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Short communication

Heterogeneity of ammonia-oxidizing community structures in a pilot-scale drinking water biofilter

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ABSTRACT

Drinking water biofilters have been widely used for ammonia removal. Knowledge about the structure of ammonia oxidizing communities can aid in understanding of nitrification process. Terminal restriction fragment length polymorphism (TRFLP) analysis of *amoA* genes in combination with cloning and sequencing analysis were used to investigate spatial heterogeneity of ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) communities in a pilot-scale granular activated carbon (GAC)-sand dual media filter. The results illustrate the diversity of AOB communities on GAC samples and their changes along the filter depth. Moreover, *Nitrosomonas*-like microorganisms were the dominant AOB species in GAC samples. However, AOA was not detected in the biofilter. This work could add some new insights into the nitrification in drinking water biofilters.

1. Introduction

Ammonia is usually present in source water used for drinking water, especially in regions with intense anthropogenic activities. The presence of ammonia in drinking water might lead to several water quality problems during distribution (Kihn et al., 2000; van der Wielen et al., 2009). Ammonia can be effectively removed through a two-step nitrification process in which sequential oxidation of ammonia into nitrite and then nitrate occurs (Leemann et al., 2010). *Nitrosomonas* and *Nitrobacter* are the most common genera, known respectively as ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). However, the discovery of ammonia oxidizing archaea (AOA) has greatly changed our understanding of nitrification (Konneke et al., 2005). Recently, AOA have been found in ground water treatment processes in Japan and the Netherlands (de Vet et al., 2009; van der Wielen et al., 2009; Kasuga et al., 2010). These works suggested that AOA could be responsible for the removal of ammonia in groundwater treatment plants. However, the information on the presence of AOA in surface water treatment processes is still lacking.

Numerous works have investigated nitrifying biomass and its activity in drinking water biofilters (Niquette et al., 1998; Kihn et al., 2000; Tränckner et al., 2008). The nitrifying biomass could decrease with increasing depth in the drinking water biofilters, as the substrate concentration in the water decreased (Kihn et al., 2000). Knowledge about the structure of nitrifier community can also aid in understanding of nitrification process. However, the structure of nitrifier community in drinking water biofilters has been rarely addressed (Yapsakli et al., 2010; van den Akker et al., 2011; Wahman et al., 2011). Moreover, to the authors' knowledge, the spatial heterogeneity of nitrifier community in drinking water biofilters has not been identified in the literature before.

Granular activated carbon (GAC)-sand dual media filter could effectively remove ammonium, organic matters and turbidity (Yang et al., 2000). It seems a good option to retrofit a sand filter to a GAC-sand dual media filter, especially where space is limited for water producers to introduce external advanced treatment units. In the current study, spatial heterogeneity of AOA and AOB community structures in a pilot-scale GAC-sand dual media filter was investigated using terminal restriction fragment length polymorphism (TRFLP) analysis in combination with cloning and sequencing analysis.

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2. Materials and methods

2.1. Samples

Water and particle samples were collected from a pilot-scale GAC-sand dual media filter in down-flow mode. The filtration column was a Plexiglas cylinder (4-m length and 0.3-m-diameter), equipped with sampling ports for water, GAC, and sand (Fig. 1). From top to down, the column was filled with GAC (1-m height), sand (0.4-m height), and gravel (0.3-m height). With a hydraulic loading of 8 m/h, the pilot filter was fed with the settled water from a drinking water plant treating river water. The treatment train of the drinking water treatment plant consists of coagulation–flocculation, sedimentation, rapid sand filtration, and disinfection. Before this study, the dual media filter had been operated for more than eight months allowing for the maturation of nitrifying biomass. During this study, the pH values, oxygen concentrations, and temperatures of the influent ranged between 7.0 and 7.5, 5.0 and 7.9 mg O_2/L, and 25 and 30 °C, respectively. The ammonia concentrations were determined according to the standard methods (China Environmental Protection Agency, 2002).

2.2. TRFLP for ammonia monooxygenase A genes of AOA and AOB

The particle samples were collected from 0.2, 0.4, 1.0, and 1.2 m depth below the surface of the GAC layer, referred to as Sample A, Sample B, Sample C, and Sample D, respectively. DNA was extracted using the UltraClean DNA extraction kit (Mobio Laboratories, Carlsbad, USA). The ammonia monooxygenase A (*amoA*) gene has been widely used for the study of ammonia oxidizers (Francis et al., 2005). PCR amplification of *amoA* gene of AOB was carried out with the forward primer *amoA*-1F (5′-GGGGTTTCTACTGGTGGT-3′; 5′ end-labeled with carboxyfluorescine) and the reverse primer *amoA*-2R (5′-CCCCTCKGSAAAGCCTTCTTC-3′) (Horz et al., 2000; Ying et al., 2010). *amoA* gene of AOA was amplified using Arch-*amoA*F (5′-STAATGGTCTGGCTTAGACG-3′; 5′ end-labeled with carboxyfluorescine) and Arch-*amoA*R (5′-GCGGCCATCCATCTG-TATGT-3′) (Francis et al., 2005; Ying et al., 2010). PCR reactions were performed as follows: 95 °C for 3 min; 35 cycles of 95 °C for 45 s, 53 °C (for AOA) or 55 °C (for AOB) for 1 min, followed by 72 °C for 1 min; and finally 72 °C for 7 min (Li et al., 2011a).

