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Continuous desulfurization and bacterial community structure of an integrated bioreactor developed to treat SO_2 from a gas stream

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ABSTRACT

Sulfide dioxide (SO_2) is often released during the combustion processes of fossil fuels. An integrated bioreactor with two sections, namely, a suspended zone (SZ) and immobilized zone (IZ), was applied to treat SO_2 for 6 months. Sampling ports were set in both sections to investigate the performance and microbial characteristics of the integrated bioreactor. SO_2 was effectively removed by the synergistic effect of the SZ and IZ, and more than 85% removal efficiency was achieved at steady state. The average elimination capacity of SO_2 in the bioreactor was 2.80 g/(m^3·hr) for the SZ and 1.50 g/(m^3· hr) for the IZ. Most SO_2 was eliminated in the SZ. The liquid level of the SZ and the water content ratio of the packing material in the IZ affected SO_2 removal efficiency. The SZ served a key function not only in SO_2 elimination, but also in moisture maintenance for the IZ. The desired water content in IZ could be feasibly maintained without any additional pre-humidification facilities. Clone libraries of 16S rDNA directly amplified from the DNA of each sample were constructed and sequenced to analyze the community composition and diversity in the individual zones. The desulfurization bacteria dominated both zones. *Paenibacillus* sp. was present in both zones, whereas *Ralstonia* sp. existed only in the SZ. The transfer of SO_2 to the SZ involved dissolution in the nutrient solution and biodegradation by the sulfur-oxidizing bacteria. This work presents a potential biological treatment method for waste gases containing hydrophilic compounds.

Introduction

Sulfur dioxide is a chemical compound with the formula SO_2. SO_2 is a global, common, and serious air pollutant, and it is a major source of atmospheric pollution, which results in acid smog formation and acid rain (Mathieu et al., 2013). SO_2 and its derivatives are harmful to humans and animals (Agar et al., 2000; Altug et al., 2013; Bai and Meng, 2005; Kan et al., 2010). Numerous studies have been carried out to clarify or investigate the strength of SO_2 pollution (Fujita et al., 1991; Liu et al., 2012; Tian et al., 2012) and many countries have established stringent regulations to limit SO_2 emission into the atmosphere.

Several desulfurization techniques have been developed to eliminate SO_2 from effluent gases. Wet and dry flue gas desulfurization processes are traditional physico-chemical methods for SO_2 control. During these processes, SO_2 can be effectively removed, while high-quality gypsum is obtained.

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This material can be applied in road base construction, wallboard manufacturing, and agriculture (Chang, 1986; Kaminsky, 1983). SO_2 and NO can be simultaneously absorbed by alkaline solution with removal efficiencies of nearly 100%, thus resulting in simultaneous desulfurization and denitration (Fang et al., 2011; Jeong et al., 2001; Wang et al., 2007).

Compared with physico-chemical technologies, in which the contaminant is sometimes simply transferred from one phase to another, biological waste gas treatment technologies present the advantage of completely degrading the contaminants into innocuous or less-contaminating products (An et al., 2010; Ottengraf and Diks, 1992). Biofilters, biotrickling filters, and bioscrubbers are the major bioreactor designs usually considered in these applications (Kennes, 2012; Li, Wan et al., 2012; Ottengraf, 1987; Wan et al., 2011). Over the last two decades, researchers have focused on developing biological methods for the treatment of gaseous streams containing SO_2 (Buisman et al., 1990; Philip and Deshusses, 2003; Selvaraj et al., 1997; Selvaraj and Sublette, 1995). The performance of a biotrickling filter followed by biological post-treatment unit has been investigated in SO_2 treatment from flue gases. Notably, 100% of SO_2 can be removed by the biotrickling filter at a concentration range of 300–1000 ppmv. A post-treatment unit can effectively treat the effluent from the biotrickling filter and produce elemental sulfur (Philip and Deshusses, 2003). Lee and Sublette developed a method for the simultaneous removal of SO_2 and NOx from cooled flue gas through contact with cultures of *Desulfovibrio desulfuricans* and *Thiobacillus denitrificans* as culture-in-series or in a co-culture in a single contacting stage (Lee and Sublette, 1991). SO_2 can be converted to elemental sulfur for disposal or by-product recovery. The sulfate-reducing bacterium (SRB) *D. desulfuricans*, which is immobilized by co-culture with floc-forming heterotrophs, is dominant during the conversion. Anaerobically digested municipal sewage solids serve as the carbon and energy source for these cultures. The maximum specific activity for SO_2 reduction is 9.1 mmol of SO_2/(h·g) in terms of dry weight of *D. desulfuricans* biomass (Selvaraj et al., 1996; Selvaraj and Sublette, 1995). Various immobilized cell bioreactors, *e.g.*, a stirred tank with SRB flocs, and columnar reactors with cells immobilized in either kappa-carrageenan gel matrix or polymeric porous beads, were investigated to maximize the productivity of the bioreactor for SO_2 reduction. The columnar reactor with mixed SRB cells immobilized in polymeric beads exhibited the highest sulfite conversion rates, in the range of 16.5 mmol/(hr·L) (with 100% conversion) to 20 mmol/(hr·L) (with 95% conversion) (Selvaraj et al., 1997) . SO_2 with temperature of 50°C–60°C has been successfully purified from waste steams through thermophilic biofiltration, which utilized the metabolic activity of thermophilic organisms (Zhang et al., 2015).

