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Temporal variation of microbial population in a thermophilic biofilter for SO₂ removal

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ABSTRACT

The performance of a biofilter relies on the activity of microorganisms during the gas contaminant treatment process. In this study, SO₂ was treated using a laboratory-scale biofilter packed with polyurethane foam cubes (PUFC), on which thermophilic desulfurization bacteria were attached. The thermophilic biofilter effectively reduced SO₂ within 10 months of operation time, with a maximum elimination capacity of 48.29 g/m³/hr. Temporal shifts in the microbial population in the thermophilic biofilter were determined through polymerase chain reaction-denaturing gradient gel electrophoresis and deoxyribonucleic acid (DNA) sequence analysis. The substrate species and environmental conditions in the biofilter influenced the microbial population. Oxygen distribution in the PUFC was analyzed using a microelectrode. When the water-containing rate in PUFC was over 98%, the oxygen distribution presented aerobic–anoxic–aerobic states along the test route on the PUFC. The appearance of sulfate-reducing bacteria was caused by the anaerobic conditions and sulfate formation after 4 months of operation.

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Introduction

Sulfur dioxide (SO₂) emission from industrial processes, especially fossil fuel combustion, is widely regarded as a risk to the atmospheric environment and human health (Weerasinghe, 2010). High levels of SO₂ are particularly dangerous in the presence of particulate matter, because it slowly adsorbs onto fine atmospheric particles and can be transported very deep into the lungs, therefore staying there for a long time (Nasir and Brahmaiah, 2015). Various technologies, including physical–chemical (scrubbing, adsorption, incineration, and masking) and biological methods have been applied in SO₂ removal (Ralebitso-Senior et al., 2012). Low operating costs, convenient maintenance, and environmental friendliness are the advantages of bio-techniques for off-gas treatment (Van Groenestijn and Hesselink, 1993; Yang et al., 2008). The bioreactor principle

is based on passing the polluted air stream through a porous packed bed on which pollutant-degrading microorganisms form an active biofilm on the surface of the packing materials. The pollutant in the air stream is transferred from the gas phase into the biofilm, where it can be biodegraded (Mohammad et al., 2007). Recently, Saravanan et al. (2015) found that xylene vapor could be removed up to 99% by using two biofilters which were packed with pressmud, proving the effectiveness of biofilters in treating polluted air.

Microorganisms in a bioreactor play important roles in the gas contaminant treatment process. Several groups of microorganisms, primarily bacterial species, are responsible for the degradation of air pollutants in biofilters (Reynolds and Grafton, 1999). *Thiobacillus thioparus*, *Bacillus* sp., and *Pseudomonas* sp. have been applied widely in the removal of sulfur-containing compounds from waste streams (Degorce-Dumas et al., 1997;

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Ho et al., 2008; Ryu et al., 2009). Various factors influence the growth kinetics of participating microorganisms, including substrate concentration, moisture content, temperature, oxygen transfer, pH, and availability of mineral nutrients (Ottengraf, 1986; Sakuma et al., 2008; Beuger and Gostomski, 2009; Jiang et al., 2012).

The microbial population growth rate depends on the temperature in the biofilter, which in turn affects biodegradation kinetics. William and Miller (1992) studied the influence of temperature on microorganism activity and found that biological activity roughly doubles for each 10°C rise in temperature of the biofilter bed. When the temperature in the biofilter was less than 25°C or over 50°C, a marked decrease occurred in the hydrogen sulfide (H₂S) removal efficiency, which was caused by a decline in sulfur oxidation bacteria (Yang and Allen, 1994). The application of thermophilic microorganisms for the treatment of off-gas at over 50°C would increase elimination rates and the desired efficiency would be achieved at shorter reaction times or a smaller reactor volume (Kong et al., 2001). Another advantage of thermophilic operation is a decrease in the biomass yield and its accumulation in the biofilter bed, which normally causes bed clogging and pressure drop through the bed. Various studies on thermophilic operation of gas phase bioreactors (45°C–75°C) for the treatment of odor compounds have been reported recently (Cox et al., 2001; Mohammad et al., 2007; Zhang et al., 2015).

