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Thermophilic biofilter for SO₂ removal: Performance and microbial characteristics

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HIGHLIGHTS

- SO₂ was effectively removed using a thermophilic biofilter.
- Biofilter reached 90% of removal efficiency and 50.67 g/m³/h of elimination capacity.
- Biofilter performance relied on the water content of the packing materials.
- Desulfurization and thermophilic bacteria were abundant in the microbial community.
- SO₂ transfer involved biodegradation, adsorption, and dissolution.

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ABSTRACT

A bench-scale thermophilic biofilter was applied to remove SO₂ at 60 °C in the present study. The SO₂ concentration in the inlet stream ranged from 100 mg/m³ to 200 mg/m³. An average SO₂ removal efficiency of 93.10% was achieved after developing acclimated organisms that can degrade SO₂. The thermophilic biofilter effectively reduced SO₂, with a maximum elimination capacity of 50.67 g/m³/h at a loading rate of 51.44 g/m³/h. Removal efficiency of the thermophilic biofilter was largely influenced by the water containing rate of the packing materials. The SO₂ transfer in the biofilter included adsorption by the packing materials, dissolution in liquid, and microbial degradation. The main product of SO₂ degradation was SO₄^{2−}. The temporal shifts in the bacterial community that formed in the biofilter were determined through polymerase chain reaction–denaturing gradient gel electrophoresis and DNA sequence analysis. These shifts revealed a correlation between biofilter performance and bacterial community structure.

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1. Introduction

SO₂-containing off-gases, which are usually generated during fossil fuel combustion, threaten human health by triggering asthma and respiratory diseases (Weerasinghe, 2010). SO₂ is a toxic gas that releases a pungent, irritating, and rotten smell. SO₂ emission not only causes health risks to people but also instigates acid rain in the atmosphere (Smith et al., 2001). The primary sources of SO₂ emissions include the thermal conversion processing of liquid and solid fuels in power plants, refineries, coal- and oil-fired boilers, and paper pulp production (Kachur et al., 2003; Querol et al., 1999).

Several desulfurization techniques for effluent gases have been developed to mitigate the pollution generated by SO₂. These

techniques include the gas-phase conversion of SO₂, absorption of SO₂ in liquids, and adsorption of SO₂ onto solid adsorbents (Atanes et al., 2012). SO₂ and NO can be simultaneously absorbed by Fe^{II}EDTA combined with a Na₂SO₃ or urea solution. Simultaneous desulfurization and denitration is possible, with a removal efficiency of nearly 100% (Fang et al., 2011; Wang et al., 2007). Compared with conventional physical–chemical methods, biological technologies, such as biofilters, bioscrubbers, and biotrickling filters are more widely applied to purify waste gas because of their high efficiency, cost effectiveness, convenient operation, and small footprint (Van Groenestijn and Hesselink, 1993). Various sulfur-oxidizing bacteria, including autotrophic and heterotrophic bacteria, have been used to remove sulfur-containing malodorous gases. Mesophilic bacteria, including *Acidithiobacillus thiooxidans* (Lee et al., 2006), *Thiobacillus thioparus* (Ramirez et al., 2011), *Bacillus* sp. (Nakada and Ohta, 1999), and *Pseudomonas* sp. (Omri et al., 2011), are sulfide oxidizers, whereas *Sulfolobus* spp. are thermophilic and autotrophic sulfur oxidizers (Dopson et al., 2006).

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Desulfotomaculum orientis has been shown to grow on SO_2 and produces H_2S , whereas SO_2 is utilized as an electron acceptor by *Desulfovibrio desulfuricans* (Sublette and Gwozdz, 1991). Studies have also biofiltered SO_2 from waste gaseous streams. SO_2 with a concentration of 1000 ppm v/v. can be effectively treated using a laboratory-scale biofilter (Deshmane et al., 1993). A two-stage process that consists of a biotrickling filter and a biological post-treatment unit has been applied to remove SO_2 from flue gases. The effluent from the biotrickling filter can be effectively treated by the post-treatment unit and produce elemental sulfur (Philip and Deshusses, 2003). The performance of bioreactors in treating sulfur-containing compounds depends on the activity of the sulfur bacteria formed in the bioreactors. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) have been successfully applied to explore the structure and diversity of microorganisms in bioreactors used to treat sulfur-containing compounds (Li et al., 2012).

