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Characteristics of Bacterial Communities in Biological Filters of Full-Scale Drinking Water Treatment Plants

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The taxonomic and functional characteristics of bacterial communities in the pre-chlorinated rapid filters and ozonated biological activated carbon (BAC) filters were compared using Illumina MiSeq sequencing of the 16S rRNA gene and community-level physiological profiling (CLPP) based on sole-carbon-source utilization patterns. Both the rapid filters and BAC filters were dominated by *Rhizobiales* within *α-proteobacteria*, but other abundant orders and genera were significantly different in both types of filter. *Firmicutes* were abundant only in the intermediate chlorinated rapid filter, while *Acidobacteria* were abundant only in the BAC filters. Bacterial communities in the rapid filter showed high utilization of carbohydrates, while those in the BAC filters showed high utilization of polymers and carboxylic acids. These different characteristics of the bacterial communities could be related to the different substrates in the influents, filling materials, and residual disinfectants. Chlorination and ozonation inactivated the existing bacteria in the influent and formed different bacterial communities, which could be resistant to the oxidants and effectively utilize different substrates produced by the oxidant, including *Phreatobacter* in the rapid filters and *Hyphomicrobium* in the BAC filters. *Bradyrhizobium* and *Leptothrix*, which could utilize compounds adsorbed on the GAC, were abundant in the BAC filters. Ozonation increased taxonomic diversity but decreased functional diversity of the bacterial communities in the BAC filters. This study provides some new insights into the effects of oxidation processes and filling materials on the bacterial community structure in the biological filters of drinking water treatment plants.

Keywords: Bacterial community, substrate utilization, rapid filter, biological activated carbon filter, biological filter, drinking water

Introduction

Biological filtration refers to the process of removing both particulate matter and biodegradable organic matter (BOM) from water [1]. Biological filters can remove organic compounds through the fixed biofilm that develops on various media such as sand, anthracite, granular activated carbon (GAC), or membranes [2]. The biologically active rapid filter and the biological activated carbon (BAC) filter have been widely used for decades in drinking water treatment plants [1–3]. The rapid filter is usually filled with sand or anthracite, and is considered the most economical way to remove particles and BOM with the same filter unit [1]. However, many previous studies have demonstrated that the BAC filter is a more appropriate process to remove BOM than the rapid filter [4, 5]. Generally, the combination of ozonation and GAC filter is referred to as the BAC process [3]. Ozonation converts high-molecular-weight refractory organic matter into low-molecular-weight BOM, and this increased BOM can be effectively removed by the bacteria attached to the BAC filter [1, 4, 6–8].

Biological filtration has many advantages for drinking water treatments. First of all, it is important to increase the biostability of drinking water by reducing the quantity of BOM which can cause bacterial regrowth in distribution systems [1, 3–5]. It can reduce the disinfection by-product

(DBPs) precursors and chlorine demand, which can help to maintain stable residual chlorine in the distribution system [1, 3]. Biological filtration can also remove various biodegradable micropollutants including taste and odor compounds [1–3, 9]. Furthermore, an active biofilm can extend the lifetime of a GAC filter by bio-regeneration [3].

However, there is a lack of information about which microorganisms are involved in the functions [2]. It is necessary to identify the attached microorganisms and characterize the microbial communities of various biological filters in order to determine the role of microorganisms and enhance the removal of biodegradable contaminants. Especially, the change of microbial community in the biological filters after the oxidation process is important to produce biologically stable drinking water in full-scale water treatment plants. Bacteria have a unique substrate preference [10, 11], so the change of microbial composition in biological filters can result in removal of different BOM in water. Both chlorination and ozonation can increase the BOM concentration, but they can produce different kinds of substrates [8, 12, 13]. Without the dominance of bacteria utilizing new substrates produced by those oxidants, biological filters cannot effectively remove the increased BOM, which can cause bacterial regrowth in the distribution system [14, 15]. In addition, understanding the microbial community in a biological filter is useful in terms of public health because the biofilm in a biological filter can be a source of microbial contamination in the distribution systems [16]. It is also possible to screen pathogenic or disinfection resistant microorganisms and adopt appropriate measures to control them in advance [17, 18].

Recently, many researchers have used molecular biological methods to evaluate microbial community structures in the aquatic ecosystem. In particular, next-generation sequencing (NGS) is a culture independent and high-throughput method of analyzing the structure of an entire microbial community [10, 19, 20]. In addition, community-level physiological profiling (CLPP) based on sole-carbon-source utilization (SCSU) patterns has deepened understanding of the metabolic ability and functional characteristics of microbial communities [21]. In this study, therefore, Illumina MiSeq sequencing of the 16S rRNA gene and CLPP were used to investigate the characteristics of bacterial communities attached to the biological filters in full-scale drinking water treatment plants.

The main purposes of this study were 1) to compare the differences in the taxonomic and functional characteristics of bacterial communities in rapid filters and BAC filters which received pre-chlorinated water and ozonated water, respectively; 2) to investigate the relationship between the taxonomic composition and substrate utilization patterns of bacterial communities; and 3) to elucidate how the different oxidation processes, *i.e.*, chlorination vs ozonation, effect on the bacterial communities of biological filters.

