



## Simultaneous removal of hydrogen sulfide and toluene in a bioreactor: Performance and characteristics of microbial community

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### Abstract

We investigated the correlation between performance and the bacteria community composition by H<sub>2</sub>S and toluene co-treatment. Operation of the bioreactor was divided into four stages, in which the inlet concentration of toluene and H<sub>2</sub>S were gradually increased. In Stage I, toluene was the sole target compound with an average removal efficiency of 86.49%. After adding H<sub>2</sub>S in Stage II, removal efficiency of toluene decreased immediately and recovered gradually to 85.96%. When the inlet concentration of toluene and H<sub>2</sub>S was increased in Stage III and Stage IV, respectively, the average removal efficiency for toluene increased continuously from 86.31% to 87.24%. The elimination capacities of toluene increased with increasing inlet loading rates of toluene and H<sub>2</sub>S. Results of the PCR-DGGE analysis showed a turnover growth and decline of the microbial populations in the bioreactor. In Stage I, the dominant toluene-degrading bacteria mainly contained *Pseudomonas* sp. strain PS+ and *Hydrogenophaga* sp. In Stage IV, however, the dominant toluene-degrading bacteria was aciduric bacteria (*Clostridium populeti*). The dominant microbial community in the bioreactor enhanced the elimination capacity of toluene, and adding H<sub>2</sub>S changed the environment of microbial growth, thus resulted in an evolution of dominant microorganisms. Analyses of microbial community and their activities provides valuable information to efficiently enhance simultaneous removal of toluene and H<sub>2</sub>S in the bioreactor.

**Key words:** off-gas treatment; bacterial community; hydrogen sulfate; toluene

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### Introduction

Air pollution is a growing environmental issue and global policies aim to reduce the emission of volatile and gaseous compounds. Volatile organic compounds (VOCs) and odorous compounds have gained much attention because of their direct impact on human health and the environment (Jenkin et al., 2008). Air pollution emissions from publicly owned treatment works (POTWs) are a mixture of VOCs such as toluene and odorous compounds, for example hydrogen sulfide (H<sub>2</sub>S) (Liang et al., 2007). Many studies have focused on the elimination of either toluene or H<sub>2</sub>S from off-gases, and bioreactors are universally used because of their simplicity and cost effectiveness (Dorado et al., 2008; Duan et al., 2006; Groenestijn and Kraakman, 2005; Morgan-Sagastume and Noyola, 2006; Pedersen and Arvin, 1997). Various physicochemical parameters and characteristics of microbial communities have been investigated to optimize bioreactor performance (Auria et al., 2000; Morales et al., 2003).

Investigating performance and the microbial community in treating toluene vapor in a biofilter revealed that bacteria

numbers increased while moulds and actinomyces decreased with increasing initial moisture content of packing material (Sun et al., 2002). Specific 16S rRNA probes were used to analyze the toluene-degrading population in a laboratory-scale biofilter for waste gas treatment. Results indicated that *P. putida* was responsible for a significant part (65%) of the toluene degraded by the entire community (Møller et al., 1996). Sercu et al. (2006) studied the microbial composition of the most active and abundant bacteria in H<sub>2</sub>S-removing biofilter by denaturing gradient gel electrophoresis (DGGE). Dominant *ribotypes* were sequenced and identified as the possible key players in H<sub>2</sub>S removal.

Recent research has found that toluene and H<sub>2</sub>S can be removed simultaneously in a single bioreactor. Cox and Deshusses (2002) investigated co-treatment effects of H<sub>2</sub>S and toluene in a biotrickling filter, which indicated that H<sub>2</sub>S did not affect toluene degradation under either neutral or acidic conditions. Liang et al. (2007) used biological activated carbon as a biofilter packing material to treat off-gas with toluene and H<sub>2</sub>S, and demonstrated effective simultaneous treatment of toluene and H<sub>2</sub>S at low pH. However, H<sub>2</sub>S is usually oxidized by *Thiobacillus* species,

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which exhibit optimum activity at acidic pH (Islander et al. 1991), and toluene-degrading bacteria such as *P. putida*, which exhibit optimum activity at neutral-pH. Therefore, although co-treatment of toluene and H<sub>2</sub>S in a bioreactor has been achieved, little is known on the characteristics of microbial community for simultaneous removal of toluene and H<sub>2</sub>S. The aim of this study was to investigate the correlation between bioreactor performance and community structure by polymerase chain reaction (PCR)-DGGE.

