Performance and Bacterial Community Diversity of a Full-Scale Biofilter Treating Leachate Odor in a Sanitary Landfill Site

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Abstract Odors, such as the malodorous and toxic hydrogen sulfide (H_2S), are released during leachate collection, storage, and treatment. A full-scale biofilter was applied to treat H_2S emitted from a leachate equalization basin in a sanitary landfill site. The inlet concentration of H_2S was 26.3–213.0 mg m^{-3} . In steady state, total removal efficiency was over 90 % in summer and over 80 % in winter. The maximum elimination capacity achieved 9.1 $\text{gm}^{-3}\text{h}^{-1}$ at a loading rate of 10.5 $\text{gm}^{-3}\text{h}^{-1}$. The biofilter was effective at reducing H_2S . Factors on the level of H_2S inlet concentration and performance of the biofilter were investigated. The H_2S inlet load and removal efficiency relied on ambient and biofilter temperature, respectively. The water containing rate and relative humidity presented seasonal variation, according to which the interval period of irrigation could be optimized. The main product of H_2S degradation was sulfate, and

sulfur also could be observed from the biofilter. Spatial and temporal shifts in bacterial community composition in the biofilter were determined by polymerase chain reaction-denaturing gradient gel electrophoresis followed by DNA sequence analysis. The present study revealed a correlation between biofilter performance and bacterial community structure, especially in a real application case.

Keywords Biological deodorization · Hydrogen sulfide · Leachate equalization basin · Landfill site · PCR-DGGE · DNA sequence analysis

1 Introduction

Odor control has been the focus of research for many years. Compared with conventional physical–chemical methods, biological technologies, such as biofilters, bioscrubbers, and biotrickling filters, have been widely applied due to their efficiency, cost effectiveness, and environmental acceptability (Deshusses 1997; Kennes and Thalasso 1998; Burgess et al. 2001).

Leachate generated from sanitary landfill is a complex wastewater, which contains a considerable amount of organic compounds, minerals, and heavy metals. In the treatment of leachate, odors are generated in high concentrations due to organic matter decomposition and anaerobic fermentation, especially when insufficient oxygen is available. Sulfides, e.g., H_2S , produce a strong rotten egg smell that humans

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can smell even at low concentration. The emission of H_2S not only causes odor complaints, but it may also pose a potential health and safety risk to people living and working near the sanitary landfill (Brosseau and Heitz 1994; Elias et al. 2002; Panza and Belgiorno 2010). Due to its predominance, H_2S removal has been extensively studied (Li et al. 1998; Duan et al. 2007; Dumont et al. 2008; Park et al. 2011; Ramírez et al. 2011; Namgung et al. 2012). Biofilter design, packing material selection, and parameters (e.g., inlet load, empty bed residence time, temperature, pH, nutrient concentrations, water content, and air relative humidity) have been investigated intensively in relation to optimizing biofilter performance (Aurai et al. 1998; Filho et al. 2010; Hernández et al. 2012). Most previous research has, however, focused on purification of H_2S generated from domestic or industrial wastewater, while those emitted from leachate are seldom mentioned.

The microorganisms in a biofilter are responsible for the decomposition of pollutants in odors, and monitoring their populations is an effective way to optimize biofilter performance. To gain biological insights into the process, microflora has been investigated using culture-independent methods. Polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (PCR-DGGE), a molecular biological method, has been successfully applied to explore bacterial diversity in various environmental samples and population shifts in response to environmental variation in bioreactors (Maron et al. 2005; An et al. 2006; Ding et al. 2008). Obtaining information on microbial community composition, its evolution, and phylogenetic analysis of the most abundant species are the main advantages of this technique. Although this method has been applied to describe spatial and temporal population changes in microbial communities in the context of biological treatment of odors in laboratory, little is known about their application on actual treatment systems. Temperature, pH, nutrient concentrations, and moisture content are important parameters influencing the activity of microorganisms involved in biodegradation, and biofilters are often faced with variation in the mentioned above factors.

A full-scale biofilter was established in a Beijing landfill site to lessen the high concentration of H_2S emitted from the leachate equalization basin. The aims of this study were to: (1) monitor the performance of the biofilter with varied H_2S load, temperature, and

moisture content; (2) analyze spatial and temporal population variation in microbial communities on packing media's biofilm in the biofilter using conventional microbiological and PCR-DGGE methods; and (3) clarify the relationship between microbial characteristics and H_2S removal, especially in a real application case.

