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Ammonia biofiltration and community analysis of ammonia-oxidizing bacteria in biofilters

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ABSTRACT

Biological removal of ammonia was investigated using compost and sludge as packing materials in laboratory-scale biofilters. The aim of this study is to characterize the composition of ammonia-oxidizing bacteria (AOB) in two biofilters designed to remove ammonia. Experimental tests and measurements included analysis of removal efficiency and metabolic products. The inlet concentration of ammonia applied was 20–100 mg m^{-3}. Removal efficiencies of BFC and BFS were in the range of 97–99% and 95–99%, respectively. Periodic analysis of the biofilter packing materials showed ammonia was removed from air stream by nitrification and by the improved absorption of NH_3 in the resultant acidity. Nitrate was the dominant product of NH_3 transformation. Changes in the composition of AOB were examined by using nested PCR, denaturing gradient gel electrophoresis (DGGE) and sequencing of DGGE bands. DGGE analysis of biofilter samples revealed that shifts in the community structure of AOB were observed in the experiment; however, the idle phase did not cause the structural shift of AOB. Phylogenetic analysis revealed the population of AOB showed *Nitrosospira* sp. remains the predominant population in BFC, while *Nitrosomonas* sp. is the predominant population in BFS.

1. Introduction

Biofiltration has proven to be a suitable technique for the degradation of volatile organic compounds (VOCs), and toxic or odorous compounds such as H_2S and NH_3. The first proposition to use biological methods to treat odorous compounds was as early as 1923. Bach thought of using a biologically active biofilter to control emissions of H_2S from a waste water treatment plant (Bellis, 1997). Biofilters have also been used at solid waste processing plants and food processing plants for several decades (Sheridan et al., 2002). Due to its more efficient and less expensive, there is a growing interest in the applications of biofiltration techniques in a variety of industries.

Reactor design, filter materials, and other factors involved in biofilter function (e.g., water content, temperature, pH, O_2 concentration, and salt concentration) have been investigated (Chen et al., 2005; Joshi et al., 2000; Morgan-Sagastume and Noyola, 2006; Smet et al., 2000). It has been suggested that more work on monitoring microbial populations and studying on microbial ecology in bioreactors is needed. Recently, molecular techniques have been used to the composition of the microbial community during biofiltration and to monitor the biofilter colonisation by specific degradative populations (Friedrich et al., 2002; Ho et al., 2008). In our study, the biofilter was designed to remove ammonia through the process of nitrification, i.e., the oxidation of ammonia into nitrate.

Chemolithoautotrophic ammonia-oxidizing bacteria (AOB), which convert ammonia to nitrite, play an important role in the global cycling of nitrogen. *Nitrosomonas europaea* is perhaps the most easily isolated and cultured ammonia-oxidizing species, but several culture-based studies have indicated that *Nitrosospira* species are common in terrestrial habitats (Kowalchuk and Stephen, 2001). It is well known that ammonia-oxidizing bacteria consist of two monophyletic groups based on comparison of 16S rDNA sequences from cultured strains. One of them involves strains of *Nitrosococcus oceanus* within the γ-subdivision of the *Proteobacteria*, and the other contains the genera *Nitrosomonas* and *Nitrosospira* (the latter now encompasses the genera *Nitrosolobus* and *Nitrosovibrio*) within the β-subdivision of the *Proteobacteria* (Kowalchuk et al., 1997). Several recent studies have used 16S rDNA sequence information to assess the diversity and distribution of the β-subdivision ammonia-oxidizing bacteria in natural environments (Kowalchuk et al., 1997; O'Mullan and Ward, 2005).

This study examined the population and structure of AOB from biofilters. The first objective was to determine the effectiveness of the biofiltration technology in removing ammonia from

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contaminated air. The second objective was to characterize β-subdivision ammonia-oxidizing bacterial populations over time in biofilters. In addition, whether biofilter performance could be credited to particular species, or whether different packing materials had different bacterial composition was determined. Denaturing gradient gel electrophoresis (DGGE) of specific groups of β-subdivision AOB, coupled with sequence analysis, was used to monitor and evaluate microbial community dynamics during a 170-day experiment in biofilters.

2. Methods

2.1. Biofilter system

Two identical bench-scale biofilter columns packed with either compost (BFC) or sludge (BFS), respectively, were used (Fig. 1). The biofilter system consisted of a gas source, a gas flow control unit (flow meters and valves), mixing cell, and parallel biofilters. The biofilter column was constructed by transparent, rigid, plexiglass tubing with an inner diameter of 150 mm. It was divided into three equal sections, each section being filled with the packing material to a bed depth of 150 mm, so that the effective volume of the biofilter was 8 L. Each of the biofilter section was provided with a PVC mesh to maintain the filtering material in position and to also enhance the radial distribution of gases between adjacent filter sections. Sections were flanged and each section could be dismantled to replace and sample the filter material and to clean the filter columns before and after use.