## 1 Materials and methods

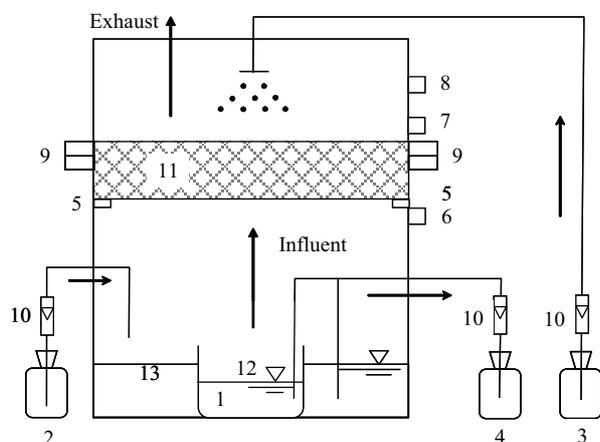
### 1.1 Experimental setup and operational conditions

This study was carried out in a laboratory-scale bioreactor made of a Plexiglas column with 0.32 m in height and 0.15 m in diameter (Fig. 1). Polyurethane foam 0.045 m thick was packed in the bioreactor as a carrier of microbial growth. Two sampling ports for measuring inlet or outlet gas concentrations were set at the bottom and top of the carrier, respectively. Two containers, one filled with toluene and one with H<sub>2</sub>SO<sub>4</sub>, were placed at the bottom of the bioreactor for H<sub>2</sub>S generation. The inlet H<sub>2</sub>S concentration was controlled by adjusting the dripping rate of Na<sub>2</sub>S solution using a peristaltic pump, and the inlet toluene concentration was varied by changing the container surface. The bioreactor operated for 84 days, which was divided into four stages and a step increase in inlet toluene and H<sub>2</sub>S concentrations was implemented.

Stage I (day 1–32): operating term was 32 days, toluene was the only pollutant in the inlet, and its concentration was 2.7–3.0 g/m<sup>3</sup>.

Stage II (day 33–51): operating term was 19 days, the inlet concentration of toluene was increased to 3.0–3.4 g/m<sup>3</sup>, and H<sub>2</sub>S of 0.05–0.07 g/m<sup>3</sup> was added into the inlet gas.

Stage III (day 52–day 63): operating term was 12 days, the inlet concentration of toluene was continuously increased to 4.0–4.2 g/m<sup>3</sup>, and the concentration of H<sub>2</sub>S was increased to 0.16–0.20 g/m<sup>3</sup>.



**Fig. 1** Schematic diagram of the bioreactor. (1) liquid toluene; (2) sodium sulfide solution; (3) nutrient tank; (4) collection of waste liquid; (5) the support for carrier; (6), (7), (8) sampling port; (9) flange; (10) peristaltic pump; (11) carrier; (12) container for liquid toluene; (13) container for sulfate solution.

Stage IV (day 64–day 84): operating term was 21 days, the inlet concentration of toluene was retained in 3.9–4.1 g/m<sup>3</sup>, and the concentration of H<sub>2</sub>S was increased to 0.35–0.38 g/m<sup>3</sup>.

To quickly start the bioreactor, the microorganism from a biofilter treated xylene off-gas as the inoculum was seeded on polyurethane foam carrier. The nutrient solution was regularly sprayed on the surface of the carrier to maintain microorganism activity. The nutrient solution had the following composition: (per liter water): 540 mg KH<sub>2</sub>PO<sub>4</sub>, 1380 mg K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 1000 mg NaCl, 25 mg MgSO<sub>4</sub>, 20 mg CaCl<sub>2</sub>, 5 mg FeSO<sub>4</sub>·4H<sub>2</sub>O, 2 mg MnSO<sub>4</sub>·7H<sub>2</sub>O, and 1 mL/L trace element solution. The solution pH was adjusted to 7.0 by adding 0.1 mol/L of NaOH or 0.1 mol/L of HCl. The bioreactor was continuously operated at ambient temperature between 18 and 30°C.