2 Materials and Methods

2.1 Landfill Site and Odor Control System

A full-scale biofilter was established to eliminate odors emitted from a leachate equalization basin in a landfill site in Beijing. The sanitary landfill site was built in 2006 and has been operational for 5 years. Each day, more than 150 m^3 of leachate is purified by a treatment process that consists of upflow anaerobic sludge blanket + membrane bioreactor + reverse osmosis. The results of the odor composition analysis indicated that H_2S was the predominant odorous compound. Therefore, H_2S was selected as the major target pollutant for removal.

Odors from the equalization basin were collected by pipes and introduced into the biofilter treatment system by a blower. After treatment, the purified gas was discharged into the atmosphere (Fig. 1a). The effective volume of the biofilter was 1.5 m^3 . Inert polyurethane foam cubes 1.0 cm^3 in size and with a density of 19 kg/m^3 and porosity of 97 % were used as the packing material in the biofilter. The total flow rate was $90 \text{ m}^3/\text{h}$, corresponding to an empty bed residence time of 60 s. The sampling ports were located at the inlet and outlet of the treatment system for determination of experimental compound concentrations in untreated and treated gas, respectively. A thermal insulation building was installed to maintain suitable temperature for the biofilter in winter and to protect the biofilter from rain and solarization in summer (Fig. 1b).

2.2 Biofilter Start-up

Initially, the biofiltration column was seeded with aerobic microbial cultures of H_2S degraders obtained from a previous H_2S -degrading bioreactor in our laboratory for a fast start-up. The cultures on the bioreactor packing material served as an inoculum. Approximately 100 g of

Fig. 1 **a** Schematic diagram of the biofilter. 1 leachate equalization basin, 2 blower, 3 inlet stream sampling point, 4 mineral nutrients tank, 5 biofilter, 6 and 7 sampling points, 8 sprayer, 9 meter pump, 10 temp/RH meter, 11 packing material. **b** Thermal insulation building housing the biofilter

packing material was cut into pieces and soaked in 20 L of nutrient solution containing 0.2 gL^{-1} of glucose, 0.5 gL^{-1} of K_2HPO_4 , 5.0 gL^{-1} of KH_2PO_4 , 0.1 gL^{-1} of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0 gL^{-1} of NH_4Cl , and 2.0 gL^{-1} of Na_2S (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 20 L nutrient solution at 30°C and 160 rpm for 7 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 4,000 rpm for 20 min (Biofuge Stratos, Heraeus, Germany). The concentrated cells were resuspended in 50 L nutrient solution and inoculated onto the biofilter packing material. Mineral nutrients containing K_2HPO_4 0.5 gL^{-1} , KH_2PO_4 5.0 gL^{-1} , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 gL^{-1} , NH_4Cl 2.0 gL^{-1} , and

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 gL^{-1} were sprayed into the biofilter at periodical intervals to maintain an adequate supply of nutrients and moisture for microorganism growth.

2.3 Chemical Analysis

Gas samples from the inlet and outlet, liquid samples from biofilter leakage, and solid samples from packing material were collected periodically to monitor the performance of the bioreactor. Gas phase H_2S was collected from the biofilter using 1 L Tedlar bags and was analyzed by gas chromatography (GC, Agilent 6890N, USA) with a flame photometric detector and HP-5 capillary column ($30 \text{ m} \times 0.32 \text{ mm} \times 0.25 \mu\text{m}$; Hewlett Packard, USA). The temperatures of the oven, injection, and detector were 50 , 100 , and 200°C , respectively. Nitrogen was used as the carrier gas. The analysis methods of ethanethiol and styrene in detail have been described in our previous report (Li and Liu 2009). Sulfate concentrations in the liquid phase were analyzed by an ion chromatogram analyzer (ICS-1000, Dionex ion chromatography system, USA). Methylene blue spectrophotometry was applied to determine sulfide concentration.

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). Gravimetry was applied to determine the water-containing rate (WCR) of packing material. Pressure drop along the biofilter was monitored by a vertical liquid column manometer (LU300, KIMO, France) connected to two ends of the biofilter.

2.4 Microbiological Analysis

2.4.1 Medium for H_2S Degraders

For microbial enumeration, 1.0 g of the polyurethane packing material (or 1.0 mL liquor samples) was taken from the sampling ports when the biofilter was in steady state. They were then mixed with 100 mL of sterile water and agitated for 10 min. The composition of the mineral medium for the H_2S degraders culture was (in grams per liter): 0.5 g of K_2HPO_4 , 9.0 g of KH_2PO_4 , 0.1 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0 g of NH_4Cl , 0.01 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 2.0 g of Na_2S . All chemicals used were provided by Beijing Aoboxing Bio-

tech Co., LTD. The number of microorganisms was expressed as colony-forming units (CFU) per gram dry packing medium. The microbial populations on packing media were assayed with molecular tools as described below.