Materials and Methods

Study Sites and Sampling

This study was conducted at three, full-scale water treatment plants (WTPs) in Seoul, South Korea (Fig. 1). All three WTPs use the surface water of the Han River as source water, and consist of conventional and advanced water treatment processes. The conventional treatment includes pre-chlorination, coagulation-sedimentation, and rapid filtration; whereas the advanced treatment includes ozonation and BAC filtration, and post-chlorination finally follows the advanced treatment. Pre-chlorination was applied at the intake of the raw water, and ozone was added after the rapid filters of the WTPs. Intermediate chlorine was added after sedimentation only at WTP2. The rapid filters of WTP1 and WTP3 are filled with sand, while WTP2 has a dual media (anthracite/sand) rapid filter. The BAC filters are filled with coal-based granular activated carbon (GAC), and the empty bed contact time (EBCT) is 15 min. The detailed design and

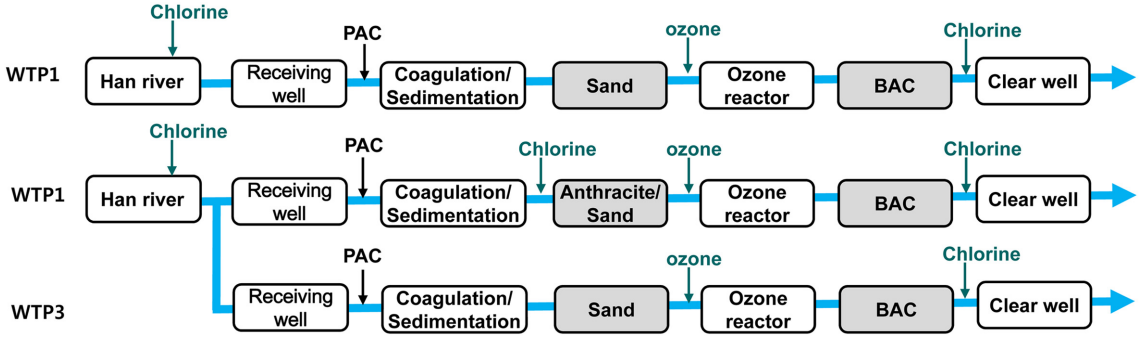

Fig. 1. Flow diagram of processes in the three drinking water treatment plants.
PAC (poly aluminium chloride): coagulant

Table 1. Design and operational parameters of biological filters and water quality of influents in three WTPs.

	WTP1	WTP2	WTP3
Capacity (m^3/day)	1,100,000	450,000	720,000
Raw water quality			
Temperature (°C)	18.7	20.9	20.4
TOC (mg/l)	2.7	2.7	2.7
pH	8.0	7.3	7.6
Disinfection dose (mg/l)			
Pre-chlorine	2.2	2.2	2.0
Intermediate chlorine	-	0.6	-
Ozone	0.4	0.5	0.6
Rapid filter			
Media/depth (m)	Sand 1.2	Anthracite 1.0/sand 0.3	Sand 1.2
Effective diameter (mm)	0.9	1.0/0.5	0.9
Influent water			
TOC (mg/l)	1.0	1.4	1.3
pH	7.1	7.1	7.3
Residual chlorine (mg/l)	0.24 (monthly)	0.50 (monthly)	0.36 (monthly)
Running time (year)	29	0.5	17
BAC filter			
Coal-based GAC (made in)	China	USA (Calgon carbon)	China
Effective diameter (mm)	0.68	0.63	0.86
Depth (m)	2.5	2.5	2.9
EBCT (min)[a]	15	15	15
Influent water			
TOC (mg/l)	1.1	1.2	1.2
pH	7.1	7.1	7.0
Running time (year)	1	0.5	1

[a]EBCT (Empty bed contact time) = bed volume of filter media/flow rate

operational parameters of the three WTPs are shown in Table 1.

The conventional processes have been operated for decades, except for WTP2; whereas the advanced treatment of the three WTPs commenced in October 2014 (WTP2, April 2015). For the purposes of this study, after one year of operation, the filling materials (media) were taken from the rapid filters and BAC filters of the three WTPs in October 2015. The media were collected from the top layer at about 1~2 cm below the surface of all the biological filters using a core sampler. The collected medium samples were then placed in a sterilized bag and mixed well, after which the taxonomic composition and substrate utilization of the attached bacterial communities in the mixed medium samples were investigated. The residual chlorine in the water samples was measured using a Hach pocket chlorine colorimeter.

Next-Generation Sequencing of Bacterial Communities

The DNA of the attached bacteria was extracted from the media of the biological filters using a Fast DNA Spin Kit for soil (MP Biomedicals), and the extracted DNA was amplified and sequenced by ChunLab Inc. (Korea).

Briefly, the extracted DNA was amplified by polymerase chain reaction (PCR) using the primers 341F and 805R, targeting the V3 to V4 regions of the 16S rRNA gene. Then, secondary amplification was performed in order to attach the Illumina NexTera barcode using an i5 forward primer and an i7 reverse primer. The condition of the PCR and the sequences of primers are shown in Table 2. The PCR products were purified using a QIAquick PCR cleanup kit (Qiagen, USA). Equal concentrations of the purified products were pooled together, and non-target products were removed with an Ampure beads kit (Agencourt Bioscience, USA). The size and quality of the products were determined by a Bioanalyzer 2100 (Agilent, USA) with a DNA 7500 chip. Mixed amplicons were pooled and sequencing was carried out with a Miseq Sequencing system (Illumina, USA).

The EzTaxon database was used for taxonomic assignment [22, 23], and similarity was analyzed using pairwise alignment. The

Table 2. Condition of PCR and sequences of primers.

	Forward	Reverse
DNA amplification	341F (5′-TCGTCGGCAGCGTCAGATGTGTATA-AGAGACAGCCTACGGGNGGCWGCAG-3′	805R (5′-GTCTCGTGGGCTCGGAGATGTGTAT-AAGAGACAGGACTACHVGGGTATCTAATCC-3′)
secondary amplification X : barcode region	i5 (5′-AATGATACGGCGACCACCGAGATC-TACAC-XXXXXXXX-TCGTCGGCAGCGTC-3′)	i7 (5′-CAAGCAGAAGACGGCATACGAGAT-XXXXXXXX-AGTCTCGTGGGCTCGG-3′)
PCR amplification conditions	initial denaturation at 95°C for 3 min 25 cycles: denaturation at 95°C for 30 sec (secondary amplification : 8 cycles) primer annealing at 55°C for 30 sec extension at 72°C for 30 sec final elongation at 72°C for 5 min	

sequences were clustered using CD-Hit7 and UCLUST8, and the operational taxonomic units (OTUs) were defined based on similarity (cutoff 97%). The two richness parameters, *i.e.*, Chao and abundance-based coverage estimators (ACE), and two diversity parameters, *i.e.*, the Shannon index and the Simpson index, were calculated using the CLcommunity program based on the CD-Hit clustering method (ChunLab Inc., Korea). To compare the bacterial community diversity between the samples, principal coordinate analysis (PcoA) and permutational analysis of variance (PERMANOVA) tests were performed using BIOiPLUG bioinformatics cloud platform using Jensen-Shannon divergence (ChunLab Inc., Korea).