### 1.2 Analytical methods

#### 1.2.1 Gas and liquid samples

Toluene gas concentration was analyzed by a chromatography unit (Agilent GC-6890, USA) equipped with a flame ionization detector (FID) and DB-FFAP capillary column (30 m × 0.32 mm × 0.25 μm Hewlett Packard, USA). Nitrogen with a flow rate of 60 mL/min was used as the carrier gas. The concentration of CO<sub>2</sub> were determined by a gas chromatography (GC) unit (Agilent 6890N, USA) equipped with a 2-m stainless-steel column packed with Porapak T and thermal conductivity detector (TCD). Helium with a flow rate of 10 mL/min was used as the carrier gas. The GC oven temperature was set to 60°C. The injection and detector temperatures were set at 100 and 200°C, respectively. Column temperature for acetic acid analysis was held at 70°C for 1 min and then increased at a rate of 20°C/min to 180°C. The injector and detector were maintained at 240 and 260°C, respectively. Nitrogen was employed as the carrier gas at a flow rate of 2.6 mL/min, which remained constant via electronic pressure control. Concentrations of SO<sub>3</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup> were determined according to the standard method (APHA, 1998). The pH value was measured by a pH meter (pH-10 Satorius, Germany).

#### 1.2.2 Microbiological analysis

DNA extraction was conducted following manufacturers instruction in the test kit (Omega, USA).

#### Polymerase chain reaction (PCR) amplification

Chains 357f (5'-CGCCCCGCGCGCGCGGGCGGGCGGGGGCACGGGGGGCCTACGGGAGGCAG-CAG-3') and 518r (5'-ATTACCGCGGCTGCTGG-3') were used to amplify the segment of 16S rDNA (Sanchez et al., 2007). The GC clamp was added to the forward primers to facilitate the DGGE. The PCR amplification reaction was performed using an MJ Research PTC-200 Peltier thermal cycler (Bio-Rad, USA) at a final volume of 50 μL. The reaction mixture contained 20 pmol of primers, 20 μmol of each dNTPs, 5 μL of 10 × buffer (TaKaRa, China), and 1.25 units of Taq DNA polymerase (TaKaRa, China). The temperature cycling conditions were 94°C for 3 min, followed by 31 cycles of 94°C for 30 sec, 55°C for

30 sec, 72°C for 1 min, and a final extension at 72°C for 10 min. Approximately 5  $\mu\text{L}$  aliquot of the PCR product was separated on a 0.8% (W/V) agarose gel at 100 V for 30 min to verify amplification prior to DGGE. The PCR product generated from each sample was separated on an 8% acrylamide gel with a linear denaturant gradient increasing from 30% to 60% using the Bio-Rad D-GENE System (USA). The DGGE was performed using 30  $\mu\text{L}$  of the PCR product in  $1 \times \text{TAE}$  buffer at 60°C and 100 V for 750 min. The resulting gel was visualized with Gel Red (Biotium USA). The digital images were captured by the Fluor-S MultiImager (Bio-Rad, USA). The relative band densities of DGGE bands (Qty) were calculated by Quantity One Software (Bio-Rad, USA).

### Sequence analysis of bands excised from DGGE

Prominent DGGE bands were selected and used for excision. The reamplified products were purified by a Gel Recovery Purification Kit (Watson Biotechnologies Inc, Shanghai, China) and cloned by the pMD18-T plasmid vector system (Ta-KaRa, China) according to the manufacturer's instructions. The cloned PCR fragments were sequenced by an ABI-Prism model 3730 automatic sequencer (PerkinElmer, USA). Gene sequence chromatographs were analyzed using the CHROMAS LITE software package version 2.01 ([http://www.technelysium.com.au/chromas\\_lite.html](http://www.technelysium.com.au/chromas_lite.html)) and the resulting sequences' closest relatives were identified by NCBI nucleotide-nucleotide BLAST (<http://www.ncbi.nlm.nih.gov/>). All 16S rRNA gene sequences were deposited in the database under the accession numbers FJ873130–FJ873147.

### 1.2.3 Statistical analysis

Bacterial diversity was estimated from the intensity and number of bands using the Shannon index ( $H$ ) described by Ding et al. (2008).

$$H = - \sum (n_i/N) \ln(n_i/N) \quad (1)$$

where,  $n_i$  is the peak height of each band,  $i$  is the number of bands in each DGGE gel profile, and  $N$  is the sum of the peak heights in a given DGGE gel profile.

