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# Drinking Water Engineering and Science Discussions

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# Technical Note: Community of bacteria attached on the PVDF MF membrane surface fouled from drinking water treatment, in Seoul, Korea

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Received: 31 January 2009 - Accepted: 11 February 2009 - Published: 2 March 2009

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Published by Copernicus Publications on behalf of the Delft University of Technology.

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## Abstract

Alpha, beta, and gamma proteobacteria comprise approximate 68, 16, and 7% of all identified bacteria. In this study, bacterial communities that had fouled polyvinylidene fluoride microfiltration membranes, which are used for drinking water treatment, over

- an 18 month period were analyzed using the 16s rRNA gene clone library method. The alpha, beta, and gamma proteobacteria were composed of mainly *Bradyrhizobium* and *Rhodopseudomonas, Ralstonia*, and *Legionella*, respectively. The presence of a relatively high amount of alpha proteobacteria was due to the oligotrophic condition of the drinking water source, the Han River, tested in this specific case study. The second
   most prominent bacteria community was the beta proteobacteria, which are typically
- found in a freshwater environment. This finding supports the notion that the drinking water source was relatively clean. Analyses of the organic foulants indicated that the they were most likely from extra cellular polymers and/or cell fractured chemicals from bacteria or micro-organisms, as identified using organic characterizing tools, including
- <sup>15</sup> 3 dimensional fluorescence excitation-emission matrix and Fourier transform IR analyses.

## 1 Introduction

A paradigm shift in the characterization of fouling has been attempted in the areas of biofouling and microbial extra-cellular polymeric substances (EPS). The development
 of such characterization techniques are especially important for the identification of microbial communities in fouled membrane, which may provide a better understanding of biofouling formation mechanisms including microorganisms and EPS. Bacterial community structure analyses have recently been performed for membranes that were fouled during wastewater and effluent treatment (Ivnitsky et al., 2007; Miura et al., 2007; Pang and Liu, 2007; Chen et al., 2004). The bacterial community was identified using both polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis

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(DGGE) and 16s rDNA gene clone library methods, in conjunction with providing phylogenetic tree information. Specific functional genes, such as the denitrifying nirK gene, were also detected in biofilms formed on biofouled membrane (Pang and Liu, 2007). Even with different trends,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -Proteobacteria were most dominant in fouled membranes that were used to treat both wastewater and advanced treated effluent, with other classes and orders also being identified to lesser extents. Compared to studies that have examined membranes fouled during wastewater treatment, bacterial community structural analysis relating to drinking water treatment has rarely been stud-

ied; bacterial community identification was conducted with biofilms formed in a drinking water distribution system (Kalmbach et al., 2000). In this case study, *Aquabacterium commune*, which is in the subclass of  $\beta$ -Proteobacteria, was found to be the dominant bacteria using the rRNA targeted probe method.

Foulants from membranes fouled during drinking water treatment have been extensively characterized, mostly using chemical characterizing tools. In these studies, <sup>15</sup> micro-organisms originated macromolecules, including colloidal organic matters (cellwall fractured and relatively hydrophilic N-acetyl amino sugars), were found to be the major source of biofouling (Leenheer et al., 2000; Kwon et al., 2005). However, the direct identification of the type of bacteria that accumulated on the fouled membranes used for drinking water treatment have been very rare; thus, this was examined in this <sup>20</sup> study..

### 2 Methods and materials

## 2.1 Bacteria community analysis

Microbial biomass was collected from the polyvinylidene fluoride (PVDF) MF membrane surface using mechanical beating with a bead beater. Genomic DNA was ex-

tracted using the AccuPrep Genomic DNA extraction kit (k-3032, Bioneer, Korea), according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplifica-





tion for 16S rRNA was performed using universal primers 9F (5'-GAG TTT GAT CCT GGC TCA G-3') and 1512R (5'-ACG GCT ACC TTG TTA CGA CTT-3') (Weisburg et al., 1991). The PCR mixture included PCR premix (AccuPower PCR PreMix kit, Bioneer, Korea), 1  $\mu$ L of 10 pmole of each primer, and 30 ng of template DNA. The reaction conditions were 94° for 10 min, followed by 34 cycles at 94° for 30 s, 55° for 30 s, and 72° 5 for 1 min, and a final extension step at 72° for 10 min. The amplicons were purified using the AccuPrep PCR purification kit (k-3034, Bioneer, Korea). The purified PCR products were ligated into the yT&A cloning vector (RBC, Twain) and transformed into HIT-DH5 $\alpha$  (RH617, RBC) cells. Transformants were selected on Luria-Bertani agar plates containing ampicilin (70  $\mu$ g/ml), 20  $\mu$ L of X-gal (40 mg/ml), and 50  $\mu$ L of IPTG 10 (200 mM). A single white colony having the recombinant plasmids was inoculated into 1ml LB broth with ampicilin (70  $\mu$ g/ml), and incubated at 37° for 12–14 h. The plasmid DNA was then extracted and purified using the AccuPrep plasmid extraction kit (Bioneer, Korea), according to the manufacturer's instructions. Clones were then seguenced using an ABI 3730xl automated DNA analyzer (Applied Biosystems, Foster 15