In general, biofiltration techniques are limited to operate under mild temperature conditions (25–35 °C) because mesophilic and oxidizing microbial populations form biofilms. However, emissions generated from boiler combustion, petroleum refinery, smelting, and composting facilities contain SO_2 at temperatures higher than 50 °C (Morales et al., 2012). Precooling the gas stream to appropriate temperatures is an effective preprocessing method (Shanchayan et al., 2006) that increases the investment and operational costs of the treatment. An alternative technique that does not require precooling treatment is thermophilic biofiltration, which utilizes the metabolic activity of thermophilic organisms. Thermophilic biofilters have advantages such as low cost and high microbial metabolism rate (Devinny et al., 1999). Elimination capacity can be increased by using thermophilic bacteria instead of mesophilic bacteria. Thermophilic bioreactors exhibit low biomass accumulation, thus alleviating the risk of clogging (Kong et al., 2001; Mohammad et al., 2007). A thermophilic biofilter for ethylacetate removal has shown a higher removal efficiency (>100 g/m³/h) than a mesophilic biofilter at 45–50 °C (Deshusses et al., 1999). Volatile compounds, including methanol, α -pinene (Kong et al., 2001), BTEX (Mohammad et al., 2007), and other organics, have been successfully treated through thermophilic biofiltration. However, only a few studies have described the use of thermophilic biotreatment for effective SO_2 purification.

The present study developed a bench-scale thermophilic biofilter that contains thermophilic desulfurization bacteria to treat SO_2 at 60 °C. The current work aims to (1) investigate the performance of a biofilter with varying SO_2 loads and water containing rates (WCRs), (2) characterize the microbial communities in the biofilter by using PCR–DGGE, and (3) determine the relationship between microbial characteristics and SO_2 removal, particularly in a thermophilic biofilter.

2. Methods

2.1. Experiments and procedures

SO_2 elimination was carried out in a thermophilic biofiltration system that comprises a biofiltration column, a SO_2 supply system, and a temperature control equipment (Fig. S1 in the Supplementary materials available on-line). A stainless column 30 mm in height and 10 mm in diameter was installed as the biofiltration column. Polyurethane foam cubes (1.0 cm³) were packed in the column for thermophilic bacteria attachment. SO_2 was supplied using a high-pressure cylinder. Gas flow rate was controlled by a calibrated mass flow meter. SO_2 gas left the high-pressure cylinder and flowed through the calibrated gas flow meter, where the flow rate was adjusted to the preferred value. Then, SO_2 mixed with a warm stream heated by a temperature-controlled oven. The mixed

gases entered the biofiltration column from the bottom and were discharged after purification. The total flow rate was 0.6 m³/h and corresponded to an empty bed residence time of 18 s. The sampling ports were located at the inlet and outlet of the biofilter to determine the concentrations before and after the treatment.

Biofilter inoculation may accelerate the startup period. The packing medium collected from the bioreactor for treating sulfur-containing compounds was soaked in a nutrient solution containing 5 g/L beef extract, 2.0 g/L K_2HPO_4 , 2.0 g/L KNO_3 , 0.01 g/L $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 1.0 g/L NaHCO_3 , 0.1 g/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.5 g/L NH_4Cl , and 5.0 g/L $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ (pH 6.5–7.0). The medium was then shaken for 30 min in an ultrasonic oscillator (KQ-250B, Kunshan, China). The liquid with suspended cells was cultured in the same nutrient solution at 60 °C and 120 rpm for 2 weeks to enrich the microorganisms that can degrade SO_2 . A mineral nutrient solution with 5 g/L beef extract, 2.0 g/L K_2HPO_4 , 2.0 g/L KNO_3 , 0.01 g/L $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 1.0 g/L NaHCO_3 , 0.1 g/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and 0.5 g/L NH_4Cl was periodically sprayed into the biofilter to supply nutrients and sustain moisture. The reused nutrient solution was drained from the bottom of the biofilter.