### 1.3 Evaluation method

As toluene was a saturated liquid, the vaporization velocity ( $Q$ ) and vaporization concentration ( $C$ ) could be controlled by adjusting volatile surface. As air movement and ambient temperature remain unchanged,  $C$  and  $Q$  tend to be constant when the volatile surface of liquid is constant. The performance of the bioreactor was evaluated

by a series of parameters, such as reduce efficiency (RE, %); inlet load (IL,  $\text{g}/(\text{m}^3 \cdot \text{hr})$ ); empty bed residence time (EBRT, sec), and elimination capacity (EC,  $\text{g}/(\text{m}^3 \cdot \text{hr})$ ). The definitions for these parameters are as follows:

$$Q = \frac{V}{T} \quad (2)$$

$$\text{RE} = \left( \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}} \right) \times 100\% \quad (3)$$

$$\text{EC} = Q \times \frac{C_{\text{in}} - C_{\text{out}}}{V_c} \quad (4)$$

$$\text{IL} = \frac{Q \times C_{\text{in}}}{V_c} \quad (5)$$

$$\text{EBRT} = \frac{V_c}{Q} \quad (6)$$

$$M = \frac{C_m}{C_t} \times 100\% \quad (7)$$

where,  $Q$  ( $\text{m}^3/\text{hr}$ ) is the vaporization velocity of toluene gas;  $V$  ( $\text{m}^3$ ) is the volume of toluene saturated liquid;  $V_c$  ( $\text{m}^3$ ) is the volume of the carrier;  $T$  (sec) is the vaporization time of saturated liquid toluene totally evaporated to gas state;  $C_{\text{in}}$  ( $\text{g}/\text{m}^3$ ) is the toluene concentration at down surface of the carrier;  $C_{\text{out}}$  ( $\text{g}/\text{m}^3$ ) is the toluene concentration at up surface of the carrier;  $M$  is the  $\text{CO}_2$  conversion rate of toluene;  $C_m$  ( $\text{g}/\text{m}^3$ ) is the measured concentration of  $\text{CO}_2$ ; and  $C_t$  ( $\text{g}/\text{m}^3$ ) is the theoretical concentration of  $\text{CO}_2$ .

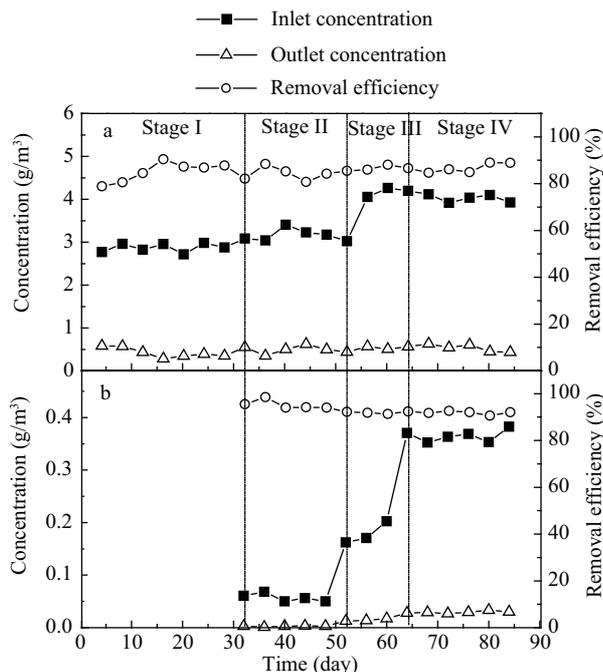
## 2 Results and discussion

### 2.1 Performance of the bioreactor

The steady operation of the bioreactor started quickly due to the adaptation of toluene-degrading bacteria initially inoculated into the bioreactor. Figure 2 shows the performance of the bioreactor, including the removal efficiencies of toluene and  $\text{H}_2\text{S}$  under different inlet concentrations of toluene and  $\text{H}_2\text{S}$ . The average removal efficiencies of toluene and  $\text{H}_2\text{S}$  in each operational stage are listed in Table 1. In Stage I, toluene was the sole pollutant. Approximately 80% of toluene could be removed within one day of toluene biodegradation. Toluene removal increased significantly with extension of operational time and reached a steady state after 10 days. The average removal efficiency of toluene was 86.49% in Stage I. However, the removal efficiency of toluene decreased immediately from 87.78% to 82.10% after adding  $\text{H}_2\text{S}$ . Twenty days later, it began to rebound and recovered gradually to 85.96%. Toluene removal was enhanced even though the concentration of toluene and  $\text{H}_2\text{S}$  increased. The average removal efficien-