The air, provided by compressor, was artificially polluted with ammonia and regulated through rotameters and humidified to prevent drying or water accumulation in the biofilter matrix. Mass flow controller was used to ensure accurate delivery of NH_3 from a concentrated source (1.5% NH_3 in N_2, Mingxing Gas Products, Hangzhou). The air and NH_3 were mixed in the mixing bottle before entering biofilters. Biofilters were operated in a upward-flow regime. The gas exiting the biofilter passed through an exhaust NH_3 trapping system to trap any NH_3.

In this study, compost and sludge were used as packing materials for biofilters. Compost was a commercial product and was mainly composed of the pig manure and agricultural by-products. The compost was amended by adding approximately 20% of perlite, which is an expanded volcanic material that is ordinarily used for increasing the porosity of potting soil to decrease the pressure drop across the filter bed. Sludge came from municipal wastewater treatment plant at Cixi (Zhejiang Province, China). The dehydrated digested sludge was dried and amended by adding approximately 20% granular activated carbon, by volume, to improve the adsorption process and to minimize fluctuation of pollutant concentration.

2.2. Procedure

The operating conditions of two biofilters were as follows listed in Table 1. During the experiment, three different periods, in which biofilters loaded with gradually increased NH_3 concentrations, were carried out. At period 1 (day 0–60), the inlet concentration of ammonia applied was 20–60 mg m^{-3}. At period 2 (day 60–90), an idle phase was conducted to examine the response of biofilters to non-use periods, to evaluate whether the biofiltration process can withstand such situations. During this period, there was no airflow through the biofilters at all. At period 3 (day 90–170), biofilters were restarted after a period of one month shutdown. To avoid too low a value of ammonia loading for the packing material and too long an experimental period, from day 140 on, the influent ammonia concentration (>70 mg m^{-3}) was higher than those found in the actual cases.

The packing materials were pre-inoculated with acclimated activated sludge suspension. Briefly, compost and sludge were inoculated with nitrifying activated sludge to create a biofilter having biomass concentration of 0.5 g of biomass kg^{-1} of packing materials.

2.3. Sampling

For gas sampling, ammonia was transferred into aqueous solution by bubbling the gaseous influent into the solution of sulfuric acid. The gas flow rate was adjusted to 0.5 L min^{-1}. The sampling delay was 10 min (Chinese Standard method, 1998, GB/T14668-93).

Packing samples were collected from biofilters periodically throughout a 170-day period, with more frequent sampling during the first 6 weeks. When sampling, biofilters were taken down and

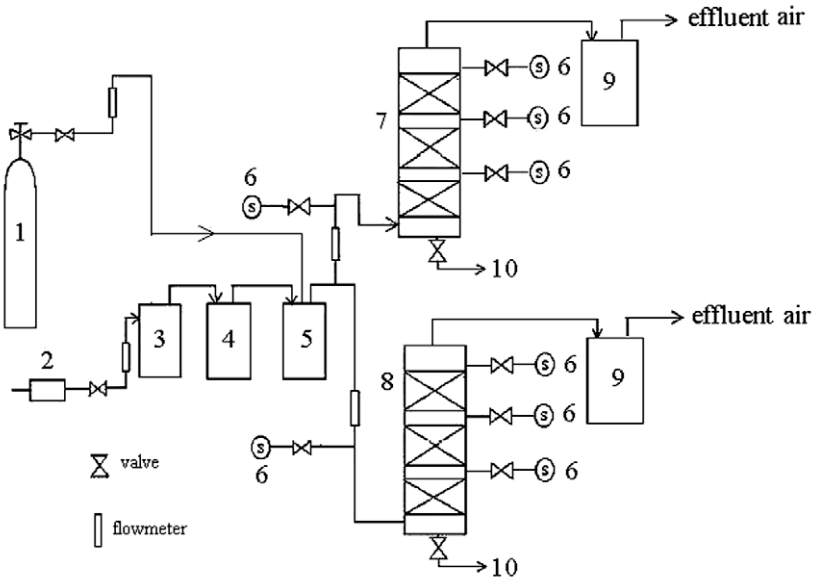

Fig. 1. A schematic diagram of the biofilter (1) NH_3 gas cylinder; (2) air pump; (3) humidifier; (4) knockout trap; (5) mixing bottle; (6) sampling port; (7) compost column; (8) sludge column; (9) exhaust NH_3 trapping system; (10) percolate waters.