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

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ABSTRACT

Microorganisms in a biofilter play important roles in the gas contaminants treatment process. In this study, thermophilic desulfurization bacteria were inoculated in a thermophilic biofilter for SO_2 treatment after acclimation and enrichment. Molecular biology techniques were applied to detect temporal variation of microbial population during acclimation and biofilter start-up period. The acclimation temperatures were 50, 55, and 60 °C. Desulfurization bacteria dominated the enrichments. During acclimation, desulfurization bacteria and thermophilic bacteria increased from 36.84% to 78.95%–52.94% and 88.24%, respectively. In addition, the microbial diversity indices of these enrichments decreased with time. Substrate species and acclimation temperature influenced the microbial structure of the enrichments. The thermophilic biofilter inoculated with enrichments could achieve a rapid start-up, and over 80% of removal efficiency could be obtained within two weeks. The maximum elimination capacity was 38.71 g m^{-3} h^{-1} at 152 mg m^{-3} inlet concentration. The total sulfur bacteria proportion increased obviously during the start-up period. Moreover, desulfurization bacteria that originally existed in inocula, e.g., *Pseudomonas putida*, *Bacillus thioparans*, *Microbacterium* sp., and *Thermoanaerobacteriaceae*, were abundant in the thermophilic biofilter for SO_2 removal.

1. Introduction

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

Compared with physical (adsorption, absorption, and condensation) and chemical (catalytic combustion, scrubbing, and oxidation) methods, biotechnology provides a more economical and sustainable approach owing to its low operational costs, high removal efficiency, and lack of secondary pollutant generation (Li and Liu, 2006; Ralebitso-Senior et al., 2012). For most applications, biological air treatment systems have traditionally relied on microorganisms, generally bacteria, found under ambient environmental conditions (Devinny et al., 1999). An integrated bioreactor with two sections, a suspended aerobic zone (SZ) and immobilized aerobic zone (IZ), was applied to treat SO_2 for six months. Microbial analysis indicated that SO_2 was degraded by *Paenibacillus* sp. in the IZ, and by *Paenibacillus* sp. and *Ralstonia* sp. in the SZ (Lin et al., 2015). Reactor stability and efficacy are maintained by functionally, compositionally, and spatiotemporally dynamic and diverse communities (Xue et al., 2013). Various factors affect the growth kinetics of participating microorganisms, including substrate concentration, moisture content, temperature, electron acceptor, pH, toxic intermediates, and availability of mineral nutrients. Any or a combination of these factors can limit biomass growth rate and substrate biodegradation (Beuger and Gostomski, 2009; Ottengraf, 1986; Sakuma et al., 2008).

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

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

However, few studies have focused on the characteristics of microbial population during acclimatization and bioreactor start-up period. SO_2, generated from the thermal conversion processing of fuels in power plants, refineries, coal- and oil-fired boilers, and paper pulp production, is harmful to the environment and the human health (Philip and Deshusses, 2003). Therefore, SO_2 was used as a target compound in the present study. Desulfurization bacteria were acclimated and enriched to inoculate a thermophilic biofilter to treat SO_2. The temporal variations of microbial population during acclimatization and biofilter start-up period were analyzed via polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), and fluorescence in situ hybridization (FISH) was applied to describe the microbial population distribution. Biofilter performance was also investigated after desulfurization bacteria inoculation. The aim of this present study is to provide data for rapid start-up and steady operation of the bioreactor, especially for the thermophilic biofilter.

2. Materials and methods

2.1. Acclimation method

Inoculum samples were collected from a bioreactor that contained SO_2 with three packing layers for off-gas treatment. From the bottom to the top layer, the operation temperatures were 60, 55, and 50 °C. The packing samples were obtained from each layer. The detail information of samples was described in Table 1. After transporting to the laboratory, the packing mediums were soaked in Luria–Bertani (LB) medium (BR, Aoboxing Biotech, Co., China). The mediums were then shaken for 1 h using ultrasonic oscillators (KQ-250B, Kunshan, China). The liquids with suspended cells were cultured in the same nutrient solution in incubator shakers (HZ-9211K, Taicang, China) at 50, 55, and 60 °C, and at 120 rpm to enrich the microorganisms.

Sulfur bacteria culture media (SCM) was used to enrich and acclimate the three inocula contained in the following components at the specified concentrations (in g L^{-1}): 5.00 $Na_2S_2O_3 \cdot 5H_2O$; 1.00 $NaHCO_3$; 2.00 K_2HPO_4; 2.00 KNO_3; 0.01 $FeCl_2 \cdot 4H_2O$; 0.10 $MgCl_4 \cdot 6H_2O$; 0.50 NH_4Cl; and 5.00 beef extract. Given that S^{2-} was converted into H_2S and was lost as gas from aerobic enrichment, $Na_2S_2O_3$ was used instead of Na_2S as the sulfur substance. The initial pH of the media was adjusted to 6.5–7.0. Agar (2%) was added as a solidifying agent. The inocula were replaced monthly with fresh media at a ratio of 10:1 and were sampled regularly to analyze the microbiological indicator.

2.2. Bioreactor setup

After the inocula were enriched at 50, 55, and 60 °C for 12 months, the inocula were mixed at a ratio of 1:1:1 to inoculate into a thermophilic biofiltration (Fig. 1). The biofiltration was a stainless column with a height of 30 cm and a diameter of 10 cm. The packing material was polyurethane foam cube with an average size of 1.0 cm^3. A high-pressure cylinder continuously supplied SO_2 to the biofiltration from the air inlet at the bottom of the column. A calibrated mass flow meter was used to control the gas flow rate, and a temperature-controlled oven was supplied to heat the SO_2 inlet. The total flow rate was 0.6 $m^3\ h^{-1}$, and the column was maintained at 60 °C through a thermostat-controlled heating belt. After inoculation, the SO_2 concentrations of the inlet and outlet were monitored online using a flue gas analyzer (rbr, Ecom-J2KN, Germany) to determine the removal efficiency. The performance of the bioreactor was evaluated by series parameters, which were defined as follows (formulas 1 and 2):

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

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

where R is the removal efficiency (%), C_{in} is the inlet concentration of SO_2 (mg m^{-3}), C_{out} is the outlet concentration of SO_2 (mg m^{-3}), EC is the elimination capacity (g $m^{-3}\ h^{-1}$), Q is the flow rate ($m^3\ h^{-1}$), and V is the volume of biofilter (m^3).

2.3. Microbiological analysis

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

2.3.1. DNA preparation and 16S rRNA gene amplification

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

Table 1
Samples information.

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

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