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The effect of injected ozone on the microbial metabolic characteristics in biofilters treating gaseous toluene

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ABSTRACT

O_3 injection technique was developed recently and successfully shown the ability of controlling biomass, allowing enhanced VOCs removal efficiency in biofilters. However, the effect of injected O_3 on microbial metabolic patterns is still unknown. In this study, six biofilters treating gaseous toluene were set up and operated continuously for 97 days with different inlet O_3 concentrations ranging from 0 to 300 mg m^{-3}. The metabolic functions and characteristics of the microbial communities in the biofilters were monitored by Biolog microplates cultivation method periodically. According to the profile of average well color development (AWCD), the metabolic activity of microorganisms in the biofilter with 300 mg m^{-3} O_3 was increased by 100%. Principle components analysis (PCA) result demonstrated significant changes in biofilters exposed to O_3, but eventually had little difference with those without O_3 after long-term operation. Among 31 carbon sources in Biolog microplate, the utilization of some non-biodegradable carbon sources including γ-hydroxybutyric acid, D-galactonic acid γ-lactone, D-mannitol, D-cellobiose and β-methyl-D-glucoside were obviously enhanced by injected O_3. The analysis on DQ-fd plot showed that the variation trends of metabolic diversity and dominance in biofilters with and without O_3 were in some extent different.

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1. Introduction

Volatile organic compounds(VOCs) are very common air pollutants (Hsu et al., 2007) which usually come from loading losses of storage tanks, leaks of piping and equipment (Khan and Ghoshal, 2000), miscellaneous coating manufacturing (An et al., 2011) and industrial manufacture process. Due to the potentially harmful effects of VOCs on human health and the environment, various efforts have been done to control and minimize VOCs emission (Delhoménie and Heitz, 2005; Li and Moe 2005; Moussavi and Mohseni 2007).

Among various technologies for VOCs treatments, biofiltration was characterized by its low cost (Jorio et al., 1998; Metris et al., 2001; Strauss et al., 2004) and minimal secondary pollution (Iranpour et al., 2005; Deng et al., 2012), which reputed it as a cost-effective and green technology. In biofilters, microorganisms adhered on the surface of packing media function as a biocatalyst for VOCs removal. Various kinds of microorganism are found in biofilters including bacteria, fungi, actinomycetes and protozoan. The metabolic activity and function of these microorganisms have a great impact on VOCs removal performance (Langlais et al., 1991). In addition, some pretreatment technologies were used to remove VOCs from air system. Li and Moe (2005) has used activated carbon to reduce inlet concentration of inhibitory compounds into a biofilter. However, no significant improvement was observed. Moreover, a photo oxidation unit using ultraviolet (UV) lamps before a biofilter converted recalcitrant VOCs and improved overall performance of the combined system (Wang et al., 2009).

For biofilters, excessive biomass accumulation may induce clogging of the filter beds. Bed clogging will reduce removal efficiency and increase pressure drop (Alonso et al., 1997; Okkerse et al., 1999). Some biomass control methods were developed to solve clogging problem. One novel technique by O_3 injection was recently reported (Xi et al., 2014). It was found that continuous exposure to low concentration O_3 could lower biomass growth rate and enhance recalcitrant VOCs removal capacity (Lee et al., 2012; Zhang et al., 2009). O_3 is regarded as a strong oxidant that can be used as a disinfectant to inactivate microorganisms or used to treat waste activated sludge (Lee et al., 2012; Mobius and Helble, 2004; Zhang et al., 2009). When high dosage is applied, microorganisms will be inactivated and their microbial activity will decrease. However, the opposite response was found in low-dosage O_3 exposure. For example, low concentration O_3 (e.g. 120 mg m^{-3}) was found to improve

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metabolic activity in a biofilter (Wang et al., 2009). However, the effects of O_3 concentration on the microbial function and metabolic characteristics of microorganisms are still unknown.

In order to understand the performance and mechanisms of biological treatment systems, methods have been developed to analyze microbial metabolic function and characteristics. Microarray technology has been applied in microbial metabolic profiles with its advantage of rapid detection, high sensitivity and high-throughput (Wang et al., 2006; Yergeau et al., 2009). Another method is to study the key enzymes and pathways of metabolism during biodegradation (Bhushan et al., 2005, 2002). Another important technique is Biolog microplate cultivation. Originally, the Biolog microplate was designed to identify bacterial isolates. With the development of this technique, Biolog microplate was considered to be a simpler and quicker method to characterize heterotrophic microbial communities (Garland and Mills 1991; Garland 1997; Jellett et al., 1996). Moll et al. (1999) observed that Biolog microplates may bring a better understanding of the relationship between microbial communities activity and experimental factors.