**Table 1** Removal efficiency and products of toluene and  $\text{H}_2\text{S}$

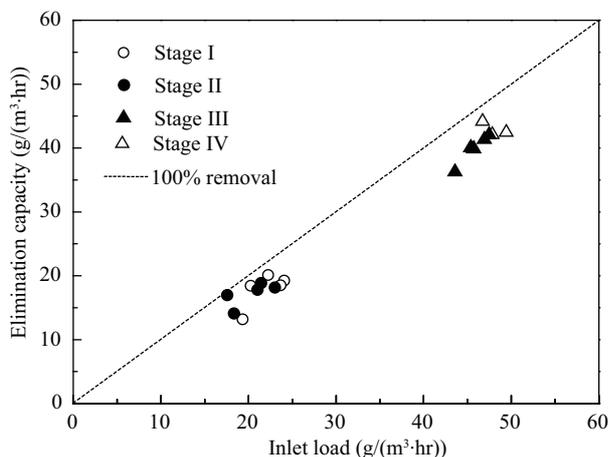
	Time	Stage I	Stage II	Stage III	Stage IV
Toluene removal	Inlet concentration ( $\text{g}/\text{m}^3$ )	2.7–3.0	3.0–3.4	4.0–4.2	3.9–4.1
	Average removal efficiency (%)	86.49	84.81	86.31	87.24
	Average acetic acid (mg/L)	84.62	145.65	114.82	109.64
$\text{H}_2\text{S}$ removal	Average sulfate (mg/L)	–	1380	1670	3680
	Inlet concentration ( $\text{g}/\text{m}^3$ )	0	0.05–0.07	0.16–0.20	0.35–0.38
	Average removal efficiency (%)	–	93.62	91.85	91.84



**Fig. 2** Profiles of toluene (a) and H<sub>2</sub>S (b) removal efficiencies and concentrations in the inlet and outlet of the bioreactor.

cies of toluene were 86.31% (Stage III) and 87.24% (Stage IV). The H<sub>2</sub>S removal was steady during this operation, and average removal efficiencies in Stage II, III and IV of operation were 93.62%, 91.85% and 91.84%, respectively.

Elimination capacity is an important parameter to evaluate bioreactor performance. The increase in toluene and H<sub>2</sub>S concentrations resulted in an increase in inlet loading rate of the bioreactor. Results in Fig. 3 show that the elimination capacity of the bioreactor for toluene removal increased with increasing inlet loading rates. In Stage I, the average inlet loading rate of toluene was 20.30 g/(m<sup>3</sup>·hr), and average elimination capacity of toluene was 17.16 g/(m<sup>3</sup>·hr). In Stage IV, the average inlet loading rate of toluene and H<sub>2</sub>S increased to 47.01 and 4.30 g/(m<sup>3</sup>·hr), respectively, and average elimination capacity of toluene and H<sub>2</sub>S achieved 41.73 and 3.91g/(m<sup>3</sup>·hr), respectively. The bioreactor removed toluene and H<sub>2</sub>S simultaneously with a high elimination capacity.



**Fig. 3** Removal capacity of toluene at different stages.

## 2.2 Characteristics of products

Biodegradation of toluene produces CO<sub>2</sub>, water, and metabolites, while bio-oxidation of H<sub>2</sub>S produces sulfate. Measuring the products can provide insight into the performance of the bioreactor. The CO<sub>2</sub> conversion rate ( $M$ ) can be used to estimate biodegradation degree of toluene. Theoretically, 7 mol of CO<sub>2</sub> will be obtained when 1 mol of toluene is degraded completely (Reaction (8)).



Besides CO<sub>2</sub>, the products of toluene degradation include metabolites and biomass. In this study, acetic acid was the main metabolite for toluene degradation. The average concentrations of acetic acid and sulfate are listed in Table 1.

