Temporal variation of microbial population in acclimation and start-up period of a thermophilic desulfurization biofilter

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ABSTRACT

Microorganisms in a biofilter play important roles in the gas contaminants treatment process. In this study, thermophilic desulfurization bacteria were inoculated in a thermophilic biofilter for SO₂ treatment after acclimation and enrichment. Molecular biology techniques were applied to detect temporal variation of microbial population during acclimation and biofilter start-up period. The acclimation temperatures were 50, 55, and 60 °C. Desulfurization bacteria dominated the enrichments. During acclimation, desulfurization bacteria and thermophilic bacteria increased from 36.84% to 78.95%–52.94% and 88.24%, respectively. In addition, the microbial diversity indices of these enrichments decreased with time. Substrate species and acclimation temperature influenced the microbial structure of the enrichments. The thermophilic biofilter inoculated with enrichments could achieve a rapid start-up, and over 80% of removal efficiency could be obtained within two weeks. The maximum elimination capacity was 38.71 g m⁻³ h⁻¹ at 152 mg m⁻³ inlet concentration. The total sulfur bacteria proportion increased obviously during the start-up period. Moreover, desulfurization bacteria that originally existed in inocula, e.g., *Pseudomonas putida*, *Bacillus thioarans*, *Microbacterium* sp., and *Thermoanaerobacteriaceae*, were abundant in the thermophilic biofilter for SO₂ removal.

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1. Introduction

Off-gas generated from most industrial and agricultural processes threatens human and ecological health and causes environmental pollution, such as odor nuisance, health effects, crop damage, smog formation, and global warming (Devinny et al., 1999; van Groenestijn and Hesselink, 1993).

Compared with physical (adsorption, absorption, and condensation) and chemical (catalytic combustion, scrubbing, and oxidation) methods, biotechnology provides a more economical and sustainable approach owing to its low operational costs, high removal efficiency, and lack of secondary pollutant generation (Li and Liu, 2006; Ralebitso-Senior et al., 2012). For most applications, biological air treatment systems have traditionally relied on microorganisms, generally bacteria, found under ambient environmental conditions (Devinny et al., 1999). An integrated bioreactor with two sections, a suspended aerobic zone (SZ) and immobilized aerobic zone (IZ), was applied to treat SO₂ for six

months. Microbial analysis indicated that SO₂ was degraded by *Paenibacillus* sp. in the IZ, and by *Paenibacillus* sp. and *Ralstonia* sp. in the SZ (Lin et al., 2015). Reactor stability and efficacy are maintained by functionally, compositionally, and spatiotemporally dynamic and diverse communities (Xue et al., 2013). Various factors affect the growth kinetics of participating microorganisms, including substrate concentration, moisture content, temperature, electron acceptor, pH, toxic intermediates, and availability of mineral nutrients. Any or a combination of these factors can limit biomass growth rate and substrate biodegradation (Beuger and Gostomski, 2009; Ottengraf, 1986; Sakuma et al., 2008).

Three important time-dependent conditions generally exist for biofilters: start-up response, response to varying loads, and long-term performance. “Start-up” time is the acclimation time required to establish an optimal biological removal. Depending on ambient and site conditions, this start-up procedure may last for weeks or months (Devinny et al., 1999). The start-up period depends on the amount of initial microbial population that can degrade the pollutants; thus, inoculation from an adapted population is desirable (Shareefdeen and Singh, 2005). As an inoculum source, sewage/activated sludge can be trickled over or inoculated directly into a support medium. Alternatively, inocula can be

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cultured in feed reactors to specific biomass concentrations before introducing waste gas. A six times-faster biofiltration start-up of volatile organic solvents can be achieved through inoculation with adapted microorganisms (Saake and Hübner, 1989). Wright et al. found that acclimation in compost biofilters is faster in treating gasoline vapors when the biofilters are inoculated with culture grown on gasoline (Wright et al., 1997). Leson and Smith reached a similar conclusion, suggesting that inoculation hastens acclimation but does not affect the ultimate removal efficiency (Leson and Smith, 1997).