2.2. SO_2 transfer experiment and procedure

SO_2 transfer was investigated using the same biofiltration column. Briefly, 25 g of polyurethane foam cubes was packed in the column. The total flow rate, inlet SO_2 concentration and temperature were 0.6 m³/h, 600 mg/m³ and 60 °C, respectively. The experiment was conducted in triplicate. In test A, the WCR of the packing material was lower than 1%. In test B, the nutrient solution (components are described in Section 2.1) was sprayed into the packing layer, resulting in 80–85% WCR and pH 6.8. In test C, microorganisms (1.6×10^7 CFU/g) were inoculated into the packing layer. The WCR and pH were similar to those in test B. Gases with SO_2 passed through the biofiltration column. Sampling ports were set at the bottom and top of the column to determine the treated and untreated SO_2 in the gas stream.

2.3. Chemical analysis

SO_2 was monitored on line with a flue gas analyzer (rbr, Ecom-J2KN, Germany). An ion chromatography analyzer (ICS-1000, Dionex ion chromatography system, USA) was used to analyze SO_4^{2-} concentrations in the liquid phase. Gravimetry was applied to determine the WCR of the packing material. The temperature and relative humidity in the biofilter were recorded using a Dewpoint thermohygrometer (WD-35612, OAKTON, Germany). A pH meter (pH-3C, Shanghai, China) was used to detect the pH of the liquid.

2.4. Microbiological analysis

2.4.1. Medium for SO_2 degraders

Bacteria were incubated in a nutrient agar (BR, Aoboxing Biotech, Co., China) at 60 °C for 24 h. The sulfur bacteria were cultivated in the medium containing 2.0 g/L K_2HPO_4 , 2.0 g/L KNO_3 , 0.01 g/L $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 1.0 g/L NaHCO_3 , 0.1 g/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.5 g/L NH_4Cl , and 5.0 g/L $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ at 60 °C for 3 d. Cell counts are reported as colony-forming units (CFUs).

2.4.2. DNA extraction

Total DNA was directly extracted by Activated Sludge DNA Automatic Plate form for Magnetic System-16 (TanBead, Taiwan). Subsequently, 1.0% agarose gel electrophoresis (Bio-Rad, USA) was applied to determine DNA bands. DNA extraction was performed as previously described (Li et al., 2012).

2.4.3. PCR amplification and DGGE analysis

PCR was carried out using a C1000TM thermal cycler (Bio-Rad, USA). Specific universal primers, namely, F357 with a GC clamp

and R517 (F357GC: 5'-CGCCGCGCGCGCGCGGGCGGGCGGGGCGGCGGCGGGGGCTACGGGAGGCAGCAG-3' and R517: 5'-ATTACCGCGGCTGCTGG-3'), were used to amplify the V3 regions of the bacterial 16S ribosomal RNA gene of the microbial samples collected from the thermophilic biofilter. Each 50 μL reaction volume comprised 5 μL of extracted DNA mixed with 5 μL of 10 $\times$ Ex Taq buffer (Mg^{2+} Plus), 4 μL of each dNTPs, 1 μL of the primers, and 1.5 U of Ex Taq polymerase (TaKaRa, Dalian, China). The reaction condition of PCR was similar to that described by Li et al. (2011).

Approximately 15 μL of the PCR products was transferred into each well of 6–12% polyacrylamide gels containing the vertical gradient of the denaturant from 40% to 70%. Electrophoresis was performed for 960 min at a constant voltage of 65 V and a temperature of 60 $^{\circ}\text{C}$. The gels were stained with SYBER Green. Digital images were captured with a Fluor-S MultiImager (Gel Doc XR, Bio-Rad, USA) and analyzed with Quantity One Software (Bio-Rad, USA).

