

Microbial community in a full-scale drinking water biosand filter

Shuo Feng¹, Chao Chen¹, Qingfeng Wang², Zhiyu Yang¹, Xiaojian Zhang¹ and Shuguang Xie^{2*}

¹School of Environment, Tsinghua University, Beijing, 100084, China

²College of Environmental Sciences and Engineering, The Key Laboratory of Water and Sediment Sciences (Ministry of Education), Peking University, Beijing, 100871, China

*Corresponding Author email : xiesg@pku.edu.cn

Publication Info

Paper received:
15 June 2012

Revised received:
12 August 2012

Accepted:
29 August 2012

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

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, *Nitrospira*, Nitrification, *Sphingomonas*, Sand filter

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)

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

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

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, COD_{Mn} 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 COD_{Mn} and ammonia removal rates were 7.9 and 68.1%, respectively.

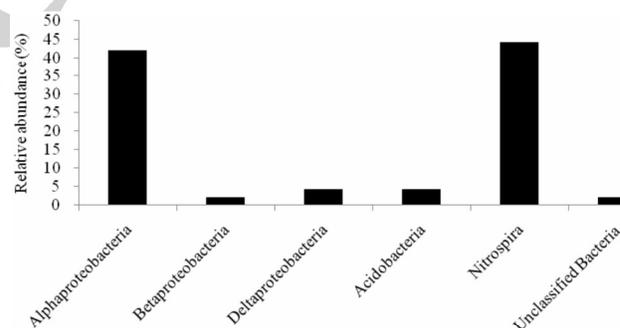
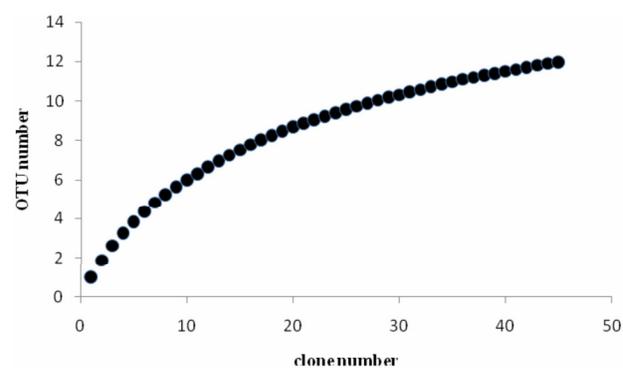
Clone library analysis: DNA from biosand sample was extracted using the UltraClean DNA extraction kit (Mbio Laboratories). 16S rRNA genes were amplified using bacterial primers 27F (5'-GAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTGTTCAGACTT-3'). The detailed procedure of clone library construction has 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).

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 reached 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 *Nitrosospira*) and nitrite-oxidizing bacteria (*Nitrobacter* and *Nitrospira*) (Leemann *et al.*, 2010; Yapsakli *et al.*, 2010). In this study, *Nitrosomonas*, *Nitrosospira* and *Nitrobacter* were not detected, although members of genus *Nitrospira* were abundant in the biosand filter. Although the detection of *Nitrospira* 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 *Nitrospira* 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 *Nitrospira* 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 *Nitrospira* species might play roles in nitrite oxidization.

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 (Ammendolia *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 (Cycon *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.

Acknowledgment

This work was financially supported by Major Science and Technology Program for Water Pollution Control and Treatment (2009ZX07423-003).

References

- Ammendolia, M.G., L. Bertuccini, F. Minelli, S. Meschini and L. Baldassarri: A *Sphingomonas* bacterium interacting with epithelial cells. *Res. Microbiol.*, **155**, 636-646 (2004).
- Berg, K.A., C. Lyra, K. Sivonen, L. Paulin, S. Suomalainen, P. Tuomi and J. Rapala: High diversity of cultivable heterotrophic bacteria in association with cyanobacterial water blooms. *ISME J.*, **3**, 314-325 (2009).
- Burrell, P.C., J. Keller and L.L. Blackall: Microbiology of a nitrite-oxidizing bioreactor. *Appl. Environ. Microbiol.*, **64**, 1878-1883 (1998).
- Cycon, M., A. Zmijowska and Z. Piotrowska-Seget: Biodegradation kinetics of 2,4-D by bacterial strains isolated from soil. *Cent. Eur. J. Biol.*, **6**, 188-198 (2011).
- De Vet, W.W.J.M., R. Kleerebezem, P.W.J.J. van der Wielen, L.C. Rietveld and M.C.M. van Loosdrecht: Assessment of nitrification in groundwater filters for drinking water production by qPCR and activity measurement. *Water Res.*, **45**, 4008-4018 (2011).
- Feng, S., X.J. Zhang, Q.F. Wang, R. Wan, C. Chen and S.G. Xie: Heterogeneity of ammonia-oxidizing community structures in a pilot-scale drinking water biofilter. *Int. Biodeterior. Biodegrad.*, **70**, 148-152(2012).
- Fonseca, A.C., R.S. Summers and M.T. Hernandez: Comparative measurements of microbial activity in drinking water biofilters. *Water Res.*, **35**, 3817-3824 (2001).
- Gorlach, K., R. Shingaki, H. Morisaki and T. Hattori: Construction of eco-collection of paddy field soil bacteria for population analysis. *J. Gen. Appl. Microbiol.*, **40**, 509-517 (1994).
- Gusman, V., Z. Jelesic, M. Mihajlovic-Ukropina, D. Medic, G. Pavlovic and B. Radosavljevic: Isolation of *Sphingomonas paucimobilis* from drinking water using novel automated system. *Healthmed*, **4**, 1068-1071 (2010).
- Huang, Y., L. Zou, S.Y. Zhang and S.G. Xie: Comparison of bacterioplankton communities in three heavily polluted streams in China. *Biomed. Environ. Sci.*, **24**, 140-145 (2011).
- Hussain, S., M. Devers-Lamrani, N. El Azhari and F. Martin-Laurent: Isolation and characterization of an isotrotron mineralizing *Sphingomonas* sp strain SH from a French agricultural soil. *Biodegradation*, **22**, 637-650 (2011).
- Ko, Y.S., Y.J. Lee and S.H. Nam: Evaluation of a pilot scale dual media biological activated carbon process for drinking water. *Korean J. Chem. Eng.*, **24**, 253-260 (2007).
- Koskinen, R., T. Ali-Vehmas, P. Kampfer, M. Laurikkala, I. Tsitko, E. Kostyal, F. Atroshi and M. Salkinoja-Salonen: Characterization of *Sphingomonas* isolates from Finnish and Swedish drinking water distribution systems. *J. Appl. Microbiol.*, **89**, 687-696 (2000).
- Lee, D.G., J.H. Lee and S.J. Kim: Diversity and dynamics of bacterial species in a biofilm at the end of the Seoul water distribution system. *World J. Microbiol. Biotechnol.*, **21**, 155-162 (2005).
- Leemann, A., B. Lothenbach, H. Siegrist and C. Hoffmann: Influence of water hardness on concrete surface deterioration caused by nitrifying biofilms in wastewater treatment plants. *Int. Biodeterior. Biodegrad.*, **64**, 489-498 (2010).
- Li, X., G. Upadhyaya, W. Yuen, J. Brown, E. Morgenroth and L. Raskin: Changes in the structure and function of microbial communities in drinking water treatment bioreactors upon addition of phosphorus. *Appl. Environ. Microbiol.*, **76**, 7473-7481 (2010).

- Liao, L., X.W. Xu, C.S. Wang, D.S. Zhang and M. Wu: Bacterial and archaeal communities in the surface sediment from the northern slope of the South China Sea. *J. Zhejiang Univ. Sci. B*, **10**, 890-901 (2009).
- Magic-Knezev, A., B. Wullings and D. Van der Kooij: *Polaromonas* and *Hydrogenophaga* species are the predominant bacteria cultured from granular activated carbon filters in water treatment. *J. Appl. Microbiol.*, **107**, 944-953 (2009).
- Niemi, R.M., I. Heiskanen, R. Heine and J. Rapala: Previously uncultured beta-proteobacteria dominate in biologically active granular activated carbon (BAC) filters. *Water Res.*, **43**, 5075-5086 (2009).
- Norton, C.D. and M.W. LeChevallier: A pilot study of bacteriological population changes through potable water treatment and distribution. *Appl. Environ. Microbiol.*, **66**, 268-276 (2000).
- Ryan, M.P. and C.C. Adley: *Sphingomonas paucimobilis*: A persistent Gram-negative nosocomial infectious organism. *Hosp. Infect.*, **75**, 153-157 (2010).
- Schloss, P.D. and J. Handelsman: Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl. Environ. Microbiol.*, **71**, 1501-1506 (2005).
- Schramm, A., D. de Beer, M. Wagner and R. Amann: Identification and activities *in situ* of *Nitrosospira* and *Nitrospira* spp. as dominant populations in a nitrifying fluidized bed reactor. *Appl. Environ. Microbiol.*, **64**, 3480-3485 (1998).
- Simoës, L.C., M. Simoës and M.J. Vieira: Biofilm interactions between distinct bacterial genera isolated from drinking water. *Appl. Environ. Microbiol.*, **73**, 6192-6200 (2007).
- Tranckner, J., B. Wricke and P. Krebs: Estimating nitrifying biomass in drinking water filters for surface water treatment. *Water Res.*, **42**, 2574-2584 (2008).
- Urfer, D. and P.M. Huck: Measurement of biomass activity in drinking water biofilters using a respirometric method. *Water Res.*, **35**, 1469-1477 (2001).
- Vaz-Moreira, I., O.C. Nunes and C.M. Manaia: Diversity and antibiotic resistance patterns of *Sphingomonadaceae* isolates from drinking water. *Appl. Environ. Microbiol.*, **77**, 5697-5706 (2011).
- Villarreal, J.V., T. Schwartz and U. Obst: Culture-independent techniques applied to food industry water surveillance - A case study. *Int. J. Food Microbiol.*, **141**, 147-155 (2010).
- Wang, Q., G.M. Garrity, J.M. Tiedje and J.R. Cole: Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.*, **73**, 5261-5267 (2007).
- Yapsakli, K., B. Mertoglu and F. Cecen: Identification of nitrifiers and nitrification performance in drinking water biological activated carbon (BAC) filtration. *Process Biochem.*, **45**, 1543-1549 (2010).
- Zhang, D.Y., W.G. Li, S.M. Zhang, M. Liu, X.Y. Zhao and X.C. Zhang: Bacterial community and function of biological activated carbon filter in drinking water treatment. *Biomed. Environ. Sci.*, **24**, 122-131 (2011a).
- Zhang, S.Y., Q.F. Wang, R. Wan and S.G. Xie: Changes in bacterial community of anthracene bioremediation in municipal solid waste composting soil. *J. Zhejiang Univ. Sci. B*, **12**, 760-768 (2011b).
- Zhang, S.Y., Q.F. Wang and S.G. Xie: Microbial community changes in contaminated soils in response to phenanthrene amendment. *Int. J. Environ. Sci. Technol.*, **8**, 321-330 (2011c).