens*. *Pseudomonas* strains have often been detected in biofilters used for H₂S control (Chung et al., 1996; Honma and Akino, 1998). Many ecological niches exist for microbial communities to occupy in pollutant treatment, even for single contaminant treatment. The microbial analysis results demonstrated that H₂S was probably biodegraded by *O. anthropi*, *Rhizobium sp.* and *Aquamicrobium defluvii* formed in the LPB, and by *O. anthropi*, *Rhizobium sp.*, *Aquamicrobium defluvii* and *Pseudomonas fluorescens* in the UPB. Different microorganisms took part in H₂S elimination in the individual parts of the biofilter.

The microbial populations of LPB and UPB were remarkably distinct to that of INOCM. Species such as *Rhodococcus sp.*, *Brevundimonas sp.*, *Delftia sp.*, *Brucella sp.*, *Pusillimonas sp.* and uncultured bacterium, which were not detected in INOCM, emerged in LPB and UPB. Biofilters are inevitably biologically open systems. Large amounts of air carrying aerosols and dust pass through the biofilters, which introduce a large variety of microorganisms such as cells and spores. During biofilter operation, these species will thrive or fail according to their abilities to find a place in the biofilter ecosystem. *Brevundimonas sp.* was found in the microbial community in nitrobenzene bio-reduction coupled to sulfate reduction system (Huang et al., 2012). *Delftia tsuruhatensis* showed highly promising metabolic diversity for the biodegradation of various recalcitrant compounds such as phenolic compounds and chloroanilines as individual compounds or mixtures (Juarez Jimenez et al., 2012). *Pusillimonas noertemannii* was identified from aerobic activated sludge for phenols and thiocyanate removal and the bacterial community in a full-scale biofilter treating pig house exhaust air (Felföldi et al., 2010; Kristiansen et al., 2011). *Rhodococcus erythropolis* was found to desulfurize dibenzothiophene in a C–S bond targeting manner and form sulfite as final products (Yan et al., 2000). The introduction of odorants into the biofilter shifted the structure of the existing microbial populations toward a specific culture that could metabolize the target substrates. In addition to H₂S in landfill gases, hundreds of organic compounds, such as

mercaptans, thioethers, thiophene, amines, phenols, and benzene series, were detected from the odors accumulated under the HDPE film at extremely low concentrations. If the biofilter removes more than one pollutant from the air, the number of species will multiply accordingly. A diverse microbial population may thrive by utilization of complex contaminants in odors. The appearance of *Brevundimonas sp.* and *D. tsuruhatensis* in LPB as well as *Rhodococcus sp.* and *Pusillimonas sp.* in UPB was probably due to the presence of thiophene, aromatic hydrocarbon, and phenols. The appearance of new species would, in turn, increase the Shannon index of the UPB and LPB clone libraries.

3.4. Distribution of H₂S biooxidation products in the biofilter bed

The main H₂S biooxidation products are elemental sulfur and sulfate (Chung et al., 1996; Elias et al., 2002). Biodegraded products should accumulate on the packing material. Thus, the content and distribution of sulfur containing compounds in the PUFCs were observed using SEM/EDX and analyzed by ICP. Detailed images provided the sulfur containing compound crystals on the PUFCs (Fig. S2 in the Supplementary materials available on-line). Element percentages of the crystals detected by EDX were 18.08% sulfur, 22.67% calcium, and 54.59% oxygen, indicating that the crystals might be dihydrate calcium sulfate. At the same time, yellow substance deposited on the packing material could also be observed, which was supposed to be S⁰.

Results of ICP showed that only 0.987 mg g⁻¹_(PUFC) of sulfur was found on unused PUFC. However, 12.515 mg g⁻¹_(PUFC) of total sulfur accumulated at LPB and 8.754 mg g⁻¹_(PUFC) at UPB after 7 months of biofilter operation. Higher amounts of total sulfur containing compounds accumulated at LPB compared with that at UPB. H₂S concentrations declined as air passed through the biofilter bed due to its biodegradation (Fig. 3), so H₂S load were much higher in the LPB than in the UPB. This was consistent with previous findings, which found that biodegradation products were mainly produced at the first and second modules of the biofilter, where higher amounts of the contaminant were received (Elias et al., 2002). The elimination capacity of H₂S in LPB was also larger than that in UPB, indicating that when the removal of H₂S varied along the PUFC packed biofilter bed, the distribution of biodegradation products changed accordingly.

4. Conclusions

A full-scale biofilter packed with polyurethane cubes was used successfully for H₂S removal in a landfill site. This study revealed the characteristics of the microbial community structure along the biofilter bed. The concentration of the H₂S varied along the PUFC packed bed, and the distribution of microorganisms, the

removal efficiency, and the amount of biodegradation products changed accordingly. During treatment, odorants introduced into the biofilter shifted the distribution of the existing microbial populations toward a specific culture that could metabolize the target odors. A diverse microbial population may thrive by utilization of the complex contaminants found in odors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2013.07.143>.

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