The bioreactor design was based on the characteristics of the pollutants discharged in air emissions. The effectiveness of the biofilter for the treatment of waste gases largely depends on the solubility of compounds in the liquid layer of the biofilm (Wang et al., 1996). SO_2 is a hydrophilic compound. Biotrickling filters and bioscrubbers are regarded as suitable techniques for hydrophilic compound removal. Bioscrubbers adsorb pollutants in the aqueous phase, which are then treated biologically in a second stage in a liquid phase bioreactor. Waste gas treatment in biotrickling filters involves using a biological filter continuously fed with a liquid medium and packed with a synthetic carrier, on which a biofilm grows. Liquid circulation pumps are required to provide the mobile liquid phase to ensure the mass transfer or nutrient supply to the microorganisms, which will result in high energy consumption. Thus, an integrated bioreactor without a circulating pump was designed to test the feasibility of SO_2 elimination in the present study. The bioreactor consisted of two zones, one for cells in suspension and the other packed with material for the attached growth of microorganisms. The performance of the two zones was compared, and the factors contributing to SO_2 removal were investigated. Conventional microbiological methods combined with the cloning/sequencing technique were applied to characterize the community structure of the bacteria formed in individual zones. The aim of the present study was to provide a biological treatment method for waste gases containing hydrophilic compounds.

1. Materials and methods

1.1. *Integrated bioreactor setup*

A schematic of the experimental setup is shown in Fig. 1. The experiments on SO_2 elimination were continuously conducted using an integrated bioreactor 5.0 m long, 1.2 m wide, and 1.20 m high. The bioreactor has a 0.30 m high zone (suspended zone, SZ) containing a bacterial suspension and a 0.90 m zone (immobilized zone, IZ) packed with 1.0 cm^3 polyurethane foam cubes providing a porous and inert substratum for the growth of attached microorganisms. First, the gases entered the SZ, and the SO_2 in the gas could be absorbed and subsequently degraded by the bacteria in this zone. These gases then passed into the IZ, where microorganisms attached to the packing material and removed the residual SO_2. The synthetic gaseous waste stream was generated as follows. Two air compressors supplied the airflow. A small stream of pure SO_2 (Beijing Hua Yuan Gas Chemical Industry Co., Ltd.) was controlled by a mass flow meter (D08-1F; Beijing Sevenstar Electronics Co., Ltd.) and then mixed with a larger gas stream. The desired concentration of experimental compounds in the influent air stream was maintained by adjusting the rates of the two airflows. The total flow rate was 300 m^3/hr and corresponded to an empty bed residence time of 86 sec. The sampling ports were located at both the inlet and outlet of the SZ and at the outlet of the integrated bioreactor to allow measurement of the compound concentrations prior to and after the treatment. A thermally insulated building was installed to maintain a suitable temperature for the integrated bioreactor during winter and to protect it from rain and sun during summer.

The bioreactor was initially seeded with microbial cultures obtained from a bioreactor packing material used for sulfur-containing compound removal in our laboratory. Approximately 50 g of packing material was cut into pieces and soaked in 10 L of nutrient solution containing 0.2 g/L of glucose, 0.5 g/L of K_2HPO_4, 5.0 g/L of KH_2PO_4, 0.1 g/L of $MgSO_4{\cdot}7H_2O$, 2.0 g/L of NH_4Cl, and 4.0 g/L of $Na_2S_2O_3{\cdot}5H_2O$ (pH 6.5–7.0). The mixture was shaken in an ultrasonic oscillator (KQ-250B; Kunshan,

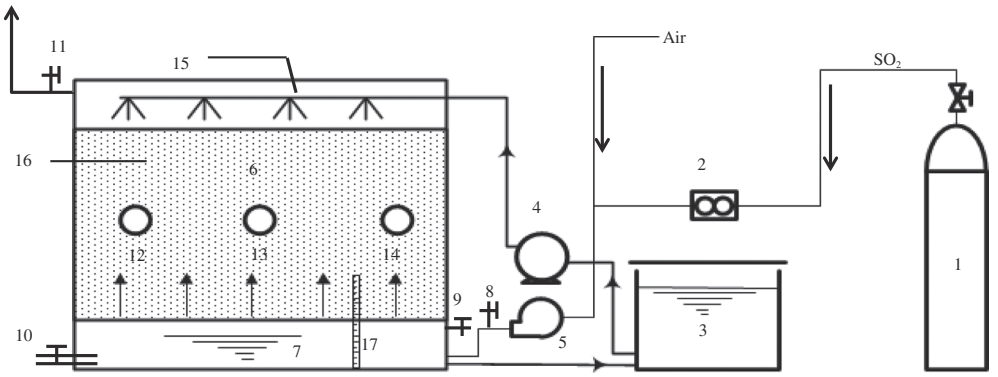

Fig. 1 – Schematic of the integrated bioreactor. (1) Sulfur dioxide cylinder; (2) mass flow meter; (3) nutrient tank; (4) meter pump; (5) blower; (6) packing material; (7) suspended zone; (8–14) sampling point; (15) sprayer; (16) immobilized zone; (17) level gauge.