Analysis of Community Level Physiological Profiling (CLPP)

Biolog EcoPlates (Biolog, Inc., USA) were used to compare the characteristics of substrate utilization by the bacterial communities attached to the biological filters. EcoPlates consist of 96-well plates containing 31 different substrates and one blank in three replications (Table 3). 10 g of each medium sample was placed in 90 ml of sterilized NaCl solution (0.85%) with sodium thiosulfate in a 250-ml Erlenmeyer flask, which was shaken at 250 rpm for 30 min to detach the bacteria. After settling large particles for 30 min, 50 ml of supernatant was taken and filtered with a 5-μm sterilized syringe filter to remove particulate matters. 150 μl of the filtrate was inoculated into each well of the EcoPlates, which were then incubated at 25°C for 7 days. The absorbance of the EcoPlates was measured at 620 nm every day using a microplate reader (Ascent Multiscan, Labsystem).

The bacterial activity (Ai) of each substrate was calculated by subtracting the absorbance value of the blank from the absorbance value obtained for each substrate. The threshold of the Ai was set at 0.1 after considering the variation of blank absorbance, so any Ai values above 0.1 were considered to be positive. The average bacterial activity of the substrates in the EcoPlates was expressed as Average Well Color Development (AWCD), as shown in the following equation [24].

$$AWCD = \Sigma Ai/31 = \Sigma(ODi\text{-}C)/31$$

where,

ODi: the mean absorbance value of the triplicate wells of each substrate.

C: the mean absorbance value of the triplicate blank wells.

Ai: the difference between the absorbance of each substrate and the blank absorbance

For functional diversity, Richness (R), the Shannon index (H′) and the Simpson index (D) were calculated using an Ai above 0.1 on the fifth day, as expressed by the following equation [24, 25]:

$$\text{Richness (R)} = \text{number of substrates with } Ai > 0.1$$
$$\text{Shannon index (H')} = -\Sigma pi\ (\ln pi)$$
$$\text{Simpson index (D)} = \Sigma(pi)^2$$
$$pi = Ai/n,\ n = -\Sigma Ai$$

where,

n: sum of Ai values of 31 substrates

pi: the ratio of the Ai value of each substrate to the sum of the Ai values of the 31 substrates

The 31 organic substrates in the EcoPlates were categorized into the following 6 groups according to Insam [26]: (1) amines, (2) amino acids, (3) carbohydrates, (4) carboxylic acids, (5) polymers, (6) phenolic compounds (Table 3).

Results and Discussion

The Taxonomic Composition of the Bacterial Communities

A comparison was made of the taxonomic composition of the bacterial communities in two rapid filters and three BAC filters. In the case of the rapid filter of WTP3, the concentration of the extracted DNA was too low to acquire sequencing data. The relative abundance of the bacterial

Table 3. Substrate utilization after 5 days by attached bacteria in the rapid filters and BAC filters of three WTPs.

Category	Substrate	WTP1		WTP2		WTP3	
		Sand	BAC	Anthracite/Sand	BAC	Sand	BAC
Carbohydrates	D-Cellobiose	++++	-	-	+	-	-
	α-D-Lactose	-	-	-	-	-	-
	β-Methyl-D-glucoside	+++	-	-	-	-	-
	D-Xylose	-	-	-	+	-	-
	i-Erythritol	-	-	-	-	-	-
	D-Mannitol	++++	-	-	+	+	-
	N-Acetyl-D-glucosamin	+++	++	-	+	-	-
	Glucose-1-phosphate	++	-	-	-	-	-
	D,L-α-Glycerol phosphate	+	+	-	-	-	-
	D-Galactonic acid γ-lactone	+++	-	-	-	+	-
Carboxylic acids	Pyruvic acid methyl ester	++	++	-	+	+	+++
	D-Glucosaminic acid	+	-	-	-	+	-
	D-Galacturonic acid	-	-	-	++	+	-
	γ-Hydroxybutyric acid	-	-	-	-	-	-
	Itaconic acid	-	-	-	-	-	-
	α-Ketobutyric acid	-	+	-	+	-	-
	D-Malic acid	-	+	-	+	-	-
Amino acids	L-Arginine	-	-	-	-	+	-
	L-Asparagine	+++	++	-	+	+	++
	L-Phenylalanine	-	+	-	+	-	+
	L-Serine	++	+	-	+	+	-
	L-Threonine	+	+	-	+	-	-
	Glycyl-L-glutamic acid	+++	+	-	+	-	-
Amines/amides	Phenylethyl-amine	-	-	-	-	-	-
	Putrescine	-	-	-	-	+	-
Phenolic compounds	2-Hydroxy benzoic acid	-	-	-	-	-	-
	4-Hydroxy benzoic acid	-	-	-	-	+	-
Polymers	Tween 40	-	+	-	++	-	-
	Tween 80	+	+	-	+	-	-
	Cyclodextrin	-	+	-	+	-	-
	Glycogen	+	+	-	++	-	-

Ai<0.1: -, 0.1< Ai <1.0: +, 1.0<Ai<1.5: ++, 1.5<Ai<2.0: +++, Ai>2.0: ++++.

communities in the five biological filter samples was shown at the phylum, class and order levels (Fig. 2).