2.4.2 DNA Extraction

Isolation of total DNA was accomplished with DNA Automatic Platform for Magnetic System-16 (TanBead, TaiWan) and DNA bands were observed by 1.0 % agarose gel electrophoresis. All samples were assayed in duplicate.

2.4.3 Polymerase Chain Reaction Amplification and DGGE Analysis

Extracted DNA was used as the template to amplify the V3 regions of the bacterial 16S ribosomal RNA gene by PCR, using universal primers F357GC (5'-CGCCCGCCGCGCGCGGCGGGCGGGCGGGG GCACGGGGGGCCTACGGGAGGCAGCAG-3') and R518 (5'-ATTACCGCGGCTGCTGG-3'). The GC clamp was added to the forward primers to facilitate DGGE. The PCR was performed in 50 μ L volumes containing 5 μ L of 10 $\times$ Ex Taq buffer (Mg²⁺ Plus), 4 μ L of each dNTPs (2.5 mmol/L), 0.75 μ L of the primers, 1.25 U of ExTaq-polymerase (TaKaRa, Dalian, China), and 2 μ L of extracted DNA by MJ Research PTC-200 Peltier thermal cycler (Bio-Rad, USA).

The PCR product generated from each sample was separated on 8 % acrylamide gel with the linear denaturant gradient increasing from 30 to 60 % using the Bio-Rad D-GENE System (Bio-Rad, USA). The DGGE was performed using 30 μ L of the PCR product in 1 $\times$ TAE buffer at 60 $^{\circ}$ C and 85 V for 960 min. After staining the gel with Gel Red (Biotium, USA), bands were visualized using a UV transilluminator. The digital images were captured by a Fluor-S MultiImager (Bio-Rad, USA) and analyzed with Quantity One Software (Bio-Rad, USA).

2.4.4 Sequencing and Phylogenetic Analysis

The diversity of each sequence set was estimated using the Shannon Wiener index (H), as described in our previous report (Ding et al. 2008; Li et al. 2011).

Prominent DGGE bands were excised and dissolved in 50 μ L of Milli-Q water overnight at 4 $^{\circ}$ C. Target DNA fragments were reamplified using primer set F357/R518 without the GC clamp, thus obtaining a pure sample for cloning and sequencing. The PCR products were ligated into the pMD18-T vector (TaKaRa, Dalian, China) and transformed into *Escherichia coli* TOP10 competent cells following the manufacturer's protocols (Biomed, Beijing, China). The clones were cultured on LB medium with X-gal, IPTG, and Amp. Positive colonies were submitted for sequencing using ABI 3730XL capillary sequencers (ABI, Japan). Sequence data from 16S rRNA fragments were subjected to phylogenetic analyses. Homology searches were conducted using the GenBank of the National Centre for Biotechnology Information and the BLAST algorithm.

2.5 Calculation Formula

The performance of the bioreactor was evaluated by series parameters, which were defined as follows:

$$R = (C_{in} - C_{out}) \times 100 / C_{in}$$

$$IL = Q \times C_{in} / V$$

$$EC = Q \times (C_{in} - C_{out}) / V$$

In which, R is the removal efficiency (in percent), C_{in} is the inlet concentration of H₂S (in milligrams per cubic meter), C_{out} is the outlet concentration of H₂S (in milligrams per cubic meter), IL is the inlet load (in milligrams per cubic meter per hour), Q is the flow rate (in cubic meters per hour), V is the volume of biofilter (in cubic meters), and EC is the elimination capacity (milligrams per cubic meter per hour).

3 Results and Discussion

3.1 Performance of the Biofilter

We observed the performance of the biofilter over a period of 9 months. Inlet and outlet concentrations and removal efficiencies of H₂S in the biofilter were shown in Table 1. The outlet concentrations of H₂S changed with inlet concentrations. After an initial period of adaptation, the removal efficiency of H₂S increased gradually from 47.4 to over 85 %. Only 2 weeks of

acclimatization was required for the microorganisms in the biofilter to adapt to the high H_2S inlet concentrations, indicating that the polyurethane packing material inoculated with H_2S -degrading bacteria exhibited high biodegradation activity. The inlet concentrations of H_2S were 30.3–206.9 mgm^{-3} . In steady state, the outlet concentrations were reduced to 4.1–22.4 mgm^{-3} . The H_2S emitted from the leachate equalization basin was removed effectively by the biofilter (Table 1).