However, few studies have focused on the characteristics of microbial population during acclimatization and bioreactor start-up period. SO₂, generated from the thermal conversion processing of fuels in power plants, refineries, coal- and oil-fired boilers, and paper pulp production, is harmful to the environment and the human health (Philip and Deshusses, 2003). Therefore, SO₂ was used as a target compound in the present study. Desulfurization bacteria were acclimated and enriched to inoculate a thermophilic biofilter to treat SO₂. The temporal variations of microbial population during acclimatization and biofilter start-up period were analyzed via polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), and fluorescence in situ hybridization (FISH) was applied to describe the microbial population distribution. Biofilter performance was also investigated after desulfurization bacteria inoculation. The aim of this present study is to provide data for rapid start-up and steady operation of the bioreactor, especially for the thermophilic biofilter.

2. Materials and methods

2.1. Acclimation method

Inoculum samples were collected from a bioreactor that contained SO₂ with three packing layers for off-gas treatment. From the bottom to the top layer, the operation temperatures were 60, 55, and 50 °C. The packing samples were obtained from each layer. The detail information of samples was described in Table 1. After transporting to the laboratory, the packing mediums were soaked in Luria–Bertani (LB) medium (BR, Aoboxing Biotech, Co., China). The mediums were then shaken for 1 h using ultrasonic oscillators (KQ-250B, Kunshan, China). The liquids with suspended cells were cultured in the same nutrient solution in incubator shakers (HZ-9211K, Taicang, China) at 50, 55, and 60 °C, and at 120 rpm to enrich the microorganisms.

Sulfur bacteria culture media (SCM) was used to enrich and acclimate the three inocula contained in the following components at the specified concentrations (in g L⁻¹): 5.00 Na₂S₂O₃·5H₂O; 1.00 NaHCO₃; 2.00 K₂HPO₄; 2.00 KNO₃; 0.01 FeCl₂·4H₂O; 0.10

MgCl₄·6H₂O; 0.50 NH₄Cl; and 5.00 beef extract. Given that S²⁻ was converted into H₂S and was lost as gas from aerobic enrichment, Na₂S₂O₃ was used instead of Na₂S as the sulfur substance. The initial pH of the media was adjusted to 6.5–7.0. Agar (2%) was added as a solidifying agent. The inocula were replaced monthly with fresh media at a ratio of 10:1 and were sampled regularly to analyze the microbiological indicator.

2.2. Bioreactor setup

After the inocula were enriched at 50, 55, and 60 °C for 12 months, the inocula were mixed at a ratio of 1:1:1 to inoculate into a thermophilic biofiltration (Fig. 1). The biofiltration was a stainless column with a height of 30 cm and a diameter of 10 cm. The packing material was polyurethane foam cube with an average size of 1.0 cm³. A high-pressure cylinder continuously supplied SO₂ to the biofiltration from the air inlet at the bottom of the column. A calibrated mass flow meter was used to control the gas flow rate, and a temperature-controlled oven was supplied to heat the SO₂ inlet. The total flow rate was 0.6 m³ h⁻¹, and the column was maintained at 60 °C through a thermostat-controlled heating belt. After inoculation, the SO₂ concentrations of the inlet and outlet were monitored online using a flue gas analyzer (rbr, Ecom-J2KN, Germany) to determine the removal efficiency. The performance of the bioreactor was evaluated by series parameters, which were defined as follows (formulas 1 and 2):

$$R = (C_{in} - C_{out}) \times 100 / C_{in} \quad (1)$$

$$EC = Q \times (C_{in} - C_{out}) / V \quad (2)$$

where *R* is the removal efficiency (%), *C_{in}* is the inlet concentration of SO₂ (mg m⁻³), *C_{out}* is the outlet concentration of SO₂ (mg m⁻³), *EC* is the elimination capacity (g m⁻³ h⁻¹), *Q* is the flow rate (m³ h⁻¹), and *V* is the volume of biofilter (m³).

2.3. Microbiological analysis

Bacteria were incubated in agar-containing LB medium (BR, Aoboxing Biotech, Co., China) at 60 °C for 24 h. Sulfur bacteria were cultivated in SCM medium at 60 °C for 3 d. The culture plates were placed in a biochemical incubator (SPX-70BIII, AISITE, Tianjin, China) for thermostatic incubation, and the amount of cells was counted and reported as colony-forming units (CFUs/ml).