The microorganism community in the biofilters uses the contaminants in off-gas as food or substrate, and such contaminants serve as their energy source and building material. The microbial population relies on substrate concentration and species (Devinny et al., 1999). For H₂S treatment in a bench biofilter, high contaminant concentration can adversely affect microbial populations. H₂S is toxic to microbes at higher concentrations. Additionally, sulfuric acid is formed as H₂S is degraded, reducing the medium pH. This declining pH eventually inhibits the performance of the biofilter (Allen and Yang, 1991). When complex compounds are treated in succession, toxic shock to the microbes may interrupt degradation until an adapted microbial population develops (Baltzis and Androutsopoulou, 1994).

Microorganisms obtain energy for their growth via aerobic respiration in the presence of oxygen, which is abundant in an environment fully exposed to air. In most cases, biofilters for off-gas treatment are aerobic, making them suitable for aerobic microorganisms. Facultative anaerobic or anaerobic microorganisms are abundant in environments with an oxygen volume fraction of less than 2% (Lee et al., 2001). With the innovation of microelectrodes, detection of the oxygen distribution in microenvironments is now possible and interesting oxygen microelectrode studies have been performed, including in nitrifying/denitrifying biofilms (Stief et al., 2003) and a single floc (Tsai et al., 2008; Han et al., 2012). Microelectrode measurements provide reliable and *in situ* information on the activity of microorganisms in biofilms formed on media (Chae et al., 2012). However, little is known about oxygen detection via microelectrodes on packing media for off-gas treatment to date.

In the current study, high-temperature SO₂ was treated with a laboratory-scale biofilter containing thermophilic desulfurization bacteria. Polyurethane foam cubes (PUFC) were packed in the

thermophilic biofilter for immobilizing the microorganisms. The polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) method was applied to explore the characteristics and temporal variation of the bacterial population in the biofilter. Microelectrodes were used to analyze the oxygen distribution in PUFC. The relationship between microbial characteristics and oxygen distribution was also explored in the thermophilic biofilter.

1. Experimental

1.1. Experiment and processes

A thermophilic biofiltration system was set up for SO₂ elimination treatment (Fig. 1). The stainless steel biofiltration column had a height of 30 cm and a diameter of 10 cm. The packing material for thermophilic bacteria attachment was PUFC (1.0 cm³). SO₂ synthesis, supplementation, and control methods were described in a previous report (Zhang et al., 2015). The synthetic gas was made of SO₂ and N₂, with a SO₂ concentration of 1%. The synthetic gas was diluted by air to certain concentrations before passing into the biofilter. The total flow rate controlled by a mass flow meter, which was calibrated in advance, was 0.6 m³/hr and corresponded to an empty bed residence time of 18 sec. The biofilter was inoculated with microorganisms to accelerate the startup period. The packing medium collected from the bioreactor for treating sulfur-containing compounds was used as inoculums and soaked in a nutrient solution, which contained beef extract of 5 g/L, K₂HPO₄ of 2.0 g/L, KNO₃ of 2.0 g/L, FeCl₂·4H₂O of 0.01 g/L, NaHCO₃ of 1.0 g/L, MgCl₂·6H₂O of 0.1 g/L, NH₄Cl of 0.5 g/L and Na₂S₂O₃·5H₂O of 5.0 g/L (pH 6.5 to 7.0). The nutrient solution with 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 r/min for 4 weeks to enrich the microorganisms able to degrade SO₂. Sampling ports were set along the biofilter for sample collection. SO₂ concentrations before and after the treatment were determined by samples 1 and 3, whereas sample 2 for PUFC collection was used for microbiological analysis (Fig. 1). A mineral nutrient solution consisting of 5 g/L beef extract, 2.0 g/L K₂HPO₄, 2.0 g/L KNO₃, 0.01 g/L FeCl₂·4H₂O, 1.0 g/L NaHCO₃, 0.1 g/L MgCl₂·6H₂O, and 0.5 g/L NH₄Cl was periodically sprayed into the biofilter to supply nutrients and sustain moisture above 80%. The used nutrient solution was drained from the bottom of the biofilter.