2.4.4. Sequencing and phylogenetic analyses

Bacterial diversity was determined from the intensity and number of bands using the Shannon index (H) as previously described (Li et al., 2011). The prominent DGGE bands were excised and dissolved in 35 μL of Milli-Q water overnight at 4 $^{\circ}\text{C}$. The target DNA fragments were reamplified through PCR by 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 Luria–Bertani (LB) agar plates containing X-gal, isopropyl-beta-D-thiogalactopyranoside, and ampicillin. Positive colonies were sequenced using a 3730xL DNA analyzer (ABI, USA). The sequences were edited to exclude priming site and then compared with those available in the GenBank database by using a nucleotide–nucleotide BLAST algorithm.

3. Results and discussion

3.1. Performance of the thermophilic biofilter

The performance of the thermophilic biofilter for SO_2 removal was continuously investigated for 8 months. The flow rate and temperature were maintained at 0.6 m^3/h and 60 $^{\circ}\text{C}$, respectively. The biofilter was started by introducing the air stream containing 120 mg/m^3 SO_2 at a constant empty bed residence time of 18 s. Fig. 1a shows the variation and removal efficiencies of SO_2 . The outlet concentrations of SO_2 varied with inlet concentrations. After 3 weeks of adaptation, the removal efficiency of SO_2 gradually increased from 20% to more than 80%. The SO_2 concentration in the inlet stream ranged from 100.0 mg/m^3 to 200.0 mg/m^3 . The outlet concentrations in average decreased to 9.0 mg/m^3 in steady state. The thermophilic biofilter achieved an average SO_2 removal efficiency of 93.10%. The elimination capacity of the thermophilic biofilter evidently increased with increasing SO_2 inlet load (Fig. 1b). A maximum elimination capacity of 50.67 $\text{g}/\text{m}^3/\text{h}$ was obtained under an SO_2 inlet load of 51.44 $\text{g}/\text{m}^3/\text{h}$. SO_2 at 60 $^{\circ}\text{C}$ can be effectively purified using the developed thermophilic biofilter.

3.2. WCR and SO_2 removal

The amount of water in the packing media is a significant parameter in biofilter operation. Mineral nutrients were sprayed into the biofilter at regular intervals to sustain nutrient and moisture for microbial growth and activity. A low WCR can dry the packing material bed and reduce the removal efficiency of the biofilter.

As shown in Fig. 1a, the removal efficiency of the biofilter exhibited a remarkable periodic fluctuation. The variations in the WCRs of the packing material within one spray cycle were investigated

Fig. 1. (a) SO_2 concentrations in the bioreactor and removal efficiency. (b) Elimination capacity VS inlet load.

(Fig. S2 in the Supplementary materials available on-line). The WCR ranged from 80% to 90% after irrigation and gradually decreased to below 20% after 7 d of operation. The WCR of the packing material was lower than 20%, and the biofilter needed to be humidified again. The decreasing speed of the WCR was faster than that of the mesophilic biofilters. The operating temperature of a regular bioreactor ranges from 25 $^{\circ}\text{C}$ to 35 $^{\circ}\text{C}$, and the spray cycles usually last for 1–7 d (Lebrero et al., 2010). The water content in the packing material was transported with the gases that passed through the biofilter column. The temperature in the column was maintained at 60 $^{\circ}\text{C}$. The evaporation rate increased as the temperature increased, which consequently decreased the water content in the packing materials. The removal efficiencies evidently decreased as the WCR decreased within 1 week (Fig. S2 in the Supplementary materials available on-line). More than 99% of SO_2 removal can be achieved when the WCR reached 80–90%. The amount of the nutrients on the packing materials increased with the WCR. The growth and activity of the microorganisms in the bioreactor increase with increasing nutrient content. In addition, SO_2 is a water-soluble compound, and high WCR benefits the transfer of SO_2 from the gas phase to the liquid phase. Therefore, high removal efficiency could be reached under a high WCR in this study. The preferred water content ranges from 40% to 70% on a weight basis for most packing materials (Devanny et al., 1999; Kafle et al., 2015). In the present study, irrigation was performed once a day to maintain the optimal water content for the optimum biological activity and optimal operation of the biofilter.