- City, CA) at Xenotech (Daejeon, Korea). Sequences were analyzed using the BLAST database (www.ncbi.nlm.nih.gov). Sequence alignments of those selected clones and closely related bacterial species were performed using the Clustal W version 1.7 program (Thomson et al., 1997) available in the BioEdit software package (Hall, 1999).
- Phylogenetic analyses of cloned the nucleotide sequence were conducted using MEGA version 3.1 (Kumar et al., 2004). The tree was constructed from a matrix of pair-wise genetic distances by the neighbor-joining method. The 16S rDNA sequences reported here have been deposited in GenBank under the accession numbers FJ572662 to FJ572674.
- 25 2.2 Organic foulants characterization

The major components and complexity of organic foulants were identified using fluorescence spectrophotometer (F-2500, Hitachi, Japan), which provided 3 dimensional fluorescence excitation emission spectra. Specific functional groups of various sam-

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ples were confirmed using Fourier transform infrared (FTIR) spectrometer (FT/IR-460 plus, Jasco, Japan). The freeze dryer (Ilshin, Korea) was used to dry down the water samples into powders, and the IR spectrum was acquired with the KBr pellet (Pike, USA). Images of fouled membranes were obtained using field emission-scanning electron microscopy (FE-SEM) (Hitachi S-4700, Japan), after pretreatment with 5~6 nm Pt layering, using an Ion Sputter (Hitachi E-1030, Japan).

## 3 Results and discussion

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Excitation and emission peaks measured from 3-D fluorescence spectra with chemically desorbed foulants using both 0.1 N NaOH and 0.1 N HCl provided information on
the peaks that was different from those of the humic substances obtained from the Suwannee river humic and fulvic acids (from International Humic Substance Society), and similar peaks for protein-like substances (Fig. 1a) (Lee et al., 2006). FTIR peaks around ~1000 cm<sup>-1</sup>, which corresponded to polysaccharides/cell fractured N-acetyl amino sugar groups, were identified, as shown in Fig. 1b (Leenheer et al., 2000; Kwon et al., 2005). Along with these peaks, sticky materials, presumably EPS, were found through FE-SEM imaging (Fig. 2). Based on these three observations, it was hypothesized that the biofouling on the fouled membrane and related foulants originated from the presence of a substantial amount of micro-organisms on the membrane.

A 16S rDNA clone library was constructed for the fouled MF membrane. Phylo-<sup>20</sup> genetic analyses of 120 randomly selected clones revealed 15 operational taxonomic units (OTUs) assigned to various bacterial divisions, as listed in Tables 1 and 2. Phylogenetic analysis (Table 1 and Fig. 3) indicated that the Proteobacteria dominated on the membrane surface, where the  $\alpha$ -proteobacteria subdivision was the largest bacterial group (68% of total clones) and the  $\beta$ -proteobacteria subdivision was the second largest bacterial group (16% of total clones) found in the samples. The majority of the  $\alpha$ -proteobacterial OTUs were primarily affiliated with *Bradyrhizobium* sp., *Rhodopseudomonas* sp. and *Afipia* sp. The remaining clones in this group were closely related to



Xanthobacter sp. and Agrobacterium sp. In the  $\beta$ -proteobacteria, the most dominant OTU (11.8% of total clones) was closely related to Ralstoina sp., while the other 3 OTUs were closely related to Alcaligenes sp., Curvibacter sp. and Janthinobacterium sp.

