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# Temporal variation of microbial population in a thermophilic biofilter for $SO_2$ 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, $SO_2$ 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 $SO_2$ within 10 months of operation time, with a maximum elimination capacity of 48.29 g/m$^3$/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.



## Introduction

Sulfur dioxide ($SO_2$) 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 $SO_2$ 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 $SO_2$ 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 ($H_2S$) 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 $H_2S$ treatment in a bench biofilter, high contaminant concentration can adversely affect microbial populations. $H_2S$ is toxic to microbes at higher concentrations. Additionally, sulfuric acid is formed as $H_2S$ 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 $SO_2$ 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 $SO_2$ 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 $cm^3$). $SO_2$ synthesis, supplementation, and control methods were described in a previous report (Zhang et al., 2015). The synthetic gas was made of $SO_2$ and $N_2$, with a $SO_2$ 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 $m^3$/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, $K_2HPO_4$ of 2.0 g/L, $KNO_3$ of 2.0 g/L, $FeCl_2{\cdot}4H_2O$ of 0.01 g/L, $NaHCO_3$ of 1.0 g/L, $MgCl_4{\cdot}6H_2O$ of 0.1 g/L, $NH_4Cl$ of 0.5 g/L and $Na_2S_2O_3{\cdot}5H_2O$ 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 $SO_2$. Sampling ports were set along the biofilter for sample collection. $SO_2$ 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 $K_2HPO_4$, 2.0 g/L $KNO_3$, 0.01 g/L $FeCl_2{\cdot}4H_2O$, 1.0 g/L $NaHCO_3$, 0.1 g/L $MgCl_4{\cdot}6H_2O$, and 0.5 g/L $NH_4Cl$ 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 $SO_2$ 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.