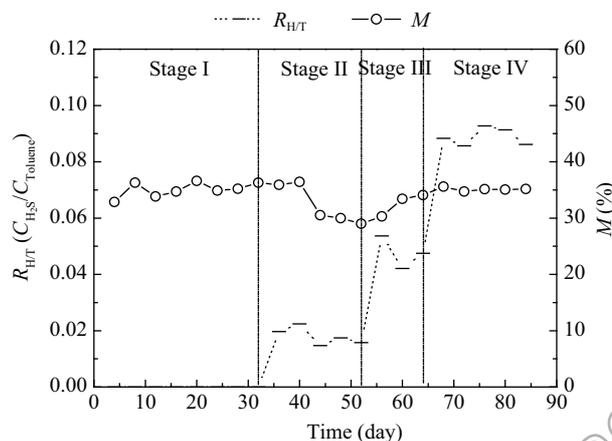
Figure 4 describes  $M$  variation with the ratio of H<sub>2</sub>S and toluene concentration ( $R_{H/T}$ ) in each stage. In most of the running time,  $M$  remained at 35%. After adding H<sub>2</sub>S, however,  $M$  decreased to 30% before gradually recovering again to 35%. The results in Fig. 4 show no evident correlation between the change of  $M$  and  $R_{H/T}$ . However,  $M$  correlated with the change of acetic acid. In Stage I, the average concentration of acetic acid was 84.62 mg/L. In Stage II, the concentration of acetic acid increased to 145.65 mg/L with decreasing  $M$ . In Stage III and IV, the concentration of acetic acid decreased to 114.82 and 109.64 mg/L, respectively, with increasing  $M$  (Table 1).

The product of H<sub>2</sub>S was sulfate during aerobic biodegradation. The concentration of sulfate increased from 1380 mg/L (in Stage II) to 3680 mg/L (in Stage IV) with increasing H<sub>2</sub>S inlet concentration (Table 1). In addition, the pH value of Stage I was 6.45. After adding H<sub>2</sub>S, the pH value of Stage II, III and IV were 2.85, 2.92 and 2.69, respectively, in the bioreactor.

## 2.3 Bacterial community composition in the bioreactor

### 2.3.1 Bacterial diversity and community composition

Traditional plate-counting used to quantify microorganisms may underestimate the actual number of bacteria as only cultivable and viable cells were detected on the agar plates used, thus possibly constituting only a part of the



**Fig. 4** Carbon dioxide conversion rate ( $M$ ) of toluene under different  $R_{H/T}$  values.  $M = C_m/C_t \times 100\%$ ;  $R_{H/T} = C_{H_2S}/C_{Toluene}$ .

total population present in the biofilters (Ho et al., 2008). The PCR-DGGE was used to generate genetic fingerprints to provide information on bacterial compositions including the culturable and nonculturable microbes in the bioreactor. The DGGE gel profile was also measured statistically by the Shannon index, which is commonly used to characterize species diversity in microbial communities.

Figure 5 shows the DGGE profiles of the bacterial communities in each stage. Lane "Original" shows community fingerprints of original inoculated bacteria isolated from a xylene-degrading bioreactor in our laboratory. Lanes I, II, III and IV present the bacterial community in Stages I, II, III and IV, respectively. The intensity of a band represents the relative abundance of the corresponding microbial species (Fang and Liu, 2002; Zhang and Fang, 2001). The corresponding Shannon indices are shown in Table 2. Because inoculated bacteria from a xylene-degrading bioreactor can also degrade toluene (Heider et al., 1998), the band patterns of inoculated bacteria were more complex in comparison with bands of Stage I. Nine intense bands were observed from Lane "Original", and its Shannon index was 2.49. Compared to Lane "Original", both band amount and Shannon index of Lane I declined slightly, with three remarkable bands and Shannon index of 2.44. Therefore, the bacterial diversity decreased in Stage I because toluene was the sole target compound. The