2.3.1. DNA preparation and 16S rRNA gene amplification

The total DNA extraction was performed by Activated Sludge DNA Automatic Plate form for Magnetic System-16 (TanBead,

Table 1
Samples information.

No.	Consortium description	Time		Culture temperature (°C)
		Incubation	Thermophilic biofilter operation	
IO-50	Inoculum	—	—	50
IO-55	Inoculum	—	—	55
IO-60	Inoculum	—	—	60
DO-50	Enrichment	6 months	—	50
DO-55	Enrichment	6 months	—	55
DO-60	Enrichment	6 months	—	60
DT-50	Enrichment	12 months	—	50
DT-55	Enrichment	12 months	—	55
DT-60	Enrichment	12 months	—	60
T-1	Consortium from thermophilic biofilter	—	1 day	60
T-2	Consortium from thermophilic biofilter	—	11 days	60

IO: Inoculum; DO: Consortium after 6 months of enrichment; DT: Consortium after 12 months of enrichment; T: Consortium collected from thermophilic biofilter.

Fig. 1. Schematic diagram of the thermophilic biofilter (1: high-pressure cylinder; 2: mass flow meter; 3: oven; 4: gas mixer; 5: air pump; 6: air inlet; 7: biofiltration column; 8: packing layer; 9: heating belt; 10: thermostat; 11: air outlet; 12: sprinkler; 13: water outlet; 14: circulating pool; and 15: water pump).

Taiwan), and the DNA bands were determined via 1.0% agarose gel electrophoresis (Bio-Rad, USA). The V3 regions of the bacterial 16S ribosomal RNA gene of the microbial samples collected from the thermophilic biofilter were amplified directly through polymerase chain reaction (PCR) using a C1000TM Thermal Cycler (Bio-Rad, USA) with specific universal primers F357 (5'-CCTACGGGAGG-CAGCAG-3') and R517 (5'-ATTACCGGGCTGCTGG-3') with the forward primer F357 attached a GC clamp (5'-CGCCCGCCGCGCGCGCGCGGGCGGGGCGGGGCGGGGCGGGG-3'). Each 50 μ L reaction volume contained 5 μ L extracted DNA, 1 μ L of each of primer (10 μ M), 4 μ L of dNTP, 5 μ L 10 $\times$ Ex Taq buffer (Mg^{2+} Plus), and 1.5 U ExTaq-polymerase (TaKaRa, Dalian, China). The temperature cycling conditions were 94 $^{\circ}$ C for 5 min, followed by 30 cycles of 94 $^{\circ}$ C for 1 min, 48 $^{\circ}$ C for 1 min, and 72 $^{\circ}$ C for 1 min. A final extension at 72 $^{\circ}$ C for 10 min was also used.

2.3.2. Denaturing gradient gel electrophoresis-16S rRNA gene analysis

Denaturing gradient gel electrophoresis (DGGE) was performed on 8% (W/V) polyacrylamide gel with 40%–60% denaturing gradient of denaturants. Electrophoresis was performed in 1 $\times$ TAE (Tris-Acetate-EDTA) buffer at 60 $^{\circ}$ C at a constant voltage of 65 V for 16 h using a Bio-Rad DCode system (DCode, Bio-Rad, USA). The gels were stained with SYBR Green I. Digital images were captured using a Fluor-S Multimager (Gel Doc XR, Bio-Rad, USA) and were analyzed using Quantity One Software (Bio-Rad, USA).

The prominent DGGE bands were excised and dissolved in 35 μ L of Milli-Q water overnight at 4 $^{\circ}$ C. The target DNA fragments were reamplified through PCR using the primer set F357/R517 without the GC clamp. The PCR products were ligated into the pEASY-T5 vector and transformed into *Escherichia coli* TOP10 competent cells (Tiangen, Beijing, China). The clones were cultured on LB agar plates that contained X-gal (80 μ g/ml), isopropyl-beta-D-thiogalactopyranoside (0.5 mM), and ampicillin (100 μ g/ml). Positive colonies were sequenced using a 3730xL DNA analyzer (ABI, USA).