3.3. Fate of SO_2

SO_3^{2-} is produced when SO_2 is dissolved in water and oxidized to SO_4^{2-} (Brown et al., 2012). The concentration of SO_4^{2-} in the

Table 1
Results of SO₂ transfer tests.

No.	WCR (%)	C _{in} (mg/m ³)	C _{out} (mg/m ³)	Te (h)	TR (g)
Test A	<1	600	600	0.5	0.07
Test B	80–85	600	600	48	9.67
Test C	80–85	600	284	–	16.04

WCR: water-containing rate, C_{in}: inlet concentration, C_{out}: inlet concentration, Te: time when C_{out} was equal to C_{in}, TR: the amount of SO₂ reduced.

Fig. 2. The tests of SO₂ transfer.

biofilter gradually increased. The pH rapidly decreased from 6.5 to 3.0 within 3 d with the formation of SO₄²⁻ and then maintained almost constant until day 7 (Fig. S3 in the Supplementary materials available on-line). SO₂ oxidation produced H₂SO₄ and decreased pH.

SO₂ is fairly soluble in water, and its solubility is 9.4 g per 100 mL of water at 25 °C. The packing material was used in the biofilter for microorganism attachment. SO₂ can also be adsorbed by the packing materials when passing through the biofilter column. Therefore, the transfer of SO₂ in the biofilter involved adsorption by the packing materials, dissolution in liquid, and microbial degradation. Three tests (A, B, and C) were compared to investigate SO₂ transfer in the thermophilic biofilter. The results are presented in Table 1 and Fig. 2.

In test A, the SO₂ concentration from the outlet stream increased with time. The concentration was similar to that from the inlet stream after 30 min of experiment, indicating that the adsorbed SO₂ by the packing material was saturated. Approximately 0.07 g of SO₂ was adsorbed by the packing material in the biofilter. A similar phenomenon occurred in Test B. That is, an equal outlet concentration of SO₂ compared with that in the inlet stream was obtained after 48 h, and this duration was longer than that in test A. Approximately 9.60 g of SO₂ was dissolved in the liquid which was higher than the SO₂ adsorbed on the packing material. The WCR of the packing material in the biofilter ranged from 80% to 85%, whereas the volume of the liquid inside the biofilter was 300 mL. Therefore, 3.2 g/100 mL of SO₂ solubility was achieved at 60 °C temperature, 0.6 m³/h flow rate, and 600 mg/m³ inlet concentration. SO₂ gradually increased and was maintained at 284 mg/m³ for 60 h in test C. The SO₂ concentration in the outlet stream was lower than that in the inlet concentration. The total degradation amount of SO₂ was 6.37 g within 48 h. The low SO₂ concentration that was maintained through microbial degradation benefited the SO₂ transfer from the gas phase into the liquid phase. The SO₂ reduction in the biofilter column involved biodegradation, dissolution, and adsorption on the packing materials. Among these processes, microbial degradation played the most important role.

Table 2
The samples collected for DGGE analysis.

Sample No.	Operation date (day)	Season	Removal efficiency (%)
LB-0 LS-0	0	Summer	–
LB-2 LS-2	58	Autumn	90.48
LB-4 LS-4	120	Winter	85.42
LB-6 LS-6	175	Spring	98.31
LB-8 LS-8	234	Spring	97.62

3.4. Microbial characteristics

3.4.1. SO₂ degraders in the biofilter

Several groups of microorganisms, primarily bacterial species, are responsible for degrading air pollutants in the biofilter. Monitoring the microbial populations that formed in the bioreactors can effectively optimize the performance of the bioreactors. Samples for microbial analysis were periodically collected (Table 2). Bacterial cells and sulfur bacterial cells in the biofilter were counted by culturing the cells on an appropriate medium as described in Section 2.4.1.