Results showed that removal capacity increased steadily with H_2S load (Fig. 2). A gradual and linear increase in elimination capacity occurred up to a load of approximately $5.4 \text{ gm}^{-3}\text{h}^{-1}$. Such elimination capacity indicated that a linear relationship existed between the removal rate and inlet load at low loading rates, and that H_2S was nearly completely removed. With further load increases, the elimination rate increased more slowly up to a critical load and then remained constant, indicating that maximum elimination capacity of the bioreactor was achieved. The bioreactor itself had a maximum H_2S elimination capacity of $9.1 \text{ gm}^{-3}\text{h}^{-1}$. This threshold was obtained at an inlet concentration of 174.5 mgm^{-3} and at a superficial gas velocity of $90 \text{ m}^3\text{h}^{-1}$. High removal efficiency was obtained at lower H_2S load (Fig. 3). The removal of H_2S decreased gradually from over 85 % to below 75 % when the inlet load increased

Fig. 2 Elimination capacity vs. H_2S inlet load

from 10.5 to over $12 \text{ gm}^{-3}\text{h}^{-1}$, respectively. When the H_2S concentration increased and the flow rate and volume remained the same, the microorganisms on the packing material could not absorb the increased H_2S in the stream, with some untreated H_2S simply passing through the biofilter and causing the decline in removal efficiency.

3.2 Factors on Biofilter Performance

3.2.1 Effect of Temperature on H_2S Inlet Load and Removal

The removal of H_2S is a microbial metabolic reaction. Environmental conditions such as temperature, pH value, and moisture content within the biofilter must

Table 1 The temperature, H_2S load, and removal efficiency (in average)

Operation time	Season	Temperature in biofilter (°C)	Concentration of H_2S (mgm^{-3})		Removal efficiency (%)
			Inlet	Outlet	
October 2009	Autumn	29.6	166.9	22.4	86.6
November 2009	Autumn	24.2	96.4	9.2	90.5
December 2009	Winter	15.4	66.4	7.1	89.3
January 2010	Winter	10.7	30.3	5.1	83.2
February 2010	Winter	13.3	38.3	7.6	80.2
March 2010	Spring	17.6	72.6	7.5	89.7
April 2010	Spring	22.3	77.5	4.2	94.6
May 2010	Spring	32.5	115.8	6.9	94.0
June 2010	Summer	34.5	196.3	15.8	92.3

Fig. 3 Removal efficiency vs. H_2S inlet load

be maintained at specified levels to enable survival and efficient biodegradation by the microorganisms. As the flow rate for odor elimination was maintained at $90 \text{ m}^3 \text{ h}^{-1}$, the inlet load of the biofilter changed with inlet concentration of H_2S . Seasonal variations in H_2S inlet concentrations were observed during the 9 months of operation. In summer, the average leachate temperature in the equalization basin was 33.5°C and the inlet concentration of H_2S was 206.3 mgm^{-3} (Fig. 4). With the decrease in temperature, the concentrations of H_2S varied accordingly. In winter, the average concentration of H_2S in the inlet gas was only 30.2 mgm^{-3} when the leachate temperature was 5.9°C .

Variation in the H_2S load was possibly due to the leachate components, weather conditions, and ambient temperatures. The leachate pollutants were comprised of dissolved organic matter, inorganic compounds (e.g., sulfate, sodium plus potassium, chloride), and xenobiotic organic compounds (Kjeldsen et al. 2002). In the absence of oxygen, leachate microbes have no dissolved oxygen available for respiration, which allows the “sulfur-reducing and sulfate-reducing” microbial community to thrive. Sulfur-reducing and sulfate-reducing bacteria derive energy from oxidation organic compounds by utilizing sulfur or sulfate present in leachates to sulfide ions. Sulfide ions then combine with hydrogen to form hydrogen sulfide. The efficiency of the sulfate to H_2S transformation may be significantly reduced by low temperatures due to the influence of temperature on the speed at which chemical reactions take place and on the

metabolism rate of sulfur-reducing and sulfate-reducing bacteria.

In addition, the solubility of H_2S in water is temperature dependent. At 30°C , the solubility of H_2S in water is 2.98 gL^{-1} , whereas 5.11 g of H_2S can dissolve in 1 L of water at 10°C (Liu et al. 2002). Thus, the solubility of H_2S almost doubles at low temperature. Less H_2S is emitted into the gas phase as more is dissolved in water. Therefore, in this study, the bioreactor received the heaviest H_2S load during summer and the lowest during winter.