At the phylum level, both the rapid filters and the BAC filters shared two dominant bacterial populations: *Proteobacteria* (58~74%) and *Bacteroidete*s (11~23%). However, *Acidobacteria* (2~15%) were abundant only in the BAC filters, while *Firmicutes* (11%) were abundant only in the rapid filter of WTP2. Some previous studies also reported the dominance of *Acidobacteria* only in the biofilm of BAC filters for drinking water treatment [17, 27]. But how *Acidobacteria* are selected in the biofilm of BAC filters is not yet understood. Meanwhile, some previous studies pointed to a high relative abundance of *Nitrospirae* in the biological filters of drinking water treatment plants [28, 29]. In this study, however, only a very low level of *Nitrospirae*, *i.e.*, average 0.07% and STD 0.13%, was detected in all of the biological filters. This finding could be attributed to the low concentration of ammonia in influent water due to oxidation by pre-chlorination.

In the subclass of *Proteobacteria*, *α-proteobacteria* (35~71%)

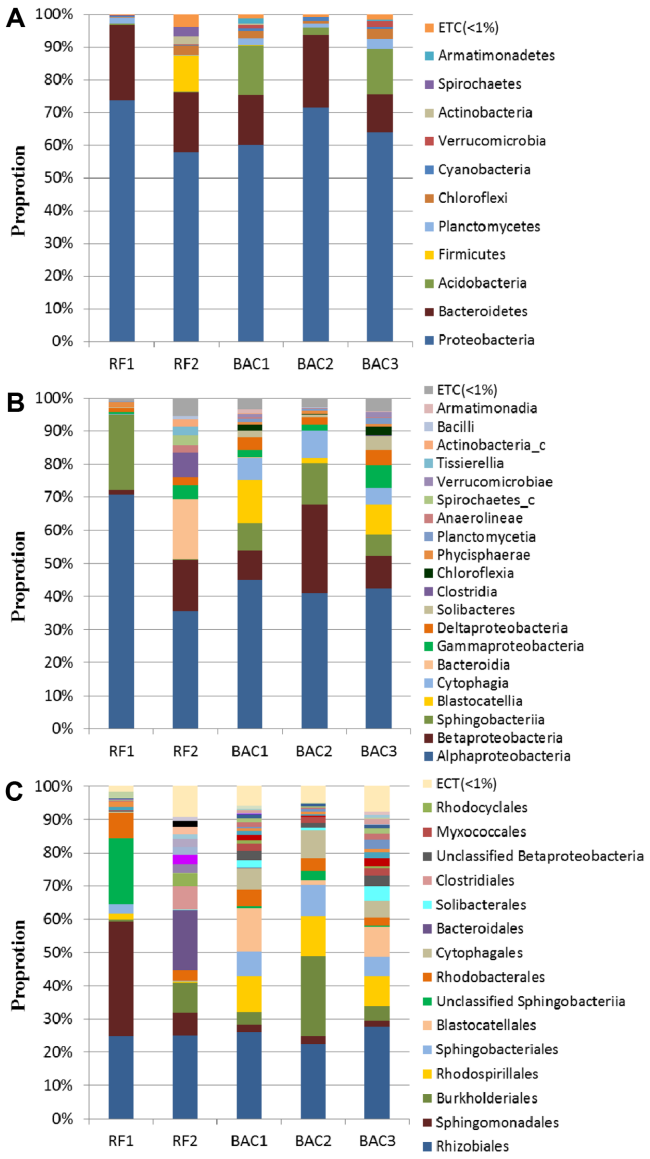

Fig. 2. Relative abundance of bacterial communities in the two rapid filters (RF) and three BAC filters at the phylum (**A**), class (**B**), and order level (**C**).

The remarks of the minor orders with a low relative abundance of <3% are not shown.

were the most dominant class in all of the biological filters, followed by *β-proteobacteria* (1~27%). *γ-proteobacteria*, a medically important group including many pathogens such as *Salmonella*, *Yersinia*, and *Vibrio* [30], showed low abundance (0.4~7%) in both types of filter. Many previous studies have also reported the dominance of *α-proteobacteria* in the biofilms of drinking water systems [18, 19, 28, 31, 32]. Generally, *α-proteobacteria* and *γ-proteobacteria* are abundant in marine ecosystems, whereas *β-proteobacteria* and *Actinobacteria* are abundant in freshwater ecosystems [11]. This dominance of *α-proteobacteria* in drinking water systems might be related to its competitiveness in the nutrient-poor conditions of drinking water treatment systems and their ability to degrade complex organic compounds, including humic substances [11]. Copiotrophic *β-* and *γ-proteobacteria* can grow rapidly in nutrient-rich conditions, while oligotrophic *α-proteobacteria* have low growth rates but can survive in chronic starvation conditions using low concentrations of substrates [33, 34]. Therefore, *α-proteobacteria* may have a disadvantage in nutrient-rich conditions such as wastewater, but they can outcompete *β-* and *γ-proteobacteria* in nutrient-poor drinking water systems [11, 33–36]. Besides *α-* and *β-proteobacteria*, the three BAC filters were dominated by *Sphingobacteriia* (6~13%), *Cytophagia* (5~8%) and *Blastocatellia* (1~13%). The rapid filters of WTP1 and WTP2 were dominated by *Sphingobacteriia* (23%) and *Bacteroidia* (18%), respectively. Also, *Clostridia* (7%), *Tissierellia* (2%) and *Bacilli* (1%) within *Firmicutes* were abundant only in the rapid filter of WTP2.

Both the rapid filters and the BAC filters showed a significant difference in bacterial composition below the order level although *Rhizobiales* within *α-proteobacteria* were the commonly abundant order (22~28%) in all of the biological filters. In particular, *Rhodospirillales*, *Blastocatellales* and *Cytophagales* were abundant orders in the BAC filters, while *Sphingomonadales* were abundant in the rapid filters. Previous studies have been reported that *Rhizobiales* were dominant in the biological filters of drinking water treatment plants, which may be related to their metabolic versatility and the ability to produce extracellular polymeric substances (EPS) that protects the bacteria from the harsh environment [18, 29, 37].