China) for 30 min. The liquid with suspended cells was then cultured in 10 L of nutrient solution at 30°C and 120 r/min for 3 days and then transferred into the same nutrient solution three times for the enrichment of the microorganisms capable of degrading SO_2. The microbial cultures were inoculated onto a biofilter packing material after resuspension in 50 L of nutrient solution. Mineral nutrient solution (2.0 g/L of KH_2PO4, 2.0 g/L of KNO_3, 0.25 g/L of NH_4Cl, 0.005 g/L of $FeCl_2{\cdot}7H_2O$, 0.5 g/L of $NaHCO_3$, 0.25 g/L of $MgCl_2{\cdot}6H_2O$, 0.2 g/L of glucose) was added into the integrated bioreactor at periodic intervals to maintain an adequate supply of nutrients and moisture for microorganism growth. Redundant nutrient solution was drained from the bottom of the bioreactor.

1.2. SO_2 transfer experiment and procedure

Two tests (Tests A and B) were conducted in a glass column to investigate the SO_2 transfer. The total volume and diameter of the glass column were 800 mL and 8 cm, respectively.

In Test A, the glass column contained 500 mL of nutrient solution without microorganisms (pH 3.0). In Test B, the glass column contained 500 mL of nutrient solution with microorganisms (pH 3.0). SO_2 passed through the liquid from the bottom of the glass column. The inlet flow rate was maintained at 2.25 L/min, and the SO_2 concentration was controlled at 102 mg/m^3. Sampling ports were placed at the top and bottom of the glass column to determine SO_2 concentrations in untreated and treated gas.

1.3. Chemical analysis

SO_2 from the inlet and outlet, liquid samples from the SZ, and packing materials from the IZ were periodically collected to monitor the bioreactor performance. SO_2 was monitored online by a SO_2 analyzer (J2KN; ECOM, Germany). The temperatures of the oven, injection, and detector were 50°C, 100°C, and 200°C, respectively. Sulfate concentrations in the liquid phase were analyzed by an ion chromatogram analyzer (ICS-1000; Dionex ion chromatography system, USA). Temperature and relative humidity (RH) in the bioreactor were measured by a Dewpoint Thermohygrometer (WD-35612; Oakton, Germany). Gravimetry was applied to determine the water content ratio (WCR) of the packing material, and a pH meter (pH-3C; Shanghai, China) was used to detect the pH values.

1.4. Microbiological analysis

For microbial enumeration, 1.0 g of the polyurethane packing material from the IZ (or 1.0 mL of liquor samples from the SZ) was obtained from the sampling ports of the bioreactor and then mixed with 100 mL of sterile water and agitated for 10 min, as described in our previous report (Li, Han et al., 2012). The bacteria were incubated in nutrient agar (BR; Aoboxing Biotech, Co., China) at 30°C for 48 hr. The medium, containing 5.0 g/L of $Na_2S_2O_3$, 2.0 g/L of KH_2PO_4, 2 g/L of KNO_3, 0.25 g/L of NH_4Cl, 0.005 g/L of $FeSO_4{\cdot}7H_2O$, 0.5 g/L of $NaHCO_3$, 0.25 g/L of $MgCl_2{\cdot}6H_2O$, and 15 g/L of agar powder, was used for the enumeration of sulfur-degrading bacteria. The cultivation temperature and time were 30°C and 48 hr, respectively. The number of microorganisms was expressed as CFU/mL of liquor or CFU/g of dry packing material.

Liquid samples from the SZ and packing material from the IZ were collected when the integrated bioreactor was in a steady state. Isolation of total DNA was achieved with a Magnetic System-16 (TanBead, Taiwan). Primers F16S-27 (5′-AGAGTTTG ATCCTGGCTCAG-3′) and R16S-1492 (5′-CGGTTACCTTGTTACG ACTTC-3′) were used to amplify the segment of eubacterial 16S rRNA, as described in a previous report (Han et al., 2012). The PCR products generated from each sample were purified by an Agarose-Gel Extraction Kit (Omega, USA). After purification, the products were ligated to 1.0 μL of pMD18-T vector (Takara, Japan) and transformed into *Escherichia coli* DH5α competent cells. Clones were cultured on LB medium with X-gal, IPTG, and Amp. All clones were selected for PCR detection, and their positive clones were submitted for sequencing using the ABI 3730DXL DNA sequencer (AB, USA). All sequences obtained from the clone libraries were analyzed by DNAMAN software, MEGA version 4.1 [24]. The representative OTU (Operational Taxonomic Unit) sequences obtained in this study were deposited in the NCBI database under accession numbers listed in Table 1. A phylogenetic tree, which includes representative sequences of each OTU and related sequences from the NCBI database, was constructed using the neighbor-joining algorithm in MEGA version 4.1 to identify the phylogenetic

Table 1 – Accession numbers of OTUs (Operational Taxonomic Unit) from all samples in GenBank database.