1.2. Chemical analysis

A flue gas analyzer (rbr, Ecom-J2KN, Germany) was used to monitor SO₂ online. An ion chromatogram analyzer was utilized to analyze sulfate concentrations in the liquid phase (ICS-1000, Dionex ion chromatography system, USA). Samples were diluted and filtered before injecting a volume of 1.0 mL. Gravimetry determined the water-containing rate (WCR) of the packing material. The temperature in the biofilter was recorded using a Dewpoint Thermohygrometer (WD-35612, OAKTON, Germany) sustained at 40°C–60°C. A pH meter (pH-3C, Shanghai, China) was used to detect the pH values.

Fig. 1 – The schematic diagram of the thermophilic biofilter. 1: high-pressure cylinder; 2: mass flow meter; 3: air pump; 4: sample 1: inlet sampling point; 5: biofiltration column; 6: packing layer; 7: thermostat; 8: heating layer; 9: sample 2: packing sampling point; 10: sample 3: outlet sampling point; 11: sprinkler; 12: circulating pool; and 13: water pump.

1.3. O₂ distribution in PUFC

The O₂ distribution on PUFC was measured using a microelectrode with a tip diameter of 10 μm (OX-10, Unisense, Denmark). PUFC with a range of WCR were investigated in the present study. O₂ determination began at point A and stopped at point B (Fig. 2). The entire detection length on the PUFC was 12,000 μm, with a step of 100 μm.

1.4. Microbiological analysis

Electron microscopy was used to observe the PUFC and biofilms of microorganisms in the packing material. Samples were fixed with 2.5% glutaraldehyde for 4 hr, washed thrice

with phosphate buffer for 15–20 min at each time, and fixed with 1% OsO₄ for 2–4 hr. Samples were then washed thrice with the same buffer prior to dehydration in a graded alcohol series (30%–100%) for 15 min. Finally, samples were dried with a critical point dryer (CPD 030) and coated with gold. Observation of samples was performed with a scanning electron microscope (HITACHI SU-8020/EDAX Inc., Japan).

The bacteria were incubated in a nutrient agar (BR, Aoboxing Biotech, Co., China) at 60°C for 24 hr. Sulfur bacteria were cultivated in a medium containing 2.0 g/L K₂HPO₄, 2.0 g/L KNO₃, 0.01 g/L FeCl₂·4H₂O, 1.0 g/L NaHCO₃, 0.1 g/L MgCl₂·6H₂O, 0.5 g/L NH₄Cl, and 5.0 g/L Na₂S₂O₃·5H₂O at 60°C for 3 days. Cell counts were reported as colony-forming units (CFUs).

Total DNA was directly extracted using an Activated Sludge DNA Automatic Plate form for Magnetic System-16 (TanBead, Taiwan). Next, 1.0% agarose gel electrophoresis (Bio-Rad, USA) was applied to determine DNA bands. PCR was conducted using a C1000TM thermal cycler (Bio-Rad, USA). Specific universal primers, such as F357 with a GC clamp and R517 (F357GC: 5'-CGCCCGCCGCGCGCGCGGGCGGGGCGGGGCGACGGGGGGC-CTACGGGAGGCAGCAG-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. Denaturing gradient gel electrophoresis (DGGE) was performed on 6% to 12% (W/V) polyacrylamide gel with 40% to 70% denaturing gradient. Electrophoresis was performed in a 1 × Tris-acetate-EDTA buffer at 60°C and a constant voltage of 65 V for 16 hr using a Bio-Rad DCode system (DCode, Bio-Rad, USA). Bacterial diversity was determined according to the intensity and number of bands using the Shannon index (*H*). Sequences were edited to exclude the priming site, and were then compared with

Fig. 2 – The schematic diagram of oxygen detection using microelectrodes.

sequences available in the GenBank database using a nucleotide–nucleotide BLAST algorithm. Detailed procedures were described in our previous report (Li et al., 2012; Zhang et al., 2015).