The MF sample also contained 5 OTUs from the  $\gamma$ -proteobacteria, the *Plancto*-5 mycetes group, the gram-positive high G+C group and Bacteroidetes group. In the  $\gamma$ proteobacteria group, clone 40 (6.8% of total clones) was closely related to Legionella sp. In the *Planctomycetes* group, clone 26 (6.8% of total clones) was closely related to Planctomycetes sp. In the high G+C group, clones 21 and 23 were closely related to Arthrobacter sp. and environmental (uncultured and unidentified) clones. One OTU from the Bacteroidetes group was related to Marinicola seohaensis (<90% similarity). 10

The  $\alpha$ -proteobacteria was found to be the most predominant group on the MF membrane surface, which was in agreement with a previous study (Chen et al., 2004). The  $\alpha$ -proteobacteria groups became the most dominant biofoulant under pressurized oligotrophic conditions. This result was expected since they are known to be well adapted to oligotrophic environments (Pinhassi and Hagström, 2000). The second largest  $\beta$ proteobacteria group on the membrane surface is typically found in a freshwater envi-

# ronment, which is consistent with drinking water sources, like the Han River.

#### Conclusions 4

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In this study, different classes and specific genes/species of bacteria existing in foulants on membranes used for drinking water treatment process were identified using 16S 20 rRNA gene clone library method, in addition to separated chemical foulants characterization, including FTIR and 3-D fluorescence spectra analyses.  $\alpha$ -,  $\beta$ -, and  $\gamma$ -Proteobacteria were found to be the dominant bacteria; however, specific bacteria found through genetic identification could not be directly compared to similar published

results with membranes that were used for drinking water plants. Despite this, the find-25 ing that  $\alpha$ - and  $\beta$ -Proteobacteria were determined to be the most dominant bacteria in the fouled membrane is consistent with the fact that they are known to be mainly found

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in pressurized and oligotrophic conditions and relatively fresh water environments, respectively. The results provided in this study will be highly beneficial for future studies in the field of membrane biofouling.

Acknowledgement. This research was supported by a grant from the National Research Laboratory Program through the Korea Science and Engineering Foundation (NOM Lab: ROA-2007-20055-0).

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**Table 1.** Summary of major bacteria from 120 colonies identified for the fouled MF membrane, as measured using the clone library method.

Phyogentic group	Major found bacteria	Occupying percentage for 120 colonies (%)
Alpha proteobacteria	<i>Bradyrhizobium</i> sp., <i>Rhodopseudomonas</i> sp. and <i>Afipia</i> sp.	68
<i>Beta</i> proteobacteria	Ralstoina sp.	16
<i>Gamma</i> proteobacteria	<i>Legionella</i> sp.	7
Planctomycetes	Planctomyces sp.	7

# **Table 2.** Phylogentic distribution of identified bacteria from the fouled MF membrane.

Sequence type (and percentage) of total clones	Phylogentic group	Most closely related bacteria	Similarity (%)
Clone 2 (35.6%)	$\alpha$ -Proteobacteria	Bradyrhizobium sp.	98–99
Clone 148 (17.8%)	$\alpha$ -Proteobacteria	Rhodopseudomonas sp.	99
Clones 37 (11.9%), 142 (0.8%)	$\alpha$ -Proteobacteria	<i>Afipia</i> sp.	98–99
Clone 147 (0.8%)	$\alpha$ -Proteobacteria	Xanthobacter sp.	99
Clone 75 (0.8%)	$\alpha$ -Proteobacteria	Agrobacterium sp.	99
Clones 87 (11.9%)	$\beta$ -Proteobacteria	Ralstoina sp.	98–99
Clone 133 (1.7 %)	$\beta$ -Proteobacteria	<i>Alcaligenes</i> sp.	99
Clones117 (1.7 %)	$\beta$ -Proteobacteria	Curvibacter sp.	96
Clone 135 (0.8%)	$\beta$ -Proteobacteria	Janthinobacterium sp.	98
Clone 40 (6.8 %)	$\gamma$ -Proteobacteria	<i>Legionella</i> sp.	94–96
Clone 38 (0.8%)	Bacteroidetes	Marinicola seohaensis	88
Clone 21 (0.8%)	High G+C	Arthrobacter sp.	99
Clone 23 (0.8%)	High G+C	Environmental clones	96
Clone 26 (6.8%)	Planctomycetes	Planctomyces sp.	94

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Fig. 1. Foulants characterization. (a) Depicts of excitation and emission of peaks from 3-D fluorescence spectrophotometer. (b) FTIR spectra of the membrane foulants.

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Fig. 2. SEM images of fouled MF membranes (×70, ×1500, ×5000).



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**Fig. 3.** Phylogenetic tree of the proteobacteria based on 16S rDNA sequences obtained from cloning analyses of fouled MF membrane. Clones obtained from this study are shown in *bold type*. The tree was derived by performing the neighbor-joining method with a Jukes-Cantor parameter in the MEGA version 3.1 programme. Bootstrap values greater than 50% (1000 replicates) are shown. *Methanococcus voltae* EU751623 was used as the out-group to root the phylogram. The scale bar represents one substitution per ten nucleotides.



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