2.3.3. Phylogenetic analysis

Bacterial diversity was determined according to the intensity and number of bands using the Shannon index (H), as previously described (Li et al., 2011). Furthermore, 16S rRNA gene sequences from DGGE bands were analyzed using the Nucleotide–Nucleotide BLAST (Blastn) database (<http://www.ncbi.nlm.nih.gov/BLAST>) and the Seqmatch program and chimera check program of the Ribosomal Database Project (<http://rdp.cme.msu.edu/>). The sequences and the closest matches of these sequences retrieved from the database were aligned using the software Clustal X version 1.8 (Thompson et al., 1997). Phylogenetic trees were constructed using the neighbor-joining method with the software MEGA 6.1. Evolutionary distances of nucleotide sequences were calculated with Kimura 2-parameter model; bootstrap values were obtained with 1000 resamplings.

2.3.4. Fluorescence in situ hybridization

The FISH technique was used to assay the functional microbiota distribution in the consortium of microorganisms after acclimation. For the FISH experiments, leachate consortium that contained microorganisms and products were fixed with 4% paraformaldehyde at 4 $^{\circ}$ C for 12 h. The fixed consortium was washed thrice with phosphate-buffered saline (pH: 7.2), and then suspended in the same saline. Moreover, 10 μ L suspension including microorganisms was spotted as a layer on gelatin-coated glass slides. The air-dried consortium were consecutively dehydrated in 50%, 80%, and 100% ethanol. A hybridization solution that contained 5 ng μ L⁻¹ of probe was applied to the slide. The FISH procedure was similar to that described by Han (Han et al., 2012). The biomass on the slide was stained with DAPI solution. The FISH images were captured using confocal laser scanning microscopy and were analyzed using the LAS AF Lit software. Table 2 shows the list of the oligonucleotide probes applied and the respective target bacteria. The distribution and relative contents of *Pseudomonas* sp. and sulfur bacteria in the consortium were detected and analyzed.

Table 2

List of 16S rRNA gene-targeted oligonucleotide probes used (Saha et al., 2012; Zhao et al., 2009).

Probes	Fluorescent label	Sequence(5'-3')	FA(%)	Specificity
Pseudo120	FITC	ACTACCAGGCAGATTCTAGGCA	30	rRNA group 1 <i>Pseudomonads</i>
Thi-d	TR	TGCTGGTACCGTCATTAGCACACGATG	35	<i>Thiobacillus denitrificans</i>

FA: Formamide concentration.

3. Results and discussion

3.1. Microbial population of inocula and enrichments

DGGE analysis of the microorganism revealed community composition distinction from the initial biofilm inocula collected from different parts of the bioreactor (Fig. 2a). A total of 39 discriminable bands (B1 to B39) were identified, and the sequences of these bands were matched with the closest relatives of the bands in the GenBank database, as listed in Table 3. The phylogenetic tree derived from neighbor-joining analysis of the partial 16S rRNA gene sequence is shown in Fig. 3.

3.1.1. Inocula

For inocula, lanes IO-50, IO-55, and IO-60 in Fig. 2a represented

the inocula at 50, 55, and 60 °C, respectively. Among these inocula, 18 bands existed under 50 °C, 16 bands at 55 °C, and 16 bands at 60 °C. Seven kinds of bacteria coexisted in the three inocula with different cultured temperatures (IO-50, IO-55, IO-60). Band 26 was closely associated with *Bacillus thiarparans* (100%), which grew chemolithoautotrophically through oxidation of thiosulfate to sulfate (Pérez-Ibarra et al., 2007). Band 27 and 32 were assigned to *Microbacterium* sp. (100%), which was a DMS degrader, and the characteristics of the isolated strain for DMS degradation under batch and continuous conditions were determined (Kayser et al., 2002). A bioreactor inoculated with *Microbacterium* sp. and *Pseudomonas putida* can indeed improve the removal of high DMS concentrations (Shu and Chen, 2009). Band 35 and 38 were related to *Tuberibacillus calidus* (100%), which was a novel aerobic thermophilic *Bacillus*, and was isolated from the microbial community in a biofilter for hydrogen sulfide and methanol removal (Hatayama et al., 2006). Band 30 was affiliated with *Thermoanaerobacteriaceae* (100%), which was isolated from thermal volcanic spring, hot spring, and high-temperature leachate, and was found with the unique capability of reducing thiosulfate to elemental sulfur in acidic solutions (Shang et al., 2013; Ueno and Tataru, 2008). These four isolates were related to the desulfurization. Band 23 was identified as *Aeribacillus pallidus* (100%), which was a thermophilic bacteria usually isolated from hot spring- or crude oil-contaminated soil and was used as bioemulsifier or aromatic-degrading bacterium (Mnif et al., 2014; Yasawong et al., 2011). Band 11 and 28 had 98% similarity with uncultured *Firmicutes* bacterium, and was isolated from a mesophilic digester; it could also be found in microaerobic reactors for biogas desulfurization (Johnson et al., 2008). Band 12 and 17 were associated with *Anoxybacillus flavithermus* (100%), which could produce thermostable α -amylase, and exhibited a unique and remarkable ability to tolerate high toluene, benzene, and p-xylene concentrations (Ozdemir et al., 2012). These seven kinds of bacteria were all thermophilic microorganisms.