At the genus level, many dominant genera in the biological filters were related to uncultured bacteria, and minor genera with a low relative abundance of <1% (ECT) accounted for the high percentage of bacterial communities. In the BAC filters, uncultured genus (FJ479296_g), *Bradyrhizobium* and *Hyphomicrobium* within *Rhizobiales* were dominant. In addition, *Aridibacter*, *Lacibacter*, *Leptothrix*, *Reyranella* and uncultured genera within *Cytophagales* (GU454944_g), *β-proteobacteria* (GQ263935_g) and *Blastocatellales* (EU335275_g) were more abundant in the BAC filters than the rapid filters. Conversely, *Phreatobacter* within *Rhizobiales* was dominant in the rapid filters, and *Sphingomonas* and *Novosphingobium* within *Sphingomonadales* were more abundant in the rapid filters compared to the BAC filters (Fig. 3).

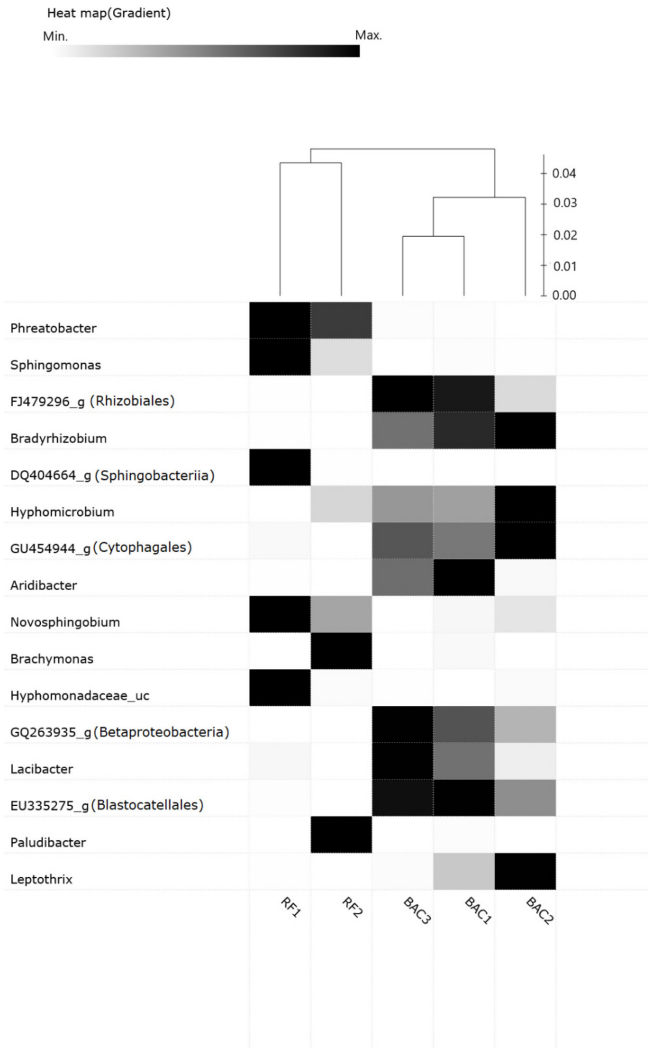

Fig. 3. Heat map showing the dominant genera of microbial communities in the rapid filters and BAC filters.
The minor genera with an average relative abundance of <1% are not shown.

The composition of the bacterial communities in the rapid filters was significantly different from that of BAC filters at the 90% confidence level (PERMANOVA, p value = 0.09). The PcoA analysis showed that three BAC filters had a similar bacterial composition while the two rapid filters had a large variation in the bacterial composition (Fig. 4). Despite this large variation, the rapid filter samples were clearly separated from the BAC samples on the first principal coordinate. Also, in the heat map analysis, the common genera in the rapid filters were quite different from those of the BAC filters, so the two rapid filters (RF-1, 2) and the three BAC filters (BAC-1, 2, 3) were clustered, respectively (Fig. 3).

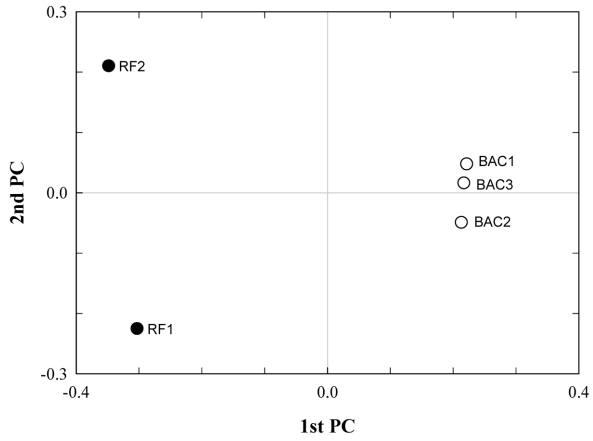

Fig. 4. Principal coordinate analysis (PcoA) of the five biological filter samples using Jensen-Shannon divergence from Illumina MiSeq sequencing.