2. Results and discussion

2.1. Performance of the thermophilic biofilter

The thermophilic biofilter operated continually for 10 months (Table 1). The inlet and outlet concentrations of SO₂ were 100–200 mg/m³ and 0–40 mg/m³, respectively. Flow rate was maintained at 0.6 m³/hr. Average removal efficiency was only 33.33% for the first week, then increased gradually. After 24 days of acclimation period, the removal efficiency reached 71.05%. SO₂ was completely removed after 5 months, and a maximum elimination capacity of 48.39 g/m³/hr was obtained under an SO₂ inlet concentration of 190 mg/m³. Results showed that SO₂ could be treated effectively in the thermophilic biofilter.

2.2. Microbial characteristics

The microbial populations and their spatial distributions inside the bioreactor can be determined and can serve as an effective tool for understanding and controlling the biological process.

2.2.1. SO₂ degraders in the biofilter

Microorganisms in the biofilter were analyzed by periodic collection of PUFC. Compared with unused PUFC (Fig. 3a), a large amount of microorganisms accumulated on the surface of PUFC that had been used for 9 months in the thermophilic biofilter (Fig. 3b). Various kinds of microorganisms, including rod-shaped and spherical bacteria, formed the biofilm (Fig. 3c).

Bacteria cells and sulfur bacteria cells in the biofilter were determined by culturing on the proper medium, as described

in Section 1.4. After inoculation, 1.52×10^{11} CFUs/mL of bacteria and 6.87×10^8 CFUs/mL of sulfur bacteria were observed in the biofilter (Table 2). A certain number of sulfur bacteria were cultured in the inoculum, and the sulfur bacteria–total bacteria (TB) ratio needed to effectively remove SO₂ was 0.45%. Similar to most biosystems, a newly installed biofilter adapts itself to the environment and nutrient supply before efficient biodegradation occurs (Cox et al., 1993). Some amount of microorganisms would disappear during the adaption period because of their inability to adapt to the new environment (Slater and Godwin, 1980). As a result, the amount of TB and sulfur bacteria decreased after inoculation. After 4 months of operation, the amount of TB was sustained from 1.84×10^{10} to 3.28×10^{10} . The amount of total sulfur bacteria (TSB) gradually increased with time after the adaptation period. The ratio of sulfur bacteria to TB markedly increased from 0.45% to 1.09% within 10 months. Microbial growth is a consequence of the uptake and metabolism of the pollutants in air. The SO₂ in the inlet stream served as an energy source and building material. Bacteria grow vigorously in an environment where food is abundant (Devinny et al., 1999; Shareefdeen and Singh, 2005). Therefore, based on the results of the current study, sulfur bacteria grew rapidly because SO₂ was the sole substrate in the biofilter.

2.2.2. Bacterial structure formed in the biofilter

DGGE was used to explore the population shifts in response to variation in the biofilter (Fig. 4a). Shannon indices were used to reflect the individual biodiversity of the bacterial groups (Fig. 4a). After the adaption period, the diversity and quantity of TB and TSB decreased with time. Microorganisms participate in the process of microbial degradation through a variety of reactions. Complex substrates lead to the increase of microbial species (Dhamwichukorn et al., 2001). SO₂ was the sole substrate in the present study, which resulted in a microbial diversity decrease in the biofilter. Large fluctuations in temperature also led to diversity variation (Kong and Wang, 2013). After

Table 1 – The performance of the thermophilic biofilter.