Sampling port	OTU	Name of OTUs in Fig. 6	Accession numbers
Immobilized zone	OUT-3-1	JULI-2	KP189378
	OUT-3-3	JULI-4	KP189370
	OUT-3-4	JULI-6	KP189372
Suspended zone	OUT-5-2	JULS-2	KP189364
	OUT-5-3	JULS-4	KP189366
	OUT-5-4	JULS-6	KP189368
	OUT-5-6	JULS-8	KP189369
	OUT-5-8	JULS-10	KP189374
	OUT-5-9	JULS-12	KP189376
	OUT-5-10	JULS-13	KP189379

affiliation of all OTUs. To determine whether the clone library number was sufficiently large to represent the diversity of an original community, we calculated the coverage of each clone library according to the equation:

$$C = 1–(n/N),$$

where n is the number of unique clones and N is the total number of examined sequences (Good, 1953). The Shannon–Wiener index (H) was used to estimate the diversity of each clone library (Ding et al., 2008).

2. Calculation equations

The performance of the bioreactor was evaluated by a series of parameters defined as follows:

$$R_T = \frac{C_{in}–C_{out}}{C_{in}} \times 100\% \quad (1)$$

$$R_s = \frac{(C_{in}–C_{Sout})}{C_{in}} \times 100\% \quad (2)$$

$$R_I = \frac{(C_{Iin}–C_{Sout})}{C_{in}} \times 100\% \quad (3)$$

$$IL_T = \frac{Q \times C_{in}}{V} \quad (4)$$

$$IL_S = \frac{Q \times C_{in}}{V_S} \quad (5)$$

$$IL_I = \frac{Q \times C_{Iin}}{V_I} \quad (6)$$

$$EC_T = \frac{Q \times (C_{in}–C_{out})}{V} \quad (7)$$

$$EC_S = \frac{Q \times (C_{in}–C_{Sout})}{V_S} \quad (8)$$

$$EC_I = \frac{Q \times (C_{Iin}–C_{Sout})}{V_I} \quad (9)$$

where R_T (%) is the total removal efficiency of the integrated bioreactor, C_{in} (mg/m^3) is the inlet concentration of SO_2, C_{Iin} (mg/m^3) is the SO_2 inlet concentration of the IZ, C_{out} (mg/m^3) is the outlet concentration of SO_2, C_{Sout} (mg/m^3) is the concentration of SO_2 output from the SZ, IL_T (mg/m^3·hr) is the total inlet load of the integrated bioreactor, IL_S (mg/m^3·hr) and IL_I (mg/m^3 · hr) are the inlet load of the SZ and IZ, Q (m^3/hr) is the flow rate, V (m^3) is the volume of the integrated bioreactor, V_S (m^3) is the volume of SZ, V_I (m^3) is the volume of IZ, EC_T (mg/(m^3·hr)) is the total elimination capacity of the integrated bioreactor, EC_S (mg/m^3·hr) is the elimination capacity of the SZ, and EC_I (mg/m^3·hr) is the elimination capacity of the IZ. In this study, C_{Iin} was the same as C_{Sout}.

$$r = \frac{q \times 100}{q_b} \quad (10)$$

where r is the percentage of sulfur bacteria in the bacterial population (%)/the ratio of sulfur bacteria to total bacteria, q_s denotes the colony-forming units of sulfur bacteria per gram dry packing medium (or per milliliter liquor) (CFU/mL or CFU/g), and q_b denotes the colony-forming units of bacteria per gram dry packing medium (or per milliliter liquor) (CFU/mL or CFU/g).

$$WCR = \frac{(m_1–m_2)}{m_1} \times 100\% \quad (11)$$

where m_1 is the mass of the moisture packing material, and m_2 is the mass of the dry packing material.

3. Results and discussion

3.1. SO_2 removal in the integrated bioreactor

The performance of the integrated bioreactor was observed for 7 months while varying the inlet concentration of SO_2. The inlet and outlet concentrations and removal efficiencies of SO_2 in the bioreactor are shown in Fig. 2. The flow rate was maintained at 300 m^3/hr. SO_2 inlet concentrations were within 35–117 mg/m^3, and the outlet concentrations were reduced to 9–79 mg/m^3 after adaptation. Over 85% of SO_2 in the inlet stream could be effectively treated during the steady stage.

Three operation modes, namely, Modes I, II, and III, were compared in this study. Under Mode I, SO_2 entered the SZ and then entered the IZ, in which SO_2 was treated by the SZ along with the IZ. By contrast, SO_2 was removed only by the SZ in Mode II. For Mode III, SO_2 directly entered the IZ without passing through the SZ. SO_2 was then treated by the IZ alone. The average removal efficiency was 81.71% in Mode I, 51.96% in Mode II, and 30.46% in Mode III (Fig. 2 and Table 2). The maximum removal efficiency could be obtained in Mode I. Results showed that the total elimination capacity of the integrated bioreactor steadily increased with SO_2 inlet load (Fig. 3a) in Mode I. With further load increases, the elimination rate increased more slowly up to a critical load and then remained constant, indicating that the maximum elimination capacity was achieved. The maximum elimination capacity of SO_2 was 4.30 g/(m^3·hr), which was achieved at an inlet concentration of 95 mg/m^3 and flow rate of 300 m^3/hr. The average elimination capacity of SO_2 was 2.80 g/(m^3·hr) in the SZ and 1.50 g/(m^3·hr) in the IZ of the integrated bioreactor. Most of the SO_2 was eliminated in the SZ. Through the synergistic function of the two zones, SO_2 could be effectively treated in an integrated bioreactor. Notably, SO_2 removal was significantly higher in the SZ than that in the IZ (Table 2).

