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Effects of Diverse Water Pipe Materials on Bacterial Communities and Water Quality in the Annular Reactor

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To investigate the effects of pipe materials on biofilm accumulation and water quality, an annular reactor with the sample coupons of four pipe materials (steel, copper, stainless steel, and polyvinyl chloride) was operated under hydraulic conditions similar to a real plumbing system for 15 months. The bacterial concentrations were substantially increased in the steel and copper reactors with progression of corrosion, whereas those in stainless steel (STS) and polyvinyl chloride (PVC) reactors were affected mainly by water temperature. The heterotrophic plate count (HPC) of biofilms was about 100 times higher on steel pipe than other pipes throughout the experiment, with the STS pipe showing the lowest bacterial number at the end of the operation. Analysis of the 16S rDNA sequences of 176 cultivated isolates revealed that 66.5% was *Proteobacteria* and the others included unclassified bacteria, *Actinobacteria*, and *Bacilli*. Regardless of the pipe materials, *Sphingomonas* was the predominant species in all biofilms. PCR–DGGE analysis showed that steel pipe exhibited the highest bacterial diversity among the metallic pipes, and the DGGE profile of biofilm on PVC showed three additional bands not detected from the profiles of the metallic materials. Environmental scanning electron microscopy showed that corrosion level and biofilm accumulation were the least in the STS coupon. These results suggest that the STS pipe is the best material for plumbing systems in terms of the microbiological aspects of water quality.

Keywords: Biofilm, drinking water distribution system, pipe material, PCR–DGGE

The drinking water produced at a water treatment plant with high quality can be deteriorated in drinking water distribution systems (DWDS) by unexpected factors such as cross-connection, backflow, corrosion, and biofilm. The biofilms that can develop within DWDS are composed of bacterial cells embedded in a matrix of extracellular polymeric substances (EPS) secreted by themselves and can exert a chlorine demand, reducing the protection afforded by residual disinfectant [34]. The highly hydrated extracellular matrix accounts for about 50~90% of the biofilm, and is made up of complex heterogeneous substances including polysaccharides, protein, lipids, and nucleic acids [9]. EPS provide a cohesive force that holds the cells together and facilitates adhesion to the surface. Actinomycetes or fungi in biofilm can cause taste and odor problems [32], and corrosion of pipes by iron bacteria can cause red water [37]. In addition, biofilm may act as a reservoir for both pathogenic and nonpathogenic organisms, some of which pose a major health risk [14, 48] and create violation of the drinking water standard, such as *E. coli* [22]. Hence, great concern has been focused on the systematic management of DWDS for improvement of water quality at the consumer's tap.

A drinking water supplying system includes pipes, fittings, coating, valves, and other adjuncts with various materials. As the material can affect bacterial attachment on the inner surface of the pipe system, it is one of the important physical factors along with surface characteristics and stability of materials [28, 33, 34]. A corrosion tubercle, which is formed by metal corrosion, not only increases the surface area for bacterial attachment but also provides bacteria with hiding places by consuming residual chlorine [20]. Biodegradable compounds dissolved from plastic pipes were reported to affect biofilm formation [45, 49]. Pipe material was also reported to affect the concentration of influenza A virus or pