Microbial community in a full-scale drinking water biosand filter

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Abstract

To remove turbidity and minimize microbiological risks, rapid sand filtration is one of main drinking water treatment processes in the world. However, after a long-term operation, sand particles will be colonized by microorganisms which can remove biodegradable organic matters and nitrogen compounds. In this study, 16S rRNA gene clone library analysis was applied to characterize the microbial community in a full-scale biosand filter used for drinking water treatment. The results indicate that phylum Nitrospirae and class Alphaproteobacteria were the dominant bacterial groups in the biosand sample collected from the upper filter layer. The dominance of Sphingomonas species might pose a microbiological risk. This work could provide some new insights into microbial community in drinking water biofilter.

Key words

Drinking water, Nitrospirae, Nitrification, Sphingomonas, Sand filter

Introduction

In China, the conventional treatment processes (coagulation –flocculation, sedimentation, rapid sand filtration, disinfection) are still widely used in drinking water treatment plants. The sand filter is mainly used to remove turbidity and minimize microbiological risks. However, after a long period of operation, microbial biomass can be enriched on sand particles. Therefore, the removal of biodegradable organic matters and nitrogen compounds occurs in the biosand filter, as the result of the activity of microbial communities that colonize the sand surfaces.

Many efforts have been made to elucidate the link of biofilter performance with microbial communities, mainly focused on the assessment of microbiological activity or biomass (Urfer and Huck, 2001; Fonseca et al., 2001; Tranckner et al., 2008). However, knowledge of microbial community structure can also contribute to a better understanding of the biological processes (Feng et al., 2012). Microorganisms in drinking water biofilters have been documented based on culture-dependent methods (Norton and LeChevallier, 2000; Ko et al., 2007; Magic-Knezev et al., 2009). Recently, molecular techniques have also been used to characterize the composition of complex microbial community in biological activated carbon filters (Niemi et al., 2009; Yapsakli et al., 2010; Zhang et al., 2011a), and trickling filters (de Vet et al., 2011 ). Li et al. (2010) used 16S rRNA gene clone library analysis to characterize the microbial community structure in a bench-scale BAC filter. However, the information about microbial community structure in biosand filter for surface water treatment is meagre. Therefore, in the current study, 16S rRNA gene clone library analysis was applied to identify the microbial community in a full-scale biosand filter used for drinking water treatment. The environmental and hygienic significances of the major bacterial groups in the biofilter were also assessed in detail.

Materials and Methods

Sampling site: Biosand sample (1 g) was collected from 0.2m depth of a biosand filter (with a sand layer of 0.7 m height)
in a drinking water treatment plant for surface water treatment in South China. Before this study, the drinking water treatment plant had been under continuous operation for nearly two decades. The sand filtration system was operated with a hydraulic loading of 6 m h⁻¹. The pH value, oxygen concentration, temperature, CODₘₚ and ammonia of the influent ranged between 7.4 and 8.0, 6.9 and 7.5 mg O₂ l⁻¹, and 11 and 32°C, 0.67-2.35 and 0.021-2.002 mg l⁻¹, respectively. According to the yearly monitoring data (July 2010-June 2011), the average CODₘₚ and ammonia removal rates were 7.9 and 68.1%, respectively.

**Clone library analysis:** DNA from biosand sample was extracted using the UltraClean DNA extraction kit (Mobio Laboratories). 16S rRNA genes were amplified using bacterial primers 27F (5'-GAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTAGAGCTT-3'). The detailed procedure of clone library construction has been noted elsewhere (Huang et al., 2011; Zhang et al., 2011b,c). Sequences with ≥ 97% identity were clustered as one operational taxonomic unit (OTU) using DOTUR program and OTU-based Shannon diversity index was calculated (Schloss and Handelsman, 2005). Selected sequences were submitted to BLAST program to obtain their closest cultured relatives. Taxonomic identities of the selected sequences was assigned using Ribosomal Database Project (RDP) II analysis tool “classifier” (Wang et al., 2007). The 16S rRNA sequences obtained in this study were submitted to GenBank under accession numbers JN887266-JN887310.

**Results and Discussion**

**Microbial community structure:** The composition of microbial community in drinking water biofilter is not well understood. Previous works based on culture-dependent methods indicated the dominance of isolates belonging to class Betaproteobacteria in BAC filters (Magic-Knezev et al., 2009; Niemi et al., 2009). Li et al. (2010) also reported the dominance of class Betaproteobacteria in a bench-scale BAC filter using clone library analysis. However, in this study, phylum Nitrospirae (44%) and class Alphaproteobacteria (42%) were the dominant bacterial groups in the biosand filter (Fig. 1).

**Table 1:** The major operational taxonomic units (OTUs) and phylogenetic affiliation

<table>
<thead>
<tr>
<th>OTU number</th>
<th>Numbers of sequences in the OTU</th>
<th>Representative member</th>
<th>Phylogenetic affiliation (Genus)</th>
<th>Nearest cultured neighbor (percent identity)</th>
<th>Source of isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>S35</td>
<td><em>Nitrospira</em></td>
<td>AJ224044.1 (99%)</td>
<td>Nitrifying fluidizedbed reactor (Schramm et al., 1998)</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>S17</td>
<td><em>Nitrospira</em></td>
<td>Y14639.1 (98%)</td>
<td>Nitrite-oxidizing bioreactor (Burrell et al., 1998)</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>S11</td>
<td><em>Sphingomonas</em></td>
<td>AM989048.1 (99%)</td>
<td>Treated drinking water (Berg et al., 2009)</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>S30</td>
<td><em>Sphingomonas</em></td>
<td>AB649022.1 (97%)</td>
<td>Paddy soil (Gorlach et al., 1994)</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>S27</td>
<td><em>Sphingomonas</em></td>
<td>JQ684237.1 (99%)</td>
<td>Permafrost soil (UnpublishedGenbank data)</td>
</tr>
</tbody>
</table>

**OTU and Phylogeny:** The recovered 45 clones could be grouped into 12 OTUs, including 5 major OTUs (with at least four sequences), 3 two-member OTUs and 4 one-member OTUs.

Moreover, members of two major OTUs belonged to genus *Nitrospira* within phylum Nitrospirae, and members of the other three major OTUs were affiliated to genus *Sphingomonas* within class Alphaproteobacteria (Table 1). Rarefaction curve for clone library almost reache asymptote (Fig. 2), indicating that the community was well sampled with low diversity (Liao et al., 2009). The OTU-based Shannon diversity index of the microbial community in the biosand filter was 2.11, much lower than that in BAC filters (2.93-3.90) (Li et al., 2010).

**Fig. 1** Composition of bacterial community in the biosand filter

**Fig. 2** Rarefaction curves indicating bacterial 16S rRNA gene richness within clone library. The observed numbers of OTUs identified by DOTUR program at 3% difference level are plotted against number of clones in library
Nitrification is a two-stage aerobic process in which ammonia is oxidized to nitrite and nitrate by two groups of bacteria, ammonia-oxidizing bacteria (Nitrosomonas and Nitrosospirae) and nitrite-oxidizing bacteria (Nitrobacter and Nitrospirae) (Leemann et al., 2010; Yapsakli et al., 2010). In this study, Nitrosomonas, Nitrosospirae and Nitrobacter were not detected, although members of genus Nitrospirae were abundant in the biosand filter. Although the detection of Nitrospirae species could confirm the occurrence of nitrification process in the biofilters, the specific primers for AOB might be necessary for elucidating the mechanism of ammonia removal (Feng et al., 2012). The closest cultured neighbor of sequence S17 was a Nitrospirae isolate (Y14639.1, 98% identity), obtained from a nitrite-oxidizing sequencing batch reactor (Burrell et al., 1998) (Table 1). Moreover, the closest cultured match of sequence S35 was a Nitrospirae isolate (AJ224044.1, 99% identity), retrieved from a nitrifying fluidized bed reactor (Schramm et al., 1998). Sequence S17 and sequence S35 only shared 92% identity, indicating that the two OTUs were distant from each other. This also suggests that two types of Nitrospirae species might play roles in nitrite oxidation.

Sequence S11 was closely related with 98% similarity to a Sphingomonas isolate (AM989048.1), obtained from treated drinking water (Berg et al., 2009). Sequence S27 had 99% similarity to a Permafrost soil Sphingomonas isolate (AY769084.1; unpublished Genbank data). The closest cultured match of sequence S30 was a paddy soil Sphingomonas isolate (AB649022.1) (Gorlach et al., 1994). Members of genus Sphingomonas have recently been linked to opportunistic pathogens involved in a variety of infections (Amendolisa et al., 2004; Villarreal et al., 2010; Ryan and Adley, 2010; Gusman et al., 2010). However, several previous works have revealed the presence of Sphingomonas species in drinking water (Simoes et al., 2007; Gusman et al., 2010; Villarreal et al., 2010; Vaz-Moreira et al., 2011), biofilms in drinking water distribution systems (Koskinen et al., 2000; Lee et al., 2005), and BAC filters (Niemi et al., 2009). To our knowledge, this was the first report on the presence of Sphingomonas species in the biosand filter. Microorganisms from genus Sphingomonas are well known for biodegradation of a variety of environmental chemicals (Cycone et al., 2011; Hussain et al., 2011). This may account for the presence of Sphingomonas species in oligotrophic water environments. Unfortunately, the ubiquity of Sphingomonas species in drinking water implied that disinfection process may not effectively inactivate these microorganisms. In this study, the dominance of Sphingomonas species in the full-scale biosand filter posed a serious microbiological risk in water supply.

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References