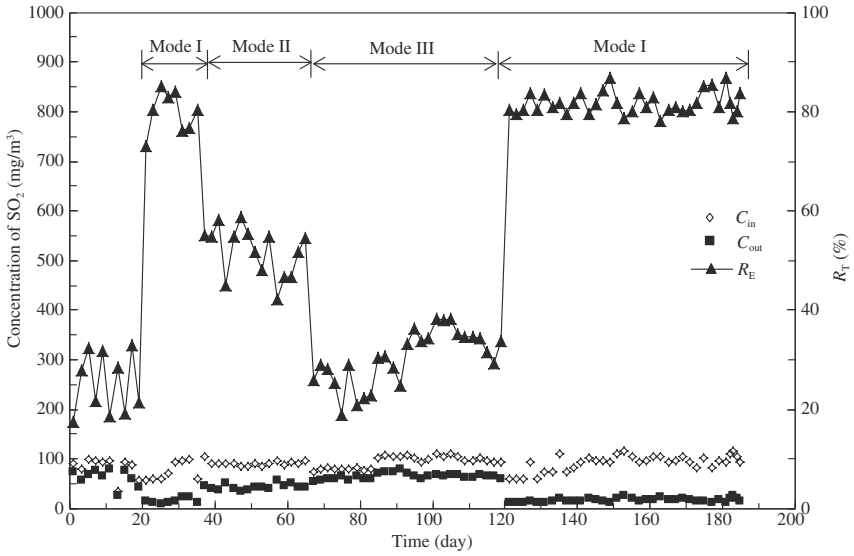

Fig. 2 – Performance of the integrated bioreactor.

SO_2 is a hydrophilic compound. The Henry's law coefficient of SO_2 and its water solubility are 365.65 kPa (20°C) and 94 g/L, respectively (Johnstone and Leppla, 1934). The SZ had a higher removal efficiency for the treatment of hydrophilic compounds.

The total removal efficiency was more than 80% when the liquid level in the SZ was above 25 cm (Fig. 4). The effective volume of the SZ (Vs) depended on the liquor level in this zone, and the load of the SZ varied with Vs. A higher removal efficiency was achieved at lower SO_2 load (Fig. 3b). The removal of SO_2 gradually decreased from greater than 80% to less than 45% when the inlet load increased from 14.6 g/(m^3·hr) to more than 36.5 g/(m^3·hr). Therefore, for the integrated bioreactor, the liquor level in the SZ clearly influenced the total removal efficiency of SO_2.

3.2. Water content ratio and SO_2 removal immobilized zone

Maintaining the moisture content of the materials packed in a biofilter is crucial for microorganisms attached to the materials, given that microorganisms require water to grow and perform metabolic activities. A very low WCR will cause the packing material to dry and decrease the biodegradation rate. In this study, the performance of the IZ in the integrated bioreactor was investigated as a function of the moisture of the packing materials. Results showed that the removal efficiency of the IZ clearly decreased with decreasing moisture content (Fig. 4b). High SO_2 removal could be achieved at high moisture content.

Table 2 – Removal efficiency of the integrated bioreactor under different operation modes.

Operation mode	Section of integrated bioreactor	Average removal efficiency
Mode I	SZ + IZ	81.71%
Mode II	SZ	51.96%
Mode III	IZ	30.46%

SZ: suspended zone; IZ: immobilized zone.

The mineral nutrients were sprayed into the integrated bioreactor at regular intervals to maintain nutrients and moisture for microbial growth and activity. Variations in the moisture content of the packing material under operation Modes I and III are shown in Fig. 5. The moisture content in Modes I and III decreased with time after irrigation. In Mode I, the WCR started at 85.40% and then slightly declined to 83.69% with an average of 84.55%. Meanwhile, the moisture in Mode III markedly dropped from 83.66% to below 25.65% after 1 week of operation. The comparison result indicated that the IZ can maintain optimum moisture for a relatively long time in operation Mode I.

In the integrated bioreactor, the SZ was installed before the IZ, and gases initially entered the SZ. The RH in the stream from the SZ could reach nearly 100%. The evaporation rate of water in the SZ may increase when gases pass through this zone. Consequently, more water will be carried off by gases, which in turn decreases the liquor level in the SZ, while increasing the moisture content in the IZ. Thus, the rate of decline for moisture content in Mode I was significantly lower than that in Mode III because of the humidification by the SZ. For most packing materials, the optimal water content is 60% (*w/w*) or higher to maintain the biological activity and ideal performance of a biofilter (Ottengraf, 1987). In this study, irrigation occurred once every 2 weeks, and the desired water content in the IZ was able to be feasibly maintained without any additional pre-humidification facilities, which would reduce water and energy consumption in real applications. The SZ served a key function not only in SO_2 elimination, but also in moisture maintenance for the IZ.