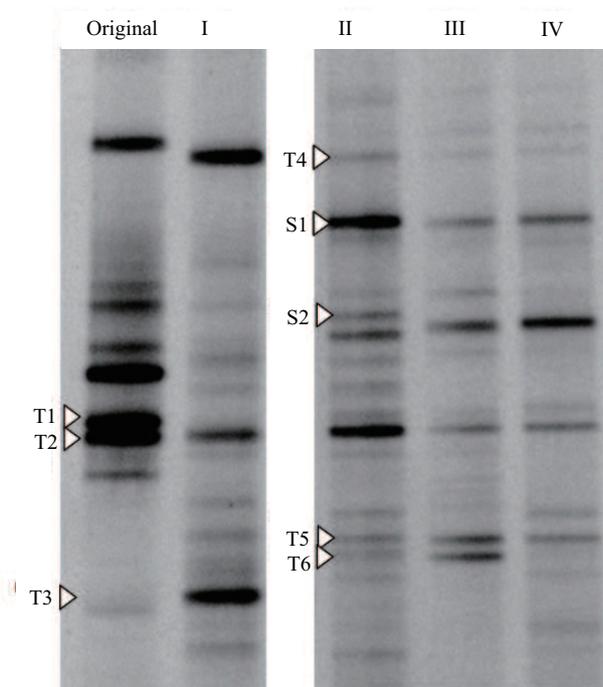
DGGE profile showed that most of the band observed in inoculated bacteria disappeared in Stage I.

After adding  $H_2S$ ,  $H_2S$ -degrading bacteria appeared and transformed  $H_2S$  into sulfate, which resulted in decreasing pH in the microbial carrier. Low pH was detrimental to the growth of toluene-degrading bacteria (Cox and Deshusses, 2002), which led to the immediate decrease of toluene removal efficiency. Thereafter, some toluene-degrading bacteria adapted to low pH, which resulted in the gradual recovery of toluene removal efficiency. The addition of  $H_2S$  promoted an abundance of bacteria population in the bioreactor; therefore, band amount increased and new bands appeared in Lane II. The Shannon index of Lane II increased to 2.85, and then dropped gradually with increasing  $R_{H/T}$  in Stage III and Stage IV. However, little difference was observed from the band patterns and band amount besides the band intensity change in Lane III and Lane IV. The increasing  $R_{H/T}$  did not influence diversity or the structure of bacterial community, although it evidently affected the concentration of functional bacteria. The amount of contaminant degraded was proportional to the number of bacteria produced in the bioreactor as kinetics of contamination is closely related to the kinetics of microbial growth.

### 2.3.2 Dominant bacteria in the bioreactor

To investigate pattern differences in detail, the discriminant bands of each Lane were extracted, reamplified, cloned, and sequenced. Seventeen sequences were recovered from DGGE bands. Their sequences were matched with the closest relative from the Genbank database. The relative quantity of bacteria was measured (Table 3) using Quantity One Software (Bio-Rad, USA), and was represented by the relative Qty. The strain name, nucleotide sequence similarity, and the relative quantity of dominant bacteria from each stage are listed in Table 3, in which six bacterial species (T1–T6) were related to toluene-degradation and two bacterial species (S1 and S2) were related to  $H_2S$ -degradation.

T1 was close to *Gordonia* sp. (98%), and had microbial characteristics of butyl benzyl phthalate degradation (Chatterjee and Dutta, 2003). T2 was identified to *Pseudomonas veronii* (100%), a long chain alkylphenol-degrading bacterium reported in previous work (Ajithkumar et al., 2003). T3 was assigned to *Pseudomonas* sp. strain PS+ (98%), as a toluene-degrading bacterium (Mattison and Harayama, 2001). T4 was related to *Hydrogenophaga* sp. (99%), which was a toluene-degrading bacterial strain (Fahy et al., 2008; Fries et al., 1997). T5 had a similarity of 99% with *Clostridium populeti*, and was assigned to a methoxylated aromatic compound-degrading bacterium, which had sulfide tolerant characteristics (Lomans et al., 2001). Belonging to *Bacteroides* sp. (100%), band T6 used to be detected from the bioreactor for the removal of benzene, toluene, ethylbenzene, and xylenes (BTEX), especially in the reactor containing polyurethane foam (Langenhoff et al., 1997; Oliveira et al., 2009). The S1 and S2 were similar to *Pseudomonas* sp. strain do-1 (100%) and *Dysgonomonas wimpennyi* (98%), respectively.



**Fig. 5** DGGE profile of bacterial community of bioreactor. Original refers to inoculum. The number above each gel column stands for the stage of operation. T1–T6 are bacterial species related to toluene-degradation. S1 and S2 are the bacterial species related to  $H_2S$ -degradation.