The performance of a biofilter is also influenced by temperature. High removal efficiencies (over 90 %) were achieved when the temperature in the biofilter was over 20°C (Fig. 5). The removal efficiency of H_2S decreased obviously with the decline in temperature. The H_2S degraders were inoculated on the packing material before biofilter operation as removal efficiency relies on their microbial activity. For mesophilic aerobic bacteria, highest grow rates are achieved at $20\text{--}40^\circ\text{C}$. Biological activity roughly doubles for each 10°C rise in temperature, up to an optimum of about 37°C . The optimum temperature for bacteria in H_2S oxidation is in the range of $20\text{--}35^\circ\text{C}$ under which they degrade H_2S into sulfate effectively. At low temperatures, the activity of H_2S degraders dropped sharply, and at the same time the metabolism of intestinal bacteria slowed down, decreasing the removal efficiency of the biofilter. In addition, we found that H_2S removal was more influenced by the inlet concentration at lower temperatures. Thus, controlling temperature is crucial to avoid thermal shock when using a biofilter in odor control. The odor treatment

Fig. 4 Changes in H_2S concentration in the inlet stream with temperature

Fig. 5 Changes in H_2S removal efficiency with temperature

system in the present study was installed in an insulated house, which maintained the temperature, especially during the cold season.

Gases were collected and sent into the biofilter by a blower, which worked 24 h a day. A large amount of heat was generated during the rotation of the motor, which heated the gases before they entered the biofilter. The insulated house and preheating of gases by the motor ensured the temperature in the biofilter was over 10 °C all year, and thus H₂S removal efficiency was maintained at over 80 % even during cold periods. It should be noted that the temperature of the biofilter varied gradually with the ambient environment and did not experience sudden change. It was likely that those species of microorganisms that acclimated and tolerated the change became active and abundant, which resulted in stable removal efficiencies. The fact that the biofilter maintained high removal efficiencies during the overall operation indicated that this biofilter was stable within a wide range of inlet loads and ambient temperatures.

3.2.2 Variation of WCR and Pressure Drop in the Biofilter Bed

Moisture content of the biofilter material is a critical factor for microorganisms, which require water to grow and carry out metabolic activities. Too little moisture content causes drying, which will reduce the biodegradation rate. There are two different biofilter water management systems for optimal water content control, namely prehumidification and surface irrigation. In the leachate equalization basin, stream evaporated through the leachate surface and transferred into the gas phase. The RH in effluent gas from the headspace of the equalization basin was often over 70 %. As a result, it was unnecessary to install a prehumidification facility before the biofilter. The mineral nutrients were sprayed into the biofilter at regular intervals to maintain nutrient and moisture for microbial growth and activity. Variations in the packing material WCR and biofilter RH within 2 weeks were shown in Fig. 6. WCR in June, WCR in December, RH in June, and RH in December decreased with time after irrigation. In June, the RH in the biofilter decreased slightly with an average of 95.3 %, whereas the WCR in the packing material dropped obviously from 86.3 to 40.5 % after 1 week of operation. Compared with June, the RH in December was lower and averaged 87.5 %. The WCR decreased

Fig. 6 WCR and RH in the biofilter

from 89.1 to 55.5 % in 7 days of operation. It then dropped below 40 % after 12 days of operation. In Beijing, spring occurs from March to May, summer from June to August, autumn from September to November, and winter from December to February. The temperature in the biofilter was 30 to 37 °C in June and 12 to 17 °C in December. The decline rate of WCR in winter was slower than that in summer due to the temperature in the biofilter. At a higher temperature, the velocity of the individual molecules increases and the total kinetic energy of the system also increases. The molecules overcome their intermolecular forces and change to a higher energy state (a gas). Thus, the rate of evaporation increased as temperature increased, which in turn decreased water content in the packing materials while increasing RH in the surrounding atmosphere.

Low water content may reduce the sorption of gaseous pollutants or the survival and metabolism of the resident microorganisms, whereas high moisture content may result in anaerobic zone development and high pressure drop (Aurai et al. 1998). The pressure drop of the biofilter bed increased dramatically to 4.7–5.2 mmH₂O following irrigation, after which it reduced gradually to 0.5–1.5 mmH₂O within 7 days. The biofilter clog brought about by excess biomass did not occur during the 9 months of operation. The polyurethane foam cubes were used as packing material in this present study. Previous research has indicated that cubic polyurethane foam is suitable packing material for malodorous gas removal, possessing high removal efficiency and low pressure drop compared with other packing materials (Ryu et al. 2010).