Characteristics of Substrate Utilization of Bacterial Community

The AWCD as an indicator of average substrate utilization by the bacterial communities was compared between the rapid filters and the BAC filters of the three WTPs (Fig. 5). Except for WTP1, the AWCD of the rapid filters was low because the attached bacterial concentration of the rapid filters was low due to the residual chlorine in the influents. However, the level of AWCD was not proportional to the attached bacterial concentration in all of the biological filters. The rapid filter of WTP1 showed the highest AWCD, although the BAC filters of WTP1 and WTP2 had over 100 times more attached bacterial biomass than the rapid filter of WTP1 (data not shown). This implies that average substrate utilization is related not only to the bacterial concentration but also to the composition of the bacterial community. Although average substrate utilization can be a good indicator of bacterial metabolic ability, it cannot provide useful information on the functional characteristics of a bacterial community. Thus, the utilization levels of each substrate group in the rapid filter and the BAC filter of WTP1 were compared, wherein both filters showed a high level of average substrate utilization. The bacterial community of the rapid filter showed a high utilization of carbohydrates and amino acids, while that of the BAC filter showed a high utilization of polymers, amino acids and carboxylic acids (Fig. 6). In the BAC filters, especially, the contributions of carbohydrates to the utilization of six substrate groups drastically decreased, while the contribution of polymers and carboxylic acids increased (Fig. 7). A higher utilization of polymers, amino acids and carboxylic

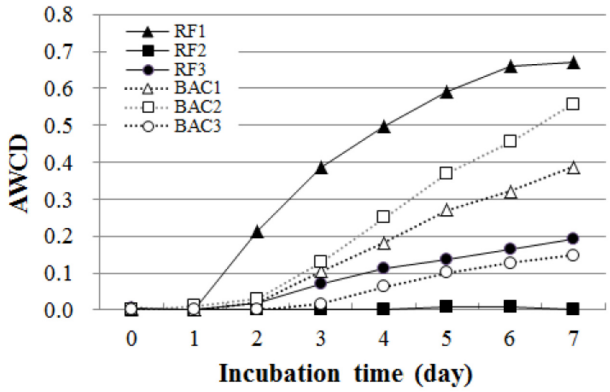

Fig. 5. Average substrate utilization by bacterial communities in the rapid filters and BAC filters of three WTPs.

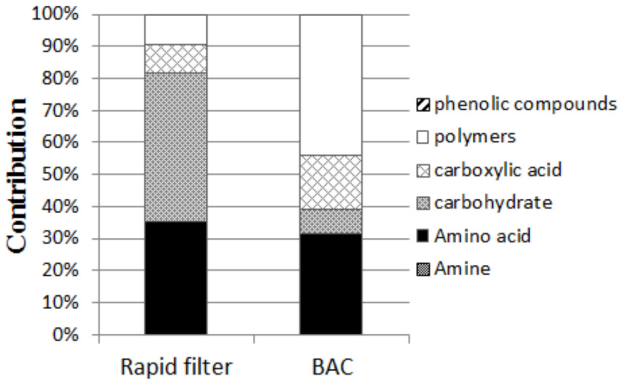

Fig. 7. The relative contribution of each substrate group to utilization of six substrate groups by bacterial communities in the rapid filter and BAC filter of WTP1.

acids was also observed in the BAC filter of WTP2 (Table 3).

Taxonomic and Functional Diversity of Bacterial Communities

The OTUs obtained by NGS sequencing ranged from 910 to 1,487 in the BAC filters, while those in the rapid filters ranged from 394 to 920. The number of OTUs of the three BAC filters was higher than that of the two rapid filters, and the highest number of OTUs was observed in the BAC filter of WTP3 (Table 4). ACE and Chao, the parameters of richness, were higher in the bacterial communities of the BAC filters than in those of the rapid filters. The Shannon indices (H') of the bacterial communities in the BAC filters ranged from 4.72 to 5.09, while those in the rapid filters ranged from 2.54 to 4.21; and the Simpson indices (D') of the bacterial communities in the BAC filters ranged from 0.017 to 0.028, while those in the rapid filters ranged from 0.051 to 0.164. These higher figures for ACE, Chao, the Shannon index, and the lower Simpson index in the BAC filters indicated that BAC filters had a more diverse bacterial composition than the rapid filters [38].

On the other hand, the bacterial communities of the rapid filters showed higher functional diversity based on the substrate utilization patterns compared to those of the BAC filters. The substrate richness and the Shannon indices of the rapid filters were higher than those of the BAC filters, while the Simpson indices of the rapid filters were lower than those of the BAC filters (Table 5). The splitting of organic matter by ozonation could remove large organic compounds and produce a greater variety of small organic compounds [6, 7, 39]. This indicates that the total numbers of organic species could increase, but the kinds of available substrate groups could decrease, especially the high-molecular-weight organic fractions. Therefore, more varied bacteria using these various small organic matters could

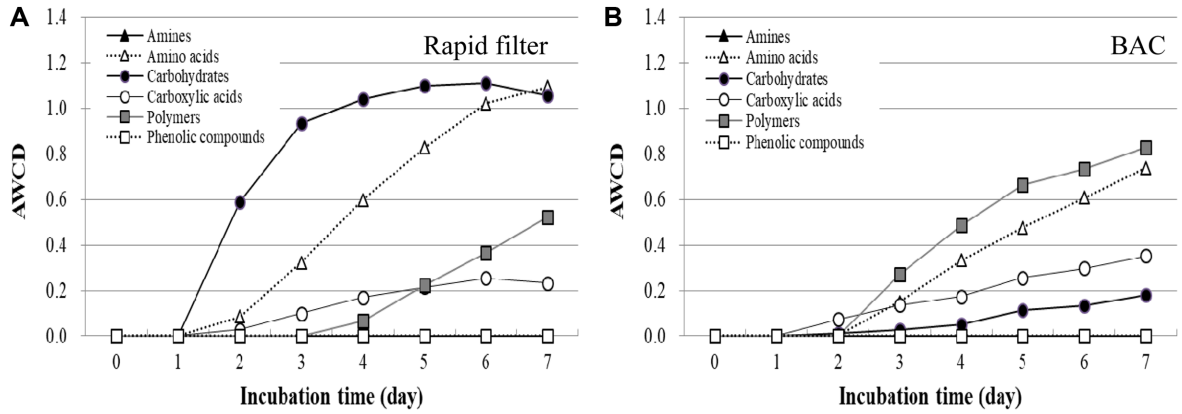

Fig. 6. Utilization of each substrate group by bacterial communities in the rapid filter (**A**) and BAC filter (**B**) of WTP1.

Table 4. Taxonomic diversity statistics for bacterial composition in the rapid filters and BAC filters.