Operation time (day)	Temperature in biofilter (°C)	Concentration of SO ₂ (mg/m ³)	Removal efficiency (%)	Elimination capacity (g/m ³ /hr)
0	50	–	–	–
7	55	132	33.33	33.62
24	50	120	71.05	30.56
55	42	175	79.10	44.57
76	42	105	86.50	26.74
90	40	117	87.25	29.80
102	40	105	91.67	26.74
120	38	161	89.09	41.00
143	42	173	98.28	44.06
158	39	126	100	32.09
179	43	158	100	40.24
193	42	170	100	43.30
212	44	190	100	48.39
233	41	190	100	48.39
253	46	176	100	44.82
274	47	161	100	41.00
295	48	190	100	48.39
308	53	190	100	48.39

Fig. 3 – Scanning electron microscope (SEM) photos. (a) Surface of unused polyurethane foam cubes (PUFC) ($\times 100$) (b) Surface of PUFC collected from thermophilic biofilter operated 9 months ($\times 100$) (c) Surface of PUFC collected from thermophilic biofilter operated 9 months ($\times 5000$).

308 days of operation, the temperature increased from 47°C to 53°C and diversity decreased from 2.69 to 2.49 and from 2.60 to 2.29 in samples Y5 and S5, respectively. The genetic

evolutionary relationships of bacterial and sulfur bacterial communities are respectively presented in Fig. 4b and c. For the bacterial community, Y1 exhibited a close evolutionary relationship to Y2, but was very different from Y5. Y2 and Y5 were collected from the biofilter after 4 and 10 months of operation, respectively. The bacterial population presented obvious temporal variations during the operation period. A similar phenomenon was observed in the sulfur bacterial community.

A total of 16 discriminable bands (B1 to B16) in Fig. 4a that represented the main function bacteria of the microbial community were identified, and their sequences were matched with their closest relatives in GenBank as listed in Table 3. The phylogenetic tree derived from neighbor-joining analysis of partial 16S rRNA gene sequences is shown in Fig. 5. Bands B10, B11, B12, B13, B15, and B16 emerged in all samples, demonstrating that the closest relatives of these bands continually existed in the biofilter. Bands B10 and B11 were closely related to *Bacillus circulans* (100%) and *Bacillus thermophilus* (100%), respectively. These microorganisms belong to *Bacillus* sp. and have the ability to remove sulfur-containing compounds under thermophilic conditions (Ryu et al., 2009). Under stressful environmental conditions, *Bacillus* can produce oval endospores to which the bacteria can reduce themselves and remain in a dormant state for very long periods of time (Madigan and Martinko, 2005). Meanwhile, Band B12 was closely associated with *Brevibacillus borstelensis* (100%), a thermophilic bacteria species widely distributed in hot springs, and can use thioanisole as sole carbon and energy source (Li et al., 2015). Bands B13, B15, and B16 belong to *Aneurinibacillus* sp., which is usually isolated from sludge in high-temperature environments. The strain could degrade sulfide via a biological denitrifying sulfide reaction (Chen et al., 2010). Thermophilic bacteria and desulfurization bacteria existed in the biofilter during the 10 months of operation.

Bands B5 and B6 were obvious in all lanes except the inoculum (Y1). Band B5 is affiliated with *Acinetobacter* sp. (99%), which is always isolated from soil and sediment samples and is capable of desulfurization (Omri et al., 2011). Band B6 is closely related to *Ralstonia* sp. (100%). Several *Ralstonia* species, such as *Ralstonia eutropha*, can degrade ethanethiol (Sedighi and Vahabzadeh, 2014).