For most packing material, optimal water content ranges between 40 and 60 % on a weight basis to maintain maximum biological activity and optimal operation of a biofilter (Aurai et al. 1998; Devinny et al. 1999). In the present study, irrigation occurred once a week in summer and once every 2 weeks in winter. It was feasible to maintain the water content of packing material in a biofilter within the desirable range (40–60 %) without any additional prehumidification facilities in the present study, which reduced water and energy consumption in real application.

3.3 Microbial Characteristics in the Biofilter

3.3.1 *H₂S* Degraders in the Biofilter

Gas-phase bioreactors utilize microbial metabolic reactions to remove contaminants from air. Within bioreactors, microorganisms are supported on packing material. Containments in odors make contact with microorganisms when they flow through the bioreactor. Thus microorganisms produce enzymes to biodegrade these odor into less hazardous products. The treatment of contaminants relies on developing better microbial communities in the bioreactor. In the present study, packing materials were collected from the biofilter in October 2009 (S1), December 2009 (S2), April 2010 (S3), and June 2010 (S4) for microbial analysis. The amounts of *H₂S* degraders on the packing material were calculated by culture on the medium, as described in Section 2.4.1. Their community diversities were assayed by PCR-DGGE and dominant groups were identified by 16S rRNA method.

The amount of *H₂S* degraders on the packing material were 9.85×10^7 CFUs g^{-1} (for S1), 9.5×10^6 CFUs g^{-1} (for S2), 2.13×10^7 CFUs g^{-1} (for S3), and 3.92×10^8 CFUs g^{-1} (for S4). Microbial growth is a consequence of uptake and metabolism of the pollutants in air. They will grow vigorously in an environment where food is abundant (Devinny et al. 1999). S1, S2, S3, and S4 were sampled in autumn, winter, spring, and summer, respectively. As the concentrations of *H₂S* in spring, summer, and autumn were far higher than that in winter, the *H₂S* degraders grew rapidly and became more abundant during those seasons. The

amount of *H₂S* degraders decreased obviously in winter due to the low inlet concentration of *H₂S*. In addition, the microorganisms required temperature for growth and activity to carry out biodegradation. The *H₂S* in the inlet stream served as an energy source and building material. Microorganisms will strive to grow and reproduce under optimal temperatures. Thus, suitable summer temperatures resulted in the abandonment of *H₂S* degraders.

3.3.2 *Microbial Community Diversity in the Biofilter*

The PCR-DGGE was used to generate genetic fingerprints for bacterial compositions including the culturable and nonculturable microbes in the biofilter (Fig. 7a). The relative diversity of the bacterial community was related to the number of bands in the gel, while the degree of abundance constituting each bacterial group was correlated to specific band intensity (Zhang and Fang 2001; Fang and Liu 2002). Shannon indices were used to reflect biodiversity of the bacterial groups

Fig. 7 **a** DGGE profile and Shannon indices of bacterial community in the biofilter. **b** Dendrogram of similarity

(Fig. 7a). Six intense bands were observed from lane S1, and its Shannon index was 2.01. S1 was sampled just after the adoption period, whereas S2, S3, and S4 were collected when the biofilter had been operational for 3, 7, and 9 months, respectively. The band patterns of lane S2, S3, and S4 were more complex in comparison to bands of lane S1, with Shannon indices of 2.07, 2.32, and 2.22, respectively. Some of the new bands observed in lane S3 and S4 did not appear in S1. In leachate odor control applications, the most prevalent contaminant is hydrogen sulfide, although other organic odorous compounds, such as ethanethiol, styrene, and BTEX, are typically present as well. If a biofilter removes more than one pollutant from the air, the number of microbial species is multiplied accordingly. The increased bacterial diversity of S2, S3, and S4 with time likely related to the complex substrate in the odors.

Distant genetic evolutionary relationships between lanes are shown in Fig. 7b. In lane S2, the bacteria community formed after 3 months of operation and presented a close inherent evolutionary relationship with lane S1. Lane S3 and S4 showed close evolutionary relationships to each other but evident distinction

to that of lane S1 and S2, indicating that bacterial community diversity in the biofilter differed significantly after 7 months of operation.

3.3.3 Dominant Groups and Their Distribution Characteristics

Eight discriminable DGGE bands (S1–S8) were excised, reamplified, cloned, and sequenced to determine identities of dominant bacterial populations. Their sequences were matched with their closest relative in GenBank. The strain name, nucleotide sequence similarity, and isolation source of dominant bacteria are listed in Table 2. These eight bacteria are frequently found in water and soil environment.