		OUT	ACE	Chao	Coverage	Shannon	Simpson
Rapid filter	1	394	461	429	0.997	2.54	0.164
	2	920	1073	1032	0.994	4.21	0.051
	3	-	-	-	-	-	-
BAC filter	1	910	972	938	0.997	4.72	0.028
	2	1267	1305	1274	0.997	5.09	0.017
	3	1487	1524	1493	0.998	5.03	0.023

grow in the BAC filters, but they could not utilize the relatively large organic matters, which resulted in an increase of the taxonomic diversity, but in a decrease of the functional diversity of substrate utilization in the BAC filters.

Factors Influencing the Bacterial Community Structure of Biological Filters

Different substrates in the influent water. The different substrates in the influent could cause differences in bacterial composition [10, 20, 40]. Pre-chlorinated water and ozonated water flowed into the three rapid filters and the three BAC filters, respectively. Both chlorine and ozone are good oxidants, but they can produce different kinds of substrates [6, 13]. Some previous studies showed changes of different fractions of DOM in the drinking water treatment process. Pre-chlorination increased the high-molecular-weight biopolymers and humic substances and low-molecular-weight neutrals, while ozonation increased the low-molecular-weight fractions including building blocks, neutrals, and organic acids [10, 41]. Biopolymers, neutrals, and organic acids are biodegradable, but their characteristics are different [42]. Biopolymers are high-molecular-weight organic matter of >20 kDa including polysaccharides and proteins. Neutrals are uncharged, small organic matter of <350 Da including alcohols, aldehydes, and ketones. Organic acids represent protic organic acids of low molecular weight of <350 Da [43]. Pre-chlorination can increase high-molecular-weight substrates by disrupting particulate organic matter, including algae in raw water [41, 44]. Algal organic matter (AOM) consists of biodegradable compounds including carbohydrates, proteins, lipids, amino acids and amine [44, 45]. Especially, the intracellular organic matter (IOM) of algae are relatively high-molecular-weight compounds compared to extracellular organic matter (EOM) [44, 46]. Therefore, the disruption of algal cells by pre-chlorination can result in an increase of high-molecular-weight substrates by releasing IOM. Although those high-molecular-weight compounds can be removed by coagulation-sedimentation [41], the pre-chlorinated influent of the rapid filters might contain a higher concentration of relatively large substrates than the ozonated influent of the BAC filters.

Table 5. Functional diversity statistics based on the substrate utilization by bacterial communities in the rapid filters and BAC filters.

		Richness	Shannon	Simpson
Rapid filter	1	15	2.5	0.09
	2	-	-	-
	3	10	2.2	0.11
BAC filter	1	14	2.4	0.10
	2	17	2.8	0.07
	3	3	1.0	0.44

Post-ozonation can break high-molecular-weight refractory dissolved organic matters and produce low-molecular-weight oxygen-containing compounds, including aldehydes, ketones, carboxylic acids and keto acids, all of which are easily biodegradable [1, 6–8, 39, 47]. On the other hand, chlorination can produce halogenated compounds, including trihalomethanes (THMs), haloacetic acids (HAAs), haloketones (HKs), and haloacetonitrils (HANs) [12, 48]. These halogenated by-products are less biodegradable than ozonated by-products although some of them can be easily biodegraded [12]. Chlorination can also convert high-molecular-weight refractory organic matter such as aromatic, lignin, and phenolic compounds to biodegradable organic matter, including aliphatic, ester, alcoholic and carboxylic compounds [49]. However, ozone is a stronger oxidant than chlorine and produces more oxidized compounds with lower molecular weights than chlorine [6, 8, 13]. For instance, ozonation can convert carbohydrates, alcohols, and aldehydes into carboxylic acids, which have the highest oxidation state of organic compounds [8, 50]. Świetlik *et al*. [13] reported that chlorine produced much fewer carboxylic acids than ozone. In this study, the utilization of carbohydrates decreased while that of

carboxylic acids increased in the BAC filter. This could be attributed to the decrease of carbohydrates and the increase of carboxylic acids in the ozonated influent of the BAC filters. Moll *et al*. [51] also reported a significant decrease of carbohydrate utilization by bacterial communities in filters with the influent of ozonated water compared to filters with the influent of non-ozonated water. Xiang *et al*. [21] reported that carboxylic acids and polymers were utilized less than other substrate groups by the bacterial community in the BAC filter with the influent of non-ozonated water.

In this study, *Phreatobacter,* the dominant genus in rapid filters, can use limited amounts of carbohydrates, alcohols and amino acids, but it cannot use carboxylic acids [52]. The other dominant bacteria in the rapid filters, *i.e*., *Sphingomonas* and *Novosphingobium,* can use various carbohydrates and degrade high-molecular-weight recalcitrant organic compounds, including chlorinated compounds and polycyclic aromatic hydrocarbons (PAH) [53–55]. Conversely, *Hyphomicrobium,* which was abundant in the BAC filters, can utilize low-molecular-weight C1 compounds including methanol, formaldehyde and formate [56]. GAC can adsorb the low-molecular-weight compounds produced by ozonation [47, 57]. Therefore, bacteria that utilize those low-molecular-weight compounds could be more likely to grow in the BAC filters than in the rapid filters. Kim *et al*. [58] reported the abundance of other methylotrophic bacteria including *Methylobacter* and *Methylosoma* which can utilize C1 compounds in BAC filter. Both *Phreatobacter* and *Hyphomicrobium* belong to the same order of *Rhizobiales*. Despite the similar proportion of *Rhizobiales*, the proliferation of completely different genera in the rapid filters and the BAC filters might be related to the substrate preference of the attached bacteria.

In the CLPP test, the utilization of polymers was higher in the BAC filters than in the rapid filters, which might be related to the abundance of the class *Cytophagia* only in the BAC filters. The family *Cytophagaceae*, *i.e*., major members of *Cytophagia,* can utilize various biopolymers including polysaccharides, proteins, and cellulose [59]. Kim *et al*. [31] also reported the abundance of *Cytophagaceae* (genus *Ohtaekwangia*) in the BAC filter.