Band 10, 20, 22, and 25 were detected from IO-50 and IO-55, but not from IO-60. Band 10, 22, and 25 had 100% similarity with *Geobacillus toebii*, which was a thermophilic hydrocarbon-degrading, heavy metal-resistant bacterium (Kim et al., 2014). Band 20 was associated with *Brevibacillus parabravis* (100%), which is a bacterium that can use organic compounds, such as polycyclic aromatic hydrocarbons, as sole carbon source and is a potential candidate for the biodegradation of low-density polyethylene (Pramila et al., 2012). Band 18, 21, 31, and 34 can only be detected from IO-50. Band 18 and 21 were identified as *Brevibacillus thermoruber* (100%) and *Brevibacillus borstelensis* (99%), respectively. As metabolic products of these thermophilic bacteria, significant amounts of sulfates were generated, which can be precipitated by supplementing the culturing media with ions, leading to insoluble salts (Hadad et al., 2005; Manachini et al., 1985). Band 31 and 34 were closely associated with *Bacillus thermophilus* (100%), which is widely distributed in soil, hot springs, and ocean sediments, and is capable of oxidizing carbon monoxide aerobically (Nazina et al., 2001). Band 8 was only found in IO-60, which was related to *Bacillus* sp. (99%) and usually involved in sulfur odor treatment (Guerrero-Barajas et al., 2011).

Fig. 2. (a) DGGE profile and Shannon indices of bacterial community, and (b) dendrogram of similarity for the bacteria.

Table 3

The closest relatives and isolation sources of dominate bacteria.

Band no.	Closest relatives	Source	Similarity
B1, B3	<i>Pseudomonas stutzeri</i> N2	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas.	100
B2, B4, B5, B7	<i>Pseudomonas putida</i> D61	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas.	100
B6	Uncultured bacterium B-91	Bacteria; environmental samples.	100
B8	<i>Bacillus</i> sp. G6	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.	99
B9	<i>Stenotrophomonas acidaminiphila</i> NK4	Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas.	99
B10, B22, B25	<i>Geobacillus toebii</i> PW12	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Geobacillus.	98
B11, B28	Uncultured <i>Firmicutes bacterium</i> EJIR08_14	Bacteria; Firmicutes; environmental samples.	98
B12, B17	<i>Anoxybacillus flavithermus</i> 3	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Anoxybacillus.	100
B13	Uncultured bacterium SIBN1075 N12D1 16B	Bacteria; environmental samples.	98
B14	<i>Brevibacillus</i> sp. KB2	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Brevibacillus.	99
B15	<i>Anoxybacillus flavithermus</i> A2	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Anoxybacillus.	99
B16	Uncultured bacterium clone UAFB TA 5216	Bacteria; environmental samples.	100
B18	<i>Brevibacillus thermoruber</i> TS 06	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Brevibacillus.	100
B19	<i>Enterobacter</i> sp. SQ6-43-8	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Enterobacter.	100
B20	<i>Brevibacillus parabrevis</i> DMB6	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Brevibacillus.	100
B21	<i>Brevibacillus borstelensis</i> B8	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Brevibacillus.	99
B23	<i>Aeribacillus pallidus</i> Billoris-VO17	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Aeribacillus.	100
B24	<i>Achromobacter xylosoxidans</i> SR50-12	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae; Achromobacter.	100
B26	<i>Bacillus thioparus</i> JB3	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.	100
B27, B32	<i>Microbacterium</i> sp. HGG-15	Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Micrococcineae; Microbacteriaceae; Microbacterium.	100
B29, B39	Uncultured <i>Firmicutes bacterium</i> CM1-134	Bacteria; Firmicutes; environmental samples.	100
B30	<i>Thermoanaerobacteriaceae</i> 91 nM	Bacteria; Firmicutes; Clostridia; Thermoanaerobacteriales; Thermoanaerobacteraceae; unclassified.	100
B31, B34	<i>Bacillus thermophilus</i> SgZ-10	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.	100
B33	<i>Geobacillus debilis</i> D33-8	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Caldibacillus.	91
B35, B38	<i>Tuberibacillus calidus</i> 606b	Bacteria; Firmicutes; Bacilli; Bacillales; Sporolactobacillaceae; Tuberibacillus.	100
B36	<i>Lactococcus garvieae</i> FT255	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Lactococcus.	100
B37	<i>Aneurinibacillus thermoaerophilus</i> Billoris-VO22	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Aneurinibacillus group; Aneurinibacillus.	100