The sequences of three DGGE bands represented by B4, B7, and B8 were related to *Pseudomonas* sp. LBUC (98 %, similarity), uncultured bacterium clone A11 (100 %, similarity), and *Lysinibacillus* sp. C250R (93 %, similarity), respectively. These dominant bacteria species originated in S1 and survived and dispersed over the 9 months operation period. Among them, band B4 (*Pseudomonas* sp. assignation) was noteworthy. Previous research isolated *Pseudomonas* sp. from

Table 2 The closest relatives and isolation sources of dominate bacteria

Band no.	Closest relatives	Source	Similarity (%)
B1	<i>L. sphaericus</i> strain BGCs117	Bacteria; Firmicutes; Bacillales; Bacillaceae; <i>Lysinibacillus</i>	98
B2	Uncultured <i>Pseudaminobacter</i> sp. clone GW-01	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae; <i>Pseudaminobacter</i> ; environmental samples	100
B3	<i>Bacillus</i> sp. Ob 06	Bacteria; Firmicutes; Bacillales; Bacillaceae; <i>Bacillus</i>	100
B4	<i>Pseudomonas</i> sp. LBUC	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; <i>Pseudomonas</i> .	98
B5	<i>Achromobacter</i> sp. QUEBA08	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae; <i>Achromobacter</i> .	98
B6	<i>Streptomyces</i> sp. AQB.SKKU 20	Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Streptomycineae; Streptomycetaceae; <i>Streptomyces</i> .	99
B7	Uncultured bacterium clone A11	Bacteria; environmental samples	100
B8	<i>Lysinibacillus</i> sp. C250R	Bacteria; Firmicutes; Bacillales; Bacillaceae; <i>Lysinibacillus</i>	98

activated sludge or soil for H₂S or dimethyl disulfide-containing odor control (Ding et al. 2008; Honma and Akino 1998; Chung et al. 1996a, b; Ito et al. 2007). The *Lysinibacillus* sp. C250R has been identified from microbial communities in soil, while uncultured bacterium clone A11 has been found in activated sludge from municipal wastewater sewage. *Lysinibacillus* sp. is frequently found in ethanethiol treatment. Band B5 and B6 were closely associated with *Achromobacter insolitus* (98 %, similarity) and *Streptomyces* sp. AQB.SKKU 20 (98 %, similarity). *Achromobacter* sp. has been reported for aerobic degradation of monoaromatic hydrocarbons (e.g., BTEX) in a bioscrubber (Nielsen et al. 2006; Farhadian et al. 2008). *Streptomyces* sp. play an important role in the degradation of organic matter, most commonly noted in geosmin-like and styrene removal (Saadoun and El-Migdadi 1998; Przybulewska et al. 2006). Little difference was observed for B5 and B6 besides the band intensity change in lane S1 to lane S4. Ethanethiol and styrene could be detected from the leachate odor in summer. The average concentrations were 0.75 mgm⁻³ for ethanethiol and 3.61 mgm⁻³ for styrene, respectively. Over 95 % of ethanethiol and styrene in odors could be reduced by the biofilter in the present study. The appearance of *Lysinibacillus* sp. and *Streptomyces* sp. was probably due to the presence of ethanethiol and styrene.

Band B3 had a similarity of 100 % with *Bacillus* sp. Ob 06. Due to their biodegradation of organic compounds and hazardous substances, *Bacillus* is widespread in water (Shokrollahzadeh et al. 2008; Zulkifly et al. 2010). It presented in lane S1, disappeared in S2, and then emerged in S3 and S4. As S2 was sampled in winter, sensitivity to the low temperature may have contributed to its absence in S2. New bands B1 and B2 appeared in lane S4 and lane S3, respectively. They were identified as *Lysinibacillus sphaericus* strain BGCs117 (98 %, similarity) and uncultured *Pseudaminobacter* sp. clone GW-01 (100 %, similarity), respectively. *L. sphaericus* strain T12-16 had been isolated in microorganisms from soil, while uncultured *Pseudaminobacter* sp. clone GW-01 was reported as a bacterium in ground water. *L. sphaericus* strain RG-1 isolated from activated sludge could biodegrade ethanethiol

(Wan et al. 2010). Thiosulfate, an intermediate of H₂S bio-oxidation, could be oxidized directly to sulfate by the *Pseudaminobacter* sp. without the formation of any detectable intermediate sulfur compounds (Deb et al. 2004; Ghosh et al. 2006; Mandal et al. 2007).