3.3. Microbial characteristics

In this study, liquor and packing materials were periodically collected from the SZ and IZ for microbial analysis. The

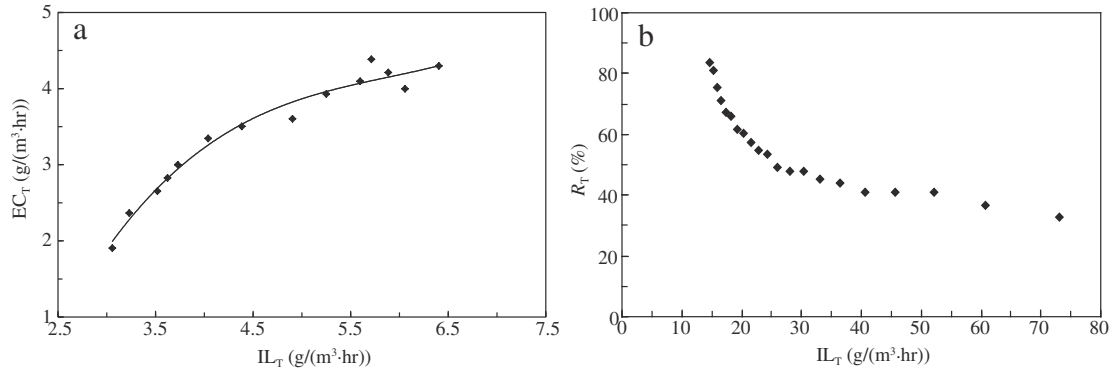

Fig. 3 – Total elimination capacity *vs.* inlet load of bioreactor (a) and total removal efficiency *vs.* inlet load of SZ (b).

amounts of total bacteria and sulfur bacteria were calculated by culture on the appropriate medium, as described in Section 1.4. The sulfur bacterial community in individual zones was assayed using the 16S rRNA method.

3.3.1. Distribution of biomass in the bioreactor

We found 3.2×10^4 CFUs/mL of total bacteria and 6.1×10^3 CFUs/mL of sulfur bacteria in the IZ in the initial period. The ratio of sulfur bacteria to the total bacteria was below 0.19. Both the total bacteria and sulfur bacteria increased with time. After 2 months of operation, the average number of total bacteria, sulfur bacteria, and their ratio were maintained at 2.4×10^6 CFUs/g, 1.2×10^6 CFUs/g, and 0.5, respectively. A similar phenomenon was observed in the bacterial population in the SZ. The number of total bacteria, sulfur bacteria, and their ratio increased from 1.7×10^4 CFUs/g, 2.9×10^3 CFUs/g, and 0.17 to 3.5×10^5 CFUs/g, 1.8×10^5 CFUs/g, and 0.51, respectively. The volume of liquor contained in the SZ was 1.5 m^3, whereas 54 kg of packing material was placed in the IZ. The total number of the sulfur bacteria present in the SZ was 2.7×10^{11} CFUs, which was significantly more than that in the IZ. Notably, the growth rate of sulfur bacteria was faster than that of total bacteria. These bacteria grew vigorously and became dominant groups in both zones. Microbial growth is a consequence of uptake and metabolism of the compounds in air (Ottengraf, 1987). The SO_2 in the inlet stream served as an energy source and building material, which in turn enabled the sulfur bacteria to grow and abound in the bioreactor.

3.3.2. Microbial structure in the bioreactor

The diversity and coverage of the 16S rDNA-based phylotypes were determined by analyzing the clones from each of the clone libraries. The samples for molecular biological analysis were collected after 181 days of bioreactor operation. A total of 47 clones were compared. We observed 13 OTUs among the 47 screened clones from the bioreactor clone library, with 4 of 23 and 9 of 24 identified from the IZ and SZ clone libraries, respectively. Coverage analysis indicated that the bioreactor libraries represented approximately 80%–92% of the total number of clones examined, providing a dependable inventory of the bacterial 16S rRNA gene sequences present in the bioreactor. The *C* values observed in all the samples were larger than 0.70, which indicates that the clone number analyzed in each sample in this study is valid for microbial diversity analysis (Han et al., 2010).

Sequences were assigned to a bacterial phylum according to their position in the phylogenetic tree (Fig. 6). The bacteria that most closely represented the OTU microorganisms were isolated from water and soil ecosystems with high sulfur content.

The sequences identified from the SZ clone library were assigned to *Paenibacillus* sp. and *Ralstonia* sp. Their percentages were 46% and 54%, respectively. For the IZ, the represented bacterial groups were *Paenibacillus* sp. (48%) and *Sphingobacterium* sp. (52%). The accession numbers of OTUs from all samples represented in the GenBank database are shown in Table 2. The diversities were 1.69 and 1.01 for the SZ and IZ bacterial populations, respectively.