The percentages of desulfurization and thermophilic bacteria were 36.84% and 78.95%, respectively. Both bacteria dominated the microbial population in the inocula. The microbial diversity indices of IO-50, IO-55, and IO-60 were 3.41, 3.10, and 3.20, respectively, whereas the amounts of microorganism were approximately 7.49×10^6 , 1.76×10^6 , and 3.04×10^5 CFU mL⁻¹, respectively. Significant differences were found in the number of bacteria and diversity among IO-50, IO-55, and IO-60. For a bioreactor, the amount and structure of microbial community were greatly correlated with the characteristics of substrates and environment for the survival of bacteria, such as the substrate species and acclimation temperature (Ottengraf, 1986). SO₂ was found as the major substrate in the inlet stream. The temperature of the bioreactor where the inocula were collected was 50–60 °C. Therefore, desulfurization and thermophilic bacteria were the main microorganisms in the inocula. Organic matter degradation bacteria can also be detected because of the volatile organic compounds in the air inflow entering the bioreactor. The stream with SO₂ entered the bioreactor from the bottom and went through the packing material bed. The stream was then exhausted from the top of the bioreactor. The SO₂ concentration declined along the packing material bed. The microorganisms were abundant at the bottom where the substrate was adequate. The temperature changed along with the packing layer,

and the microorganisms grew exponentially when the temperature was suitable. However, higher temperature limited microbial growth, resulting in higher microbial diversity of IO-50 than that of IO-55 and IO-60.

In the inocula, *Pseudomonas stutzeri* (B1, B3), *P. putida* (B2, B4, B5, B7), *Bacillus* sp. (B8), *Stenotrophomonas acidaminiphila* (B9), *G. toebii* (B10, B22, B25), *A. flavithermus* (B12, B15, B17), *Brevibacillus* sp. (B14), *B. thermoruber* (B18), *Enterobacter* sp. (B19), *B. parabrevis* (B20), *B. borstelensis* (B21), *Achromobacter xylosoxidans* (B24), *Microbacterium* sp. (B27, B32), *Thermoanaerobacteriaceae* (B30), *T. calidus* (B35, B38), and *Lactococcus garvieae* (B36) were culturable. Among them, two kinds of bacteria belonging to *Pseudomonas* notably emerged: *P. stutzeri* and *P. putida*. *Pseudomonas* sp. is usually used to remove sulfur-containing compounds with higher than 90% removal efficiency (Li et al., 2013; Omri et al., 2011).