Biolog ECO systems can reflect metabolic activities in microbial communities and their utilization capacity for different carbon sources (Stefanowicz, 2006). Each Biolog ECO plate contains 31 sole carbon sources and one control (water), the composition of which is dominated by carbohydrates (n = 9), amino acids (n = 6), carboxylic acids (n = 8) and polymers (n = 4). Thus, results of Biolog ECO-plate can determine whether the pattern of sole source utilization was caused by changes in microbial communities. For example, soil microbial functional diversity was assessed by Biolog substrate utilization ECO-plate (Lombao et al., 2015). Kong et al. (2013) also found that higher temperature can enhance microbial community activity in the operation using Biolog ECO-plate.

In this study, microbial metabolic function and characteristics of the microorganisms in biofilters with different inlet O_3 concentrations were explored using Biolog ECO microplate cultivation methods. Based on the Biolog test data, metabolic activity, metabolic pattern, carbon utilization and metabolic diversity/dominance of microorganisms in the biofilter were analyzed.

2. Materials and methods

2.1. Experimental set-up and operation of six biofilters

The experiments were carried out using six laboratory scale biofilters (BF1-BF6) with different inlet O_3 concentrations. The experimental system is shown in Fig. 1. Each biofilter has an inner diameter of 12 cm and height of 50 cm, which are packed with 250 g porous perlite (5–8 mm in diameter). And each biofilter has one filter bed layer, whose height is 23 cm and the effective volume is 2.6 l. Toluene was selected as the representative VOC, which is typical and widely used in many industries. A syringe pump (ALCBIO, ALCIP900, Shanghai, China) was used to control toluene concentration and inject toluene into gas to form mixture. Meanwhile, O_3 was produced by a generator (Shanmeishuimei, YG-2, Beijing, China) and mixed with toluene. The metering pump (Iwaki, EHC-B220R, Japan) sprayed nutrient solution (10 g/L $NaNO_3$, 0.7 g/L Na_2HPO_4, and 0.52 g/L KH_2PO_4) onto the filter beds to ensure microorganism growth.

All biofilters were operated in parallel conditions except that their inlet O_3 concentrations were different after 15 days. During operation, the temperature was maintained relatively constant at 20–30 °C. The more detailed operating conditions can be found in Table 1. During phase (16–97 days), all biofilters began to be continuously fed O_3 at different concentrations to determine the effect of O_3 injection on the metabolic profiles of the microbial communities. The biomass concentration in the inlet section and outlet sections was measured periodically.

2.2. Biolog microplate cultivation and test

The microorganisms existing on the surface of the packing medium were washed off and suspended in 0.9% NaCl by ultrasonic wave (40 kHz, 100 W, 15 s × 4). The suspension was diluted to a certain concentration with 0.05 cm^{-1} absorbance at 600 nm. Then, 150 μl suspension was added to each well in Biolog ECO plate except the blank well (pure water added) and the plate was incubated at 30 °C without agitation (Wang et al., 2009). All samples were inoculated in three replicates.

A microplate reader (Bio-Rad 550, USA) was used to detect the absorbance of each well at 595 nm. In order to evaluate the metabolic activity of each sample, the average well color development (AWCD) was calculated according to Eq. (1) (Grove et al., 2007).

$$AWCD = \frac{1}{31}\sum_{i=1}^{31}(R_{it} - R_{0t}) \tag{1}$$

in which R_{it} is the average absorbance of each carbon source at time t (cm^{-1}) and R_{0t} is the average absorbance of blank at time t (cm^{-1}).

2.3. PCA and carbon source utilization analysis

The metabolic pattern of each biofilter was expressed by the utilization of each carbon source which was indicated by the difference between optical density (OD) of each carbon-source well and the OD of control well. The principal component analysis (PCA) was conducted to show the microbial metabolic pattern in the biofilters (Buyer et al., 2001; Grove et al., 2007). PCA analysis allows the 31 carbon source in a multivariate data set to be reduced, whilst retaining two principal factors (PC1and PC2). According to the value of Biolog ECO-plate, all statistical analyses were made using the SPSS 18.0 statistical package.

The carbon sources can be assigned to groups of alcohols, amines, amino acids, carbohydrates, carboxylic acids, esters and polymers. Qualitative measures of substrates utilization by each group can be calculated using the sum of absorbency fraction (Zak et al., 1994). Then the utilization of some specific carbon sources with poor biodegradability under different O_3 concentrations were also analyzed.

2.4. Metabolic diversity and dominance analysis

In this study, the "DQ-fd plots" is used to analyze the changes in metabolic diversities and dominance in different biofilters. The DQ index was proposed by Hu et al. (1999) and described by Eqs. (2) and (3).

$$DQ = \left(\sum_{i=1}^{31}\sqrt{f_i}\right)^2 \tag{2}$$

$$f_i = \frac{R_{it}}{\sum_{n=1}^{31} R_{it}} \tag{3}$$

where f i is the OD fraction of the $i-$th carbon source. R_{it} is the average absorbance of each carbon source at time t (cm^{-1}).

f_d is the OD fraction of the dominant carbon source and can be defined as the maximum f_i for a sample.

$$fd = \frac{1}{n_j}\sum_{i=1}^{nj} f_i$$

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