Approximately 9.45×10^6 CFU/mL of bacteria and 1.09×10^6 CFU/mL of sulfur bacteria were obtained in the biofilter after inoculation (Table 3). Within the operation period, the amounts of TB and TSB gradually increased with time. The increasing rate of TB was faster than that of TSB. LB-4 was sampled in winter when the average ambient temperature decreased to 10 °C. The metabolism of microorganisms varied with temperature. The enzyme activities of microorganisms decrease by two to four times when the temperature is decreased by 10 °C (Feller and Gerday, 2003). In the present study, the number of sulfur bacteria decreased to 1.10×10^4 CFU/mL because of the low ambient temperature in winter. Desulfurization bacteria were also added into the biofilter to maintain biofilter performance.

3.4.2. Bacterial structure formed in the biofilter

DGGE was used to explore the population shifts in response to variation in the biofilter (Fig. 3a). A total of 25 discriminable bands (B1–B25) were identified, and their sequences were matched with their closest relatives in GenBank as listed in Table 4. The phylogenetic tree derived from neighbor-joining analysis of partial 16S rRNA gene sequence was showed in Fig. 4. Bands B2, B7, B11, B12, and B15 emerged in all samples. Bands B2 and B11 were closely related to *Pseudomonas fluorescens* LOCK 0961 (100%) and *Pseudomonas stutzeri* L2 (99%), respectively. These microorganisms are frequently isolated from soil and activated sludge. *Pseudomonas* sp. is usually used to remove sulfur-containing compounds with removal efficiencies higher than 90% (Li et al., 2013; Omri et al., 2011). Band B7 was closely associated with *Bacillus* sp. G334 (100%), which is usually involved in sulfur odor treatment (Nakada and Ohta, 1999; Xie et al., 2009). Band B12 belonged to *Brevibacillus borstelensis* B8 (100%), a species of thermophilic bacteria widely distributed in hot springs. *B. borstelensis* can transform thioanisoles (Chinese patent, 103013877B; Hadad et al., 2005). Band B15 was assigned to *Paenibacillus* sp. kzC1 with 100% similarity. Several *Paenibacillus* species, such as *Paenibacillus polymyxa*, can degrade sulfur-containing compounds (Giri et al., 2010; Zhang et al., 2013). Bands B13, B14, and B16 appeared at lanes of LS-0 to LS-8 but not at lanes of LB-0 to LB-8, which showed that some cultivable sulfur bacteria were not the predominant species only when determined by pure culture method. B13 was closely associated with *Citrobacter freundii* FHMDC1F2-14 (100%), a SO₄²⁻-reducing bacterial strain isolated from sediments. B14 and B16 exhibited 100% similarity to *Aeromonas* sp., which are thermostable (Gandu et al., 2013). B10 presented in LB-0, LS-6, and LS-8 had 100% similarity to *Ralstonia* sp. Gaseous ethanethiol can be

Table 3

The amount of bacteria and sulfur bacteria in the biofilter.

	LB-0	LB-2	LB-4	LB-6	LB-8
TB (CFU/mL)	9.45×10^6	3.38×10^7	1.16×10^7	5.84×10^7	1.18×10^8
TSB (CFU/mL)	1.09×10^6	5.28×10^5	1.10×10^4	4.49×10^6	7.25×10^6
R = TSB/TB × 100 (%)	11.50	1.56	0.09	7.69	6.13

TB: total bacteria; TSB: total sulfur bacteria; R: ratio.

Fig. 3. (a) DGGE profile and Shannon indices of bacterial community. (b) Dendrogram of similarity for bacteria. (c) Dendrogram of similarity for sulfur bacteria.

efficiently degraded by the pure culture of *Ralstonia eutropha* in various initial concentrations within 120 h to 168 h. (Sedighi and Vahabzadeh, 2014).