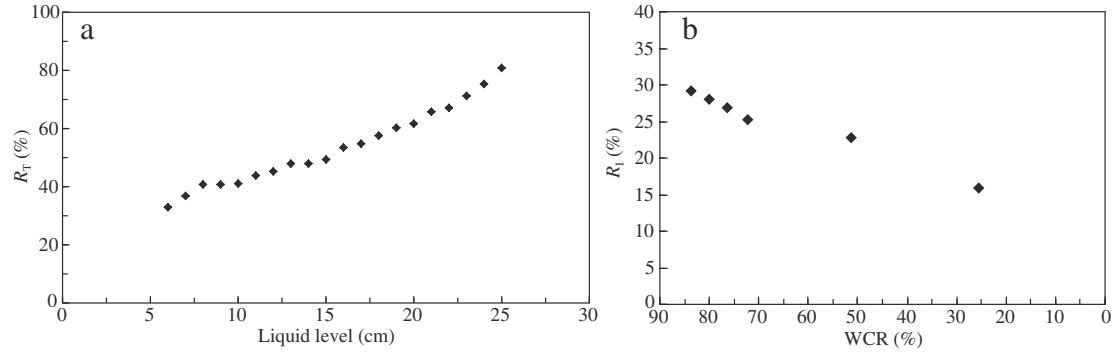

Fig. 4 – Total removal efficiency *vs.* liquid level of SZ (a) and removal efficiency of IZ *vs.* moisture content of packing material (b).

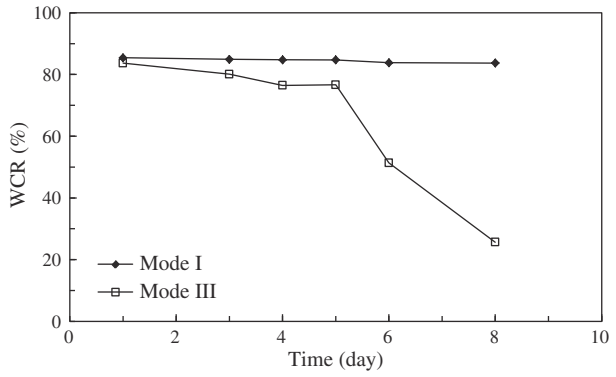

Fig. 5 – Water content ratio (WCR) of the packing material during two operation modes.

Paenibacillus polymyxa was used for modification of the surface of coal and achieved desulfurization from 3.3% to 1.12% (El-Midany and Abdel-Khalek, 2014). A thermophilic desulfurizing bacterium with desulfurization activity, *Paenibacillus* sp. strain A11-2, removed sulfur from alkylated dibenzothiophenes by converting them to alkylated mono-hydroxybiphenyls (Onaka et al., 2001). *Paenibacillus sp*. was found to dominate in a thermophilic biofilter for SO_2 removal (Zhang et al., 2015). Gaseous ethanethiol could be efficiently degraded by a pure culture of *Ralstonia eutropha* under various initial concentrations ranging from 115 to 320 mg/m^3 within 120–168 hr (Sedighi and Vahabzadeh, 2014; Sedighi et al., 2013). Both desulfurizing species were present in the SZ, but only *Paenibacillus* sp. was detected in the IZ. The sulfur bacteria were abundant in environments with plentiful SO_2 (substrate).

Sphingobacterium mizutaii was the dominant species in a membrane bioreactor for the removal of COD and NH^{4-}-N. More than 90% of NH^{4-}-N could be treated, with effluent content of less than 1.0 mg/L (Xing et al., 2011). Nutrient solution containing KNO_3 and NH_4Cl was sprayed over the packing material in the IZ once every 2 weeks, which resulted in an abundance of *S. mizutaii* in the IZ.

Microbial analysis exhibited that SO_2 was degraded by *Paenibacillus* sp. in the IZ and by *Paenibacillus sp*. and *Ralstonia sp*. in the SZ. With the synergistic effect of the desulfurization bacteria formed in each zone, SO_2 could be removed effectively in the integrated bioreactor.

3.4. Fate of SO_2

In the SO_2 removal system, the following reaction occurs (Formula 12):

$$2SO_2 + O_2 + 2H_2O \rightarrow 2SO_4^{2-} + 4H^+. \quad (12)$$

The main product of SO_2 oxidation is sulfate. The concentrations of SO_4^{2-} in the integrated bioreactor were periodically analyzed. The variation of SO_4^{2-} within one irrigation period is shown in Fig. 7. The pH in the SZ was also recorded to evaluate the production of H^+. The pH value gradually decreased from 3.01 to 1.96 with the formation of SO_4^{2-} within 21 days. The oxidation of SO_2 produced sulfuric acid and reduced the pH value.

Tests A and B were conducted to investigate SO_2 transfer in the SZ. The results of Tests A and B are shown in Fig. 8. During Test A, the outlet concentration of SO_2 rapidly increased and became equal to the level of the inlet stream (102 mg/m^3) within 150 min, which indicates that SO_2 could no longer be