**Table 2** Shannon diversity index for the DGGE gel patterns at individual stages

Sampling points	Original	I	II	III	IV
Shannon index value	2.49	2.44	2.85	2.73	2.62

**Table 3** Relative Qty of dominant bacteria at individual stages

Band	Closest relatives	Similarity (%)	Relative Qty of DGGE bands				
			Original	Stage I	Stage II	Stage III	Stage IV
T1	<i>Gordonia</i> sp.	98	13.25	1.95	2.92	2.5	3.06
T2	<i>Pseudomonas veronii</i>	100	14	5.51	6.51	3.88	3.76
T3	<i>Pseudomonas</i> sp. strain PS+	98	4.38	11.62	–	–	–
T4	<i>Hydrogenophaga</i> sp	99	–	11.62	3.78	1.46	1.58
T5	<i>Clostridium populeti</i>	99	–	4.24	2.67	4.62	5.33
T6	<i>Bacteroides</i> sp	100	–	1.72	2.12	3.33	1.04
S1	<i>Pseudomonas</i> sp. strain do-1	100	–	–	8.78	4.79	4.99
S2	<i>Dysgonomonas wimpennyi</i>	98	–	–	3.12	6.23	6.36

*Pseudomonas* sp. strain do-1 was reported as a hydrogen sulfide-removing bacterium isolated from soil (Honma and Akino, 1998), while *Dysgonomonas wimpennyi* was found in the system of sulphate-reducing consortium (Boothman et al., 2006).

Emergence of T2 in the inoculated microbial community indicated that toluene-degrading bacteria existed in the bioreactor at the beginning of operation. In Stage I, in which toluene was fed into the bioreactor as the sole target compound, another two functional bacteria (T3 and T4) with high relative Qty of 11.62 appeared as the dominant toluene-degrading bacteria. However, T3 could not be detected during Stage II to Stage IV. The relative Qty of T4 declined gradually with H<sub>2</sub>S addition. In Stage IV, T4 was not the dominant bacteria. Another bacterium related to toluene-degradation, T5, emerged in Stage I and existed throughout the four stages. The relative Qty of T5 decreased in the presence of H<sub>2</sub>S, and then increased gradually. In Stage IV, T5 became the dominant toluene-degrading bacteria with a relative Qty of 5.33. The highest relative Qty (3.33) of T6 was discovered in the Stage III, and decreased to 1.04 in the Stage IV.

Two dominant sulfide-removing bacteria, S1 and S2, were observed in Stage II. After this, the relative Qty of S1 decreased from 8.78 (Stage II) to 4.99 (Stage IV), but the relative Qty of S2 increased from 3.12 (Stage II) to 6.36 (Stage IV) with the increase of  $R_{H/T}$ .

Cloning and sequencing of the selected predominant DGGE bands showed that six functional bacteria were detected in the bioreactor, including four bacterial species (T2, T4, T6 and T6) related to toluene-degradation and two bacterial species (S1 and S2) related to H<sub>2</sub>S-degradation. Based on the above results, multiple functional bacteria could effectively remove toluene, H<sub>2</sub>S and their corresponding metabolites. This suggests that a bacterial community consisting of different types of bacteria had a capacity to adapt to changes in gas composition and concentration.

### 3 Conclusions

Results in this study demonstrated that toluene and H<sub>2</sub>S could be removed simultaneously in a single bioreactor, with high removal efficiencies achieved for both (86% for toluene and 91% for H<sub>2</sub>S). Compared to when toluene was the sole target compound, the removal efficiency of toluene decreased immediately after adding H<sub>2</sub>S. After 20

days, removal efficiency rebounded and began to recover gradually. The elimination capacity of toluene increased with increasing inlet loading rates of toluene and H<sub>2</sub>S.

The dominant microbial community in the bioreactor played a crucial role in enhancing removal efficiency and elimination capacity. However, the dominant microbial community was interrelated with pollutant composition in the off-gases. Adding H<sub>2</sub>S led to the growth of H<sub>2</sub>S-degrading bacteria and change of microenvironment for microbial growth, which resulted in the evolution of dominant microorganisms in the bioreactor. The community structure changed after H<sub>2</sub>S addition. The dominant toluene-degrading bacteria changed from adapting neutral-pH species (*Pseudomonas* sp. strain PS+ and *Hydrogenophaga* sp.) to tolerating acidic-pH species (*Clostridium populeti*).

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