Desulfurization bacteria (e.g., *Pseudomonas* sp., *Paenibacillus* sp., and *Ralstonia* sp.) and thermophilic bacteria (e.g., *Brevibacillus* sp. and *Bacillus* sp.) dominated the microbial population in the thermophilic biofilter. Microbial growth is a consequence of the uptake and metabolism of air pollutants. The SO₂ in the inlet stream functioned as an energy source and building material. During the operation, the temperature of the biofilter was maintained at 40–60 °C.

Table 4

The closest relatives and isolation sources of dominate bacteria.

Band No.	Closest relatives	Source	Similarity (%)
B1	<i>Lysinibacillus</i> sp. ES3-1	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Lysinibacillus	100
B2	<i>Pseudomonas fluorescens</i> LOCK 0961	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	100
B3	<i>Sphingobacterium</i> sp. RA-16	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Sphingobacteriaceae; Sphingobacterium	100
B4	<i>Acinetobacter lwoffii</i> 214	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter	100
B5,B12	<i>Brevibacillus borstelensis</i> B8	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Brevibacillus	100
B6	<i>Bacillus oleronius</i> DSM 9356	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus	100
B7	<i>Bacillus</i> sp. G334	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus	100
B8,B23	<i>Morganella morganii</i> LEY01	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Morganella	100
B9,B21,B25	<i>Aeromonas veronii</i> FHMDA1V1-32	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Aeromonas	100
B10	<i>Ralstonia</i> sp. N-QB-1504	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Ralstonia	100
B11	<i>Pseudomonas stutzeri</i> L2	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	99
B13,B22	<i>Citrobacter freundii</i> FHMD1F2-14	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Citrobacter; Citrobacter freundii complex	100
B14	<i>Aeromonas hydrophila</i> 4AK4	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Aeromonas	100
B15,B20	<i>Paenibacillus</i> sp. kzC1	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Paenibacillus	100
B16	<i>Aeromonas media</i> w-4	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Aeromonas	100
B17,B19	<i>Bacillus farraginis</i> TN6	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus	99
B18	<i>Bacillus subtilis</i> subsp. AG1839	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus	96
B24	<i>Planomicrobium chinense</i> ZBGKL9	Bacteria; Firmicutes; Bacilli; Bacillales; Planococcaceae; Planomicrobium	99

Fig. 4. The phylogenetic tree derived from neighbor-joining analysis of partial 16S rRNA gene sequence.

Desulfurization and thermophilic bacteria can vigorously grow in an environment with an abundant food supply and optimal temperature. Some bacteria expressed by B1, B4, and B18 in Fig. 3a originally appeared in the inoculums. However, these bacteria were not detected after 60 d of operation because they cannot adapt to the environment in the biofilter.

Shannon indices were used to reflect the individual biodiversity of the bacterial groups (Fig. 3a). The diversity of TB and TSB decreased with time and then increased when adding acclimated bacteria at 175 d of operation as observed in the case of LB-6. The reduction in diversity was probably due to SO₂ being the sole substance in the inlet streams which agreed with previous study (Van Groenestijn and Hesselink, 1993). The genetic evolutionary relationships of bacterial and sulfur bacterial communities are presented in Fig. 3b and c, respectively. For the bacterial community, LB-0 exhibited a close evolutionary relationship to LB-2 but was very different from LB-8. LB-2 and LB-8 were collected at the biofilter after 2 and 8 months of operation, respectively. The bacterial community presented temporal variations during the operation period. A similar phenomenon was observed in the sulfur bacterial community.

4. Conclusions

A thermophilic biofilter that contains desulfurization bacteria was used to remove SO₂ at 60 °C. The biofilter achieved a removal efficiency of >90% and a maximum elimination capacity of 50.67 g/m³/h in steady state. The optimal water content of the packing materials should be maintained. Irrigation was performed once a day for 30 min. SO₂ reduction involved biodegradation, dissolution, and adsorption on the packing materials. Microbial degradation dominated the process. DGGE fingerprint patterns showed that the bacterial community composition shifted in the biofilter. Desulfurization and thermophilic bacteria were also abundant. Overall, thermophilic biofiltration is a potential treatment for SO₂ at high temperatures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2014.12.074>.

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