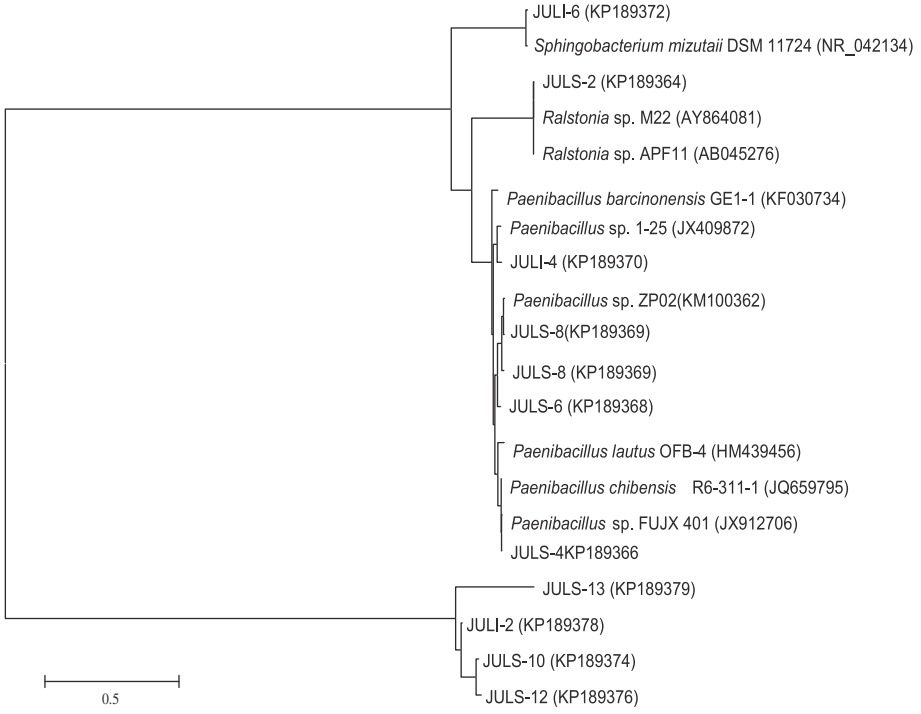

Fig. 6 – Phylogenetic tree showing the relationship of representative sequences of OTUs (Operational Taxonomic Unit) in all samples and reference sequences in GenBank. The accession numbers of OTUs from all samples represented in the GenBank database are shown in Table 2.

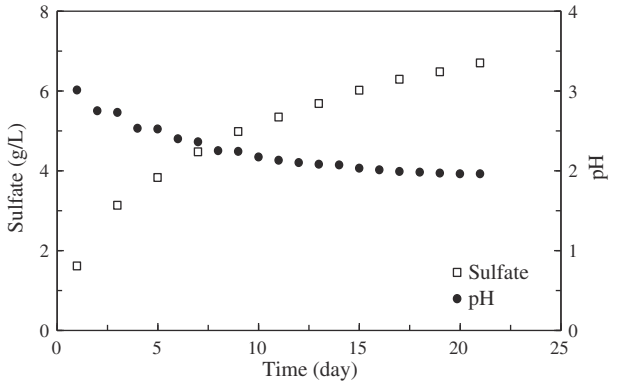

Fig. 7 – Variation of SO_4^{2-} and pH within one irrigation period.

absorbed. The decrease of SO_2 was attributed to its transfer from the gas phase to the liquid phase and its subsequent dissolution in the nutrient solution. SO_2 could be detected in the outlet stream after 76 min in Test B. The outlet concentration of SO_2 increased gradually and reached a maximum of 73 mg/m^3 at 735.5 min. This value was significantly lower than that in the inlet stream (102 mg/m^3). In Test B, nutrient solution with microorganisms was placed into a glass column. The decrease of SO_2 was not only attributed to its absorption in the nutrient solution, but also to biodegradation by sulfur-oxidizing bacteria. We can infer that the processes of SO_2 degradation occurred in two stages. First, SO_2 was transferred from the gas phase into the liquid phase. Second, SO_2 dissolved in the liquid and was then biodegraded by the sulfur-oxidizing bacteria.

4. Conclusions

SO_2 was reliably treated with high removal efficiency using an integrated bioreactor without liquid circulation pump. More than 85% of SO_2 in the inlet stream could be effectively removed, with the elimination capacity reaching a level of 4.30 g/(m^3·hr). The performance of individual zones was quite different. Compared with the IZ, the SZ had higher removal efficiency. The total removal efficiency exhibited a positive correlation with the liquid level in the SZ, whereas in the IZ, the removal efficiency was related to the WCR of the packing material. The liquid level of the SZ was maintained to stabilize the WCR of the packing material in the IZ. Microbial analysis indicated that SO_2 was degraded by *Paenibacillus* sp. in the IZ and by *Paenibacillus* sp. and *Ralstonia* sp. in the SZ. The transfer of SO_2 in the SZ involved dissolution in the nutrient solution and biodegradation by the sulfur-oxidizing bacteria. The integrated bioreactor can be potentially employed for the treatment of waste gases containing hydrophilic compounds.

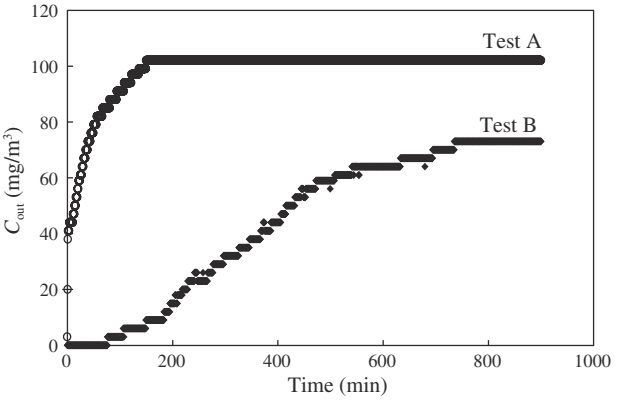

Fig. 8 – SO_2 transfer experiment. Test A: nutrient solution without microorganisms; Test B: nutrient solution with microorganisms; inlet concentration of SO_2: 102 mg/m^3.

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