3.1.2. Enrichments

Lanes DO-50, DO-55, and DO-60 in Fig. 2a represent the bacterial consortium after enrichment for 6 months. The desulfurization bacteria, e.g., *Brevibacillus* sp. (B14), *B. thermoruber* (B18), and *B. borstelensis* (B21), and the thermophilic bacteria, including uncultured *F. bacterium* (B11, B28), *B. parabrevis* (B20), and *Bacillus thermophilus* (B31, B34), were found in the microbial communities

Fig. 3. Phylogenetic tree derived from the neighbor-joining analysis of the partial 16S rRNA gene sequence.

of DO-50, DO-55, DO-60. These communities existed in original inocula and were enhanced after enrichment. The amount of these desulfurization and thermophilic bacteria increased after enrichment. Aside from these bacteria, the desulfurization bacteria *P. putida* (B2, B4, B5, B7), *P. stutzeri* (B1, B3), and *Aneurinibacillus thermoaerophilus* (B37) increased when the culture temperature was 50 °C. Meanwhile, *T. calidus* (B35, B39) and *Bacillus thio-parans* (B26) still existed. These bacteria can also be detected when cultured at 55 and 60 °C. In addition, some thermophilic microorganisms, including *Bacillus* sp. (B8), were enhanced at 55 °C, whereas B10, B22, B25, and B33, which were assigned to two *Geobacillus* sp., were enhanced at 60 °C. Compared with the consortium cultured at 50 °C, all the enhanced bacteria were thermophilic at 55 and 60 °C because of the increased temperature.

Lanes DT-50, DT-55, and DT-60 represented the consortium after 12 months of enrichment at 50, 55, and 60 °C, respectively.

When the microorganisms were cultured for 12 months, all the enhanced bacteria were desulfurized and thermophilic bacteria, such as *B. thermoruber* (B18), *B. parabrevis* (B20), *A. thermoaerophilus* (B37), and *T. calidus* (B35, B39). Meanwhile, some microorganisms were reduced because of their inability to tolerate certain sulfur compound concentrations or to resist high temperatures.

The microbial diversity indices of IO-50, IO-55, and IO-60 were 3.41, 3.16 and 3.20, respectively, whereas the microbial diversity indices of DO-50, DO-55, and DO-60 were 3.26, 2.97, and 3.15 (Fig. 2a), respectively. Microbial diversity was reduced because some bands were weakened or undetected after enrichment, e.g., B9, which was related to *S. acidaminiphila* (99%) and always existed in environments below 40 °C, thereby leading to gradual reduction in diversity during enrichment (Assih et al., 2002). A similar phenomenon occurred in DT-50, DT-55, and DT-60, where microbial diversity was reduced gradually as culture time increased. However, the proportions of desulfurization and thermophilic bacteria after enrichment were 50.00% and 83.33%, respectively, which increased compared with the inocula. The proportion for enrichment after 12 months increased to 52.94% and 88.24%, respectively, as the enrichment time grew.

The distribution and relative contents of *Pseudomonas* sp. and sulfur bacteria in the consortium of microorganisms after acclimation were analyzed by FISH (Fig. 4). Both *Pseudomonas* sp. (shown in green) and sulfur bacteria (shown in red) exhibited clustered scattering on the consortium with a particle size of 30–80 μm. The mean frame intensities of the green and red showed that *Pseudomonas* sp. and sulfur bacteria accounted for a large proportion in the total bacteria. While they did not gather alone, grew together with other bacteria.

3.2. Genetic evolutionary relationships

The genetic evolutionary relationships of bacterial communities are presented in Fig. 2b. Inocula (IO-50, IO-55, and IO-60) were associated with the IC series (IC-50, IC-55, and IC-60) because these series were the culturable bacteria in the inocula. Inocula have a distant relationship with the series of DO (DO-50, DO-55, and DO-60) and DT (DT-50, DT-55, and DT-60). Obvious distinction occurred in the microbial structure after enrichment. The microbial structure depended on the media component, and the bacteria in this study were cultured by sulfur bacteria culture medium. The microbial structure of the DO series was distinguished from that of the DT series because of the different substrates. In addition, the culture condition, e.g., temperature and time, also influenced the microbial structure. As the acclimation time increased, the changes in microbial structure became more evident. DO-50, DO-55, and DO-60 were cultured under different temperatures. Therefore, the individual microbial structures exhibited obvious differences. The microbial population formed in each consortium was closely correlated with the substrate species, acclimation temperature, and acclimation time. Compared with the other factors, substrate had a greater effect in this study.

3.3. Performance of enrichment-inoculated biofilter

Thermophilic biofilter had been carried out for 2 months to investigate the biofilter performance. The flow rate and temperature were maintained at 0.6 m³ h⁻¹ and 60 °C, respectively. The elimination of SO₂ which carried out in the biofilter system was an aerobic process. SO₂ dissolved in the water and then be oxidized to SO₄²⁻ under aerobic condition (formulas 3 and 4) (Zhang et al., 2015).