Bands B4, B7, and B14 appeared at the lane of Y1 (inoculum) but weakened with the increase in operation time. Band B4 exhibited 100% similarity to *Stenotrophomonas humi*, and B7 was similar with *Desulfotobacterium dichloroeliminans* (98%). They always existed in environments below 40°C (Lie et al., 1999; Papizadeh et al., 2011). Microbial activity is strongly influenced by temperature. Each species is adapted to control its own reactions within a certain temperature range. The temperature in the biofilter was maintained at over 40°C. As the temperature increases, each microbial species reaches a point where it can no longer be effective, and metabolic activity drops off rapidly (Wang et al., 2012). Actually, the *Stenotrophomonas humi* and *Desulfotobacterium dichloroeliminans* were killed by the heat and were not present after 4 months. Band B14 is closely associated with *Pusillimonas terrae* (99%), which is thermostable but not a desulfurization bacterium (Chénier and Juteau, 2009). SO₂ was the sole substrate with high concentration in the biofilter. SO₂ may serve as energy source, building material,

Table 2 – Total bacteria and sulfur bacteria in the biofilter.

No.	Y1	Y2	Y3	Y4	Y5
Operation time (day)	0	120	158	233	308
TB (CFU/mL)	1.52×10^{11}	2.28×10^{10}	3.28×10^{10}	1.84×10^{10}	2.54×10^{10}
TSB (CFU/mL)	6.87×10^8	6.93×10^7	1.11×10^8	1.11×10^8	2.76×10^8
R = TSB/TB × 100%	0.45	0.3	0.34	0.6	1.09

TB: Total bacteria; TSB: Total sulfur bacteria; R: Ratio.

or both to the microorganisms. When microorganisms could not use SO_2 , the high concentration of SO_2 would act like a poison to them.

Desulfurization bacteria and thermophilic bacteria dominated the microbial population in the thermophilic biofilter. The percentages of desulfurization and thermophilic bacteria were 66.7% and 80.0%, respectively. Microbial growth is a consequence of the uptake and metabolism of air pollutants. The SO_2 in the inlet stream functioned as an energy source and building material. The temperature of the biofilter was maintained at 40 to 60°C during the operation. Generally, the desulfurization and thermophilic bacteria can vigorously grow in an environment with an abundant food supply and optimal temperature, which maintains the biofilter performance.

2.3. Oxygen distribution and microbial characteristics in PUFC

2.3.1. Oxygen distribution

A dissolved oxygen (DO) microelectrode was used to investigate oxygen distributions on PUFC with 98.06% WCR (Fig. 6). DO concentrations were determined along the route that

began at position A, as shown in Fig. 6. DO concentration gradually decreased from the surface to the core of the PUFC. A minimum concentration of 0.3 mg/L could be observed at a position at 4700 μm distance from the surface, whereas the concentration in the surrounding air was 9.7 mg/L. The environment may be regarded as anoxic when the DO concentration is under 0.5 mg/L (Wilén and Balmer, 1998). Therefore, the oxygen distribution presented aerobic–anoxic–aerobic states along the test route on PUFC under the conditions of 98.06% WCR.

The oxygen transfer from air to liquid phase can be divided into two processes: surface oxygen transfer and liquid film diffusion. Surface oxygen transfer occurs at the air–water interface. The diffusion rate of oxygen in air is only one ten-thousandth of that in water (Cussler, 1997). As a polar molecule, oxygen is poorly soluble in water, inhibiting oxygen transfer through the liquid–gas surface and diffusion in liquid. Therefore, the remarkable change in DO resulted in aerobic–anoxic–aerobic states occurring in PUFC with high WCR. A similar phenomenon was found in the PUFC collected from the biofilter that had been operated for 3 months.

Fig. 4 – (a): DGGE profile and Shannon indices of bacterial community (b): dendrogram of similarity for bacteria (c): Dendrogram of similarity for sulfur bacteria. DGGE: denaturing gradient gel electrophoresis.

Table 3 – The closest relatives and isolation sources of dominate bacteria.