Filling Materials (Media)

The filling materials could affect the bacterial communities of the biological filters. In this study, *Bradyrhizobium* was abundant only in the BAC filters, although it shows versatile substrate utilization, including various carbohydrates and alcohols [52]. This dominance of *Bradyrhizobium* in the BAC filters has also been reported in other studies [10, 27, 29]. A recent study reported that the metabolic pathway associated with aromatics degradation was significantly higher in the bacterial community of the BAC filter than that of the rapid sand filter, and *Bradyrhizobium* may play an important role in aromatics biodegradation [29]. This finding may be one of the explanations for the dominance of *Bradyrhizobium* only in the BAC filters since aromatic organic matter can be accumulated in the BAC filters by adsorption [57]. Inorganic compounds such as iron and manganese, which are minor components of GAC and can be adsorbed on GAC [60], can also affect bacterial composition. *Bradyrhizobium* is a nitrogen-fixing bacterium and iron is required for the synthesis of many components related to nitrogen fixation activity [61]. Therefore, nitrogen-fixing bacteria including *Bradyrhizobium* could effectively proliferate in the iron-containing BAC filters. *Leptothrix,* which was another abundant genus only in the BAC filters, is an important bacterium for the biological oxidation of iron and manganese [62]. *Leptothrix* could have a growth advantage in the BAC filters by oxidizing those adsorbed minerals. Oh *et al*. [29] reported that the iron metabolic pathway was significantly enriched in the bacterial community of the BAC filter over that of the rapid sand filter. This finding also supports the abundance of *Leptothrix* in the BAC filters.

In this study, attached bacterial biomass was much higher in the BAC filters than in the rapid filters. GAC can support a denser attached bacterial biomass than sand or anthracite since macroporous and the irregular structure of GAC can be more suitable for bacterial attachment due to protecting bacteria from adverse environment [1]. The surface charge of GAC can also enhance bacterial attachment, and the adsorption capacity of substrates and nutrients can promote the growth of attached bacteria in the GAC filters more so than in the rapid filters [3].

Attached bacteria can produce biopolymers such as polysaccharide and proteins, and develop biofilm that is an assemblage of those EPS and microbial cells [48, 63]. Especially, *Bradyrhizobium,* which was abundant in the BAC filters, is known to produce significant quantities of EPS [64]. Therefore, the higher biomass of attached bacteria in the BAC filters could be another source of biopolymers due to the detached biofilm and the excretion of metabolites by the bacteria [27, 39, 48]. The increased biopolymers could result in the growth of polymer-utilizing bacteria in the BAC filter.

The high bacterial biomass and the dominance of EPS-producing bacteria in the BAC filters can adversely affect

biofilter performance and water quality. The EPS released by attached bacteria can cause buildup of head loss of the filters and increase of DBP formation [27]. In addition, the detachment of accumulated bacteria can increase the bacterial concentration in the effluent [65, 66]. Although these microbes can be easily disinfected by post-chlorination, some bacteria attached to GAC particles can be resistant to disinfectants [65, 67]. To ensure the safety of effluent quality from those challenges, more enhanced operational measures may be required, including increasing disinfectant dose, backwashing frequency and intensity optimization, and the installation of membrane or sand filters after BAC filters [67].

The two rapid filters of WTP1 and WTP2 showed significant differences in their bacterial composition despite having the same chlorinated influents. This could be related to the different materials of media (sand for WTP1 and anthracite for WTP2). According to a study by Gerrity *et al*. [27], the bacterial communities of the anthracite filter and the BAC filter differed significantly, although the same ozonated water flowed into both filters. Although the cause is not clearly understood, these results imply that the physical and chemical characteristics of the filling materials might be one of the important factors that influence the bacterial composition in the biological filters.

Disinfection

The difference in the bacterial composition between the rapid filters and the BAC filters could be related to disinfection resistance. The phylum *Firmicutes* were abundant only in the rapid filter of WTP2. In the case of WTP2, the average residual chlorine in the influent of the rapid filter was highest due to intermediate chlorination, so chlorine resistant bacteria could have a growth advantage in the rapid filter of WTP2. *Firmicutes* have a gram-positive cell wall structure and can form endospores, making them resistant to disinfectants [2, 68]. Previous research also showed a dramatic change in the bacterial community structure after chlorination, including the dominance of gram-positive bacteria [17, 69, 70]. *Sphingomonadaceae*, which have been detected in drinking water distribution systems due to their resistance to chlorine, were also abundant in both rapid filters [17, 71].

Actually, the bacterial composition of the biological filters is very complex and can be affected by various factors including temperature, pH, dissolved oxygen, nutrients, and the age of the biofilms [16, 20, 31, 38, 72]. Although this study is not sufficiently broad in scope to fundamentally understand the bacterial community changes in the biological filters, it can provide some insights into the effects of the substrate changes caused by oxidation processes on the bacterial community structures in the biological filters of drinking water treatment plants.

The main conclusions of this study are as follows: (1) The bacterial communities in the rapid filters and BAC filters showed significant differences in their taxonomic composition and substrate utilization due to the different substrates in the influents, filling materials and residual disinfectants. (2) Both the rapid filters and BAC filters were dominated by *Rhizobiales* within *α-proteobacteria,* but other abundant orders and genera were significantly different in both types of filter. *Acidobacteria* were abundant only in the BAC filters, while *Firmicutes* were abundant only in the intermediate chlorinated rapid filter. (3) Chlorination and ozonation could affect the characteristics of a bacterial community by inactivating the existing bacteria in the influent and producing different substrates. More various bacteria that utilize biopolymers and small ozone by-products were abundant in the BAC filters, while chlorine resistant and/or less oxidized substrate-utilizing bacteria were abundant in the rapid filters. (4) Ozonation increased taxonomic diversity, but decreased functional diversity of the bacterial communities of the BAC filters.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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