Fig. 4. Distribution and contents of the functional bacteria in the microbial consortium after acclimation. The bacteria dyed with DAPI were displayed in blue, whereas (a) the green pixels in the images represented *Pseudomonas* sp. (a) and (b) the red pixels represented sulfur bacteria. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5 shows the variation of SO_2 concentrations and removal efficiencies. The inlet concentrations of SO_2 ranged from 100.0 mg m^{-3} to 200.0 mg m^{-3} . After 11 days of adaptation, the removal efficiency of SO_2 gradually increased from 20% to more than 80%. Similar to all biosystems, a newly installed biofilter will adapt itself with respect to its microbial ecology and physiological conditions to its environment and nutrient supply of the biofilter before efficient biodegradation occurs (Cox et al., 1993). The start-up period of uninoculated biofilters usually takes two weeks to six months. The species in the inocula survive under the environmental conditions of the biofilter (e.g., pH and temperature) and withstand the inevitable variations during the start-up period. Pollutant degradation is a necessary trait for species in a biofilter, and rapid utilization of the pollutant provides the species a competitive advantage (van Groenestijn et al., 1995). In the present study, the microorganisms that can degrade SO_2 grew rapidly and proliferated during the acclimation period. Inoculation with desulphurization bacteria hastened acclimation of the thermophilic desulfurization biofilter but did not affect the removal efficiency of the biofilter.

The outlet concentrations in the steady state were from 0 mg m^{-3} to 50 mg m^{-3} . During 2 months of operating period,

Fig. 5. SO_2 concentrations in the bioreactor and removal efficiency.

removal efficiency was maintained above 80%. The results showed that the elimination capacity of the thermophilic biofilter increased obviously with the SO_2 inlet load (Fig. 6). The biofilter reached the maximum elimination capacity ($38.71 \text{ g m}^{-3} \text{ h}^{-1}$) at an inlet concentration of 152 mg m^{-3} , and flow rate of $0.6 \text{ m}^3 \text{ h}^{-1}$, SO_2 at 60°C can be effectively removed using the developed thermophilic biofilter. The bioreactor after inoculating enrichment does not only shorten the start-up period but also maintain removal capacity.

3.4. Microbial population during start-up period

Lanes T-1 and T-2 in Fig. 2a represent the consortium sampled just after inoculation and 11 days of operation, respectively. The microbial diversity decreased slightly within 11 days of operation. Moreover, 16 bands were found in Lane T-1. The bands were represented by *P. putida* (B7), *Bacillus* sp. (B8), uncultured *F. bacterium* (B11), *A. flavithermus* (B12, B15, B17), *G. toebii* (B22), *Bacillus thio-parans* (B26), *Microbacterium* sp. (B27, B32), and *Bacillus thermo-philus* SgZ-10(B34). After 11 days, 14 bands existed in Lane T-2. The desulfurization bacteria, e.g., *P. putida* (B7), *Bacillus thio-parans* (B26), *Microbacterium* sp. (B27, B32), and *Thermoanaerobacteriaceae* (B30), were abundant in the biofilter. SO_2 was the sole substrate that resulted in the increase in the proportion of sulfur bacteria from 30% to 40%, whereas some bacteria with the ability to remove

Fig. 6. SO_2 elimination capacity VS Inlet load.

VOC, such as *A. flavithermus* and *Bacillus thermophilus*, were lost after 11 days of operation. Given these desulfurization bacteria, SO₂ could be treated effectively after the start-up period.

4. Conclusions

The microbial population during acclimation and biofilter start-up period relied on substrate species and acclimation temperature. The proportion of desulfurization and thermophilic bacteria increased after acclimation. *Pseudomonas* sp. and sulfur bacteria exhibited clustered scattering in the consortium, with a particle size of 30–80 µm. The thermophilic biofilter inoculated with enrichments can hasten start-up for SO₂ removal. Over 80% removal efficiency was achieved in the steady state. The desulfurization and thermophilic bacteria *P. putida*, *Bacillus thio-parans*, and *Microbacterium* sp. dominated the thermophilic biofilter after the start-up period.

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