Depending on the nature of the leachate, the odor compounds released can consist of any combination of ammonia, amines, hydrogen sulfide, organic sulfides, mercaptans, and BTEX in a wide range of concentrations. Competition may occur when mix contaminants are treated within one bioreactor. Contaminants will compete with each other for active adsorption sites that enable contact with the microorganisms in the biofilter. If the first substrate is present in high concentration and provides abundant energy, the microorganisms that use it may compete vigorously for space and nutrients, preventing microorganisms that use the second substrate from growing. Mix contaminants may require different microorganism species and many metabolic steps for transformation. The complex composition of treated odors promoted an abundance of bacteria in the biofilter; therefore, bands increased and new ones appeared in lane S2, S3, and S4, which is in agreement with the Shannon index results gained from the DGGE profile.

The DGGE fingerprint patterns obtained in this study showed that the bacterial community composition in the biofilter was highly affected by the biodegradation of H₂S and ambient temperature, and shifted throughout the operation period. The increased abundance of band B3 after 7 months of operation and B4 after 9 months of operation corresponded well with the high H₂S removal efficiency in the biofilter. The resulting low temperature was likely detrimental to the growth of some microorganisms which would, in turn, decrease the Shannon index in B2. High microbial diversity is essential for efficient degradation potential of a biofilter because microorganism variety allows the microbial community to respond to variable conditions.

3.4 Products of H₂S Degradation

In the case of the hydrogen sulfide removal system, the following overall biological reactions occur (Eqs. 1, 2, and 3). The oxidation supplies energy to the cell

and produces odorless compounds:

The polyurethane foam cubes with 40–80 % of water content rate were packed in the biofilter and acted as packing material. H_2S dissolved in water when passing through the packing material, giving the sulfide ion S^{2-} and HS^- . Then they were bio-oxidized into elemental sulfur and sulfate by microorganisms attached growth on packing material. The process of H_2S removal in the biofilter was very complex including physical, chemical, and biological processes.

The main products of H_2S bio-oxidation are elemental sulfur and sulfate (Elias et al. 2002; Chung et al. 1996a, b). Hydrogen sulfide is slightly soluble in water and acts as a weak acid, giving the sulfide ion S^{2-} . Therefore, S^{2-} was also detected from the bioreactor when H_2S dissolved in the liquid phase. The concentrations of SO_4^{2-} and S^{2-} in the integrated bioreactor were analyzed periodically. The variation of SO_4^{2-} within one irrigation period is shown in Fig. 8. The pH in the biofilter was also recorded to evaluate the production of H^+ .

Results revealed that S^{2-} concentration remained stable constant with an average of 0.62 mg L^{-1} . The pH value decreased gradually from 6.9 to 4.1 with the formation of SO_4^{2-} within 7 days. The oxidation of H_2S produced sulfuric acid and resulted in a reduction in the pH value. Most sulfur-containing compounds presented in the form of SO_4^{2-} . At the same time, it could be observed that yellow substance accumulated on the packing material and deposited in the liquid, which was supposed to be S^0 .

Pseudomonas sp. dominated throughout the operation period. Previous research has found that the main product of H_2S biodegradation by *Pseudomonas* sp.

Fig. 8 pH and sulfate variation within one irrigation period

was sulfur (Chung et al. 1996a, b). High concentration of SO_4^{2-} probably generated in H_2S or sulfur degradation by other bacteria appeared in the biofilter. Further research is required to determine the possible role of each of the bacteria detected in H_2S transformation.

4 Conclusions

The H_2S generated from the leachate equalization basin was reduced effectively by a full-scale biofilter, and a removal efficiency of over 90 % was obtained. Temperature in the biofilter was closely related to the H_2S inlet load and removal efficiency. Preheating of the gases entering the biofilter and prevention of thermal shock via insulation maintained the temperature in the biofilter to above 10°C , even in winter.

Water content of the packing materials was controlled to avoid excessive wetting or drying of the filter bed without prehumidity facility. According to the variation of WCR and RH in the biofilter, the interval of irrigation can be selected to fulfill microbial activity and reduce pressure drop and energy consumption.

The DGGE fingerprint patterns obtained in this study showed that the bacterial community composition in the biofilter was highly affected by H_2S removal and temperature, and shifted throughout the operation period. The optimization of biofilter and characterization of microbial communities described in this present study are crucial for development of

reliable measures of biofilter performance in other applications.

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