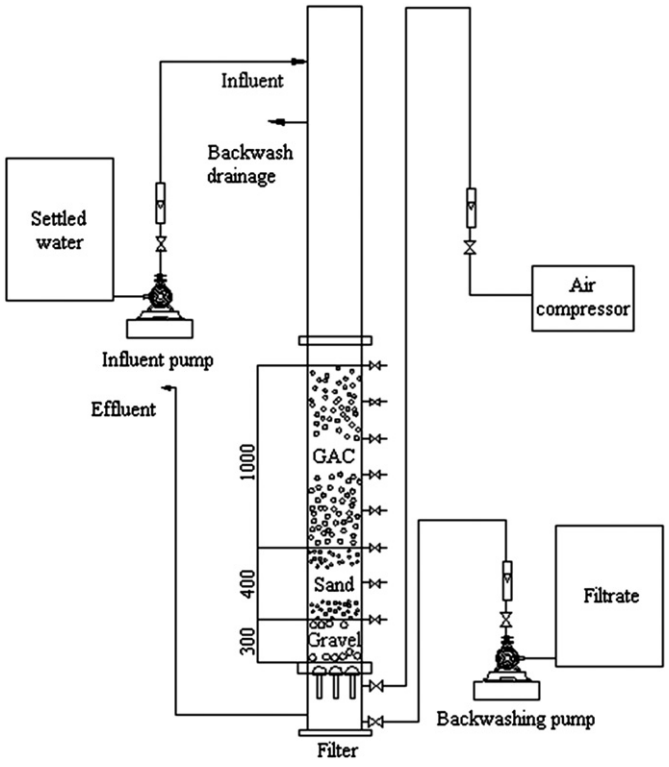

Fig. 1. Schematic of the pilot-scale GAC-sand dual media filter.

*Hae*III and *Hha*I were selected to digest PCR products purified with QIA quick PCR purification kit (Qiagen Inc., Germany) and may classify the different clone sequences into unique terminal restriction fragments (Zhang et al., 2011a). The clone sequence was first identified, the in silico cut site (*Hae*III digest) of which matched the length of the abundant fragment (*Hae*III digest). If the clone restriction enzyme (*Hha*I) cut site predicted from sequence also matched the observed length of the abundant fragment (*Hha*I), the taxonomic identity of the abundant fragment (*Hae*III digest) could be confirmed (Zhang et al., 2011a).

The fragment pattern was detected using an ABI 3730 DNA analyzer (Applied Biosystems, Foster, USA). The relative abundance of each terminal restriction fragment was determined by calculating the ratio of the area of each peak to the total area of all peaks in a given TRFLP profile. The peaks with relative abundance <1% or smaller than 50 bp were excluded from further analysis. Ribotype richness (*S*) equals to the total number of distinct fragments in a profile. The Shannon diversity index (*H*) and evenness (*E*) were calculated according to the standard method (Mills et al., 2003).

2.3. Cloning and sequencing

The PCR conditions were the same as the above-mentioned, except that the forward primer was unlabeled. The PCR products were cloned into pMD19-T vector (TaKaRa Co., Japan) following the manufacturer's instruction. The white colonies were verified by PCR with primers M13 F (5′-TGTAAAACGACGGCCAGT-3′) and M13 R (5′-AACAGCTATGACCATG-3′). Clones were sequenced at SinoGenoMax Co., Ltd. (Beijing). Clones sharing ≥98% identity were grouped into one operational taxonomic unit (OTU) using distance-based OTU and richness program (DOTUR) (Schloss and Handelsman, 2005). The nucleotide sequences were compared with those from the GenBank using BLASTn (http://www.ncbi.nlm.nih.gov). Neighbor-joining trees of the sequences in this study and the reference sequences retrieved from the GenBank were constructed using MEGA Version 4.0 with 1,000 replicates (Tamura et al., 2007). Alignment of the sequences was performed using ClustalW (http://www.ebi.ac.uk/clustalw/). The sequences reported in this study were deposited in the GenBank under accession number JN998582-JN998605.

3. Results

Although the influent ammonia concentration varied greatly during the 92-day operation, the GAC-sand dual media filter maintained an effective ammonia removal (Fig. 2). Change of ammonia concentration along the filter depth on day 92 is shown in Fig. 3. The result indicates that ammonia removal mainly occurred in GAC layer, especially in the top 0.6-m, however, negligible reduction of ammonia was observed in sand layer.

amoA gene of AOB was successfully amplified for Sample A, B and C, but not Sample D. This was consistent with the negligible reduction of ammonia in sand layer. However, all four samples showed no PCR amplification with the widely used primers for *amoA* gene of AOA. Fig. 4 illustrates the change of AOB community structure in GAC layer along the filter depth. In Sample A fragment 167 bp (*Hae*III) was predominant (with a relative abundance of 71%), and there were no other abundant fragments (less than 5%). In