Band no.	Closest relatives	Source	Similarity
B1, B13	<i>Aneurinibacillus aneurinilyticus</i> G7_2_4BCO2	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Aneurinibacillus group; Aneurinibacillus.	100%
B2	<i>Lysinibacillus fusiformis</i> BHR3P1B1	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Lysinibacillus.	100%
B3	<i>Clostridium</i> sp. M1	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium.	100%
B4	<i>Stenotrophomonas humi</i> KPS-24	Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas.	100%
B5	<i>Acinetobacter</i> sp. CSeB30_45	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter.	99%
B6	<i>Ralstonia</i> sp. AF7	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Ralstonia.	100%
B7	<i>Desulfitobacterium dichloroeliminans</i> LMG P-21439	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Desulfitobacterium.	98%
B8	<i>Clostridium symbiosum</i> JCM 1297	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae.	100%
B9	Sulfate-reducing bacterium 1-3-5-G4	Bacteria; environmental samples.	94%
B10	<i>Bacillus circulans</i> S1	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.	100%
B11	<i>Bacillus thermophilus</i> SgZ-10	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.	100%
B12	<i>Brevibacillus borstelensis</i> 56	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Brevibacillus.	100%
B14	<i>Pusillimonas terrae</i> EGY-WCP1	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae; Parapusillimonas.	99%
B15, B16	<i>Aneurinibacillus thermoaerophilus</i> SWCR50_11	Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Aneurinibacillus group; Aneurinibacillus.	99%

2.3.2. Oxygen distribution and microbial characteristics

Oxygen limitation is more likely to occur in high concentrations of easily degradable hydrophilic compounds (Devinny et al.,

1999). SO₂ is a water soluble substance. In this study, the inlet concentration in the biofilter was 100–200 mg/m³. The biofilter was packed with PUFCs, which had 40% to 90% of WCR.

Fig. 5 – The phylogenetic tree derived from neighbor-joining analysis of partial 16S rRNA gene sequences.

Fig. 6 – Dissolved oxygen (DO) concentration in PUFC.

Although oxygen concentrations in both inlet and outlet of the biofilter were 21%, oxygen deprivation may have occurred at the center of the PUFC with high WCR. Bands B8 and B9, which were closely associated with *Clostridium symbiosum* (100%) and sulfate-reducing bacterium (94%), respectively, were detected after 4 months of operation (Fig. 4a). *Clostridium* sp. is abundant in sulfide removal systems, and plays a role in sulfide-oxidizing pathways for transforming sulfate (SO_4^{2-}) to elemental sulfur (Wang et al., 2015). Both of them are anaerobic bacteria and can use SO_4^{2-} as substrate for growth. SO_2 was transformed into SO_4^{2-} , which accumulated on the PUFCs. The average SO_4^{2-} concentration in the biofilter was 33.73 g/L. Here, SO_4^{2-} served as an energy provider that was utilized by *Clostridium symbiosum* and sulfate-reducing bacterium under anoxia conditions. The growth of microorganisms was associated with the environmental conditions and substrate species. The appearance of sulfate-reducing bacterium in the present study was caused by the anaerobic conditions and SO_4^{2-} formation.

3. Conclusions

A thermophilic biofilter containing desulfurization bacteria achieved over 90% SO_2 removal efficiency and 48.39 g/m³/hr of maximum elimination capacity at steady state. The microbial population presented temporal variation and relied on the SO_2 concentration and environmental conditions formed in the thermophilic biofilter. Results of oxygen detection using micro-electrodes indicated that the oxygen concentration shifted along with depth in the PUFC. Aerobic–anoxic–aerobic states were present in PUFC with 98.06% of WCR. Aerobic bacteria and anaerobic bacteria were all found in the PUFC. The oxygen distribution also influenced the microbial population. Finally, the anaerobic conditions and SO_4^{2-} formation led to the appearance of sulfate-reducing bacteria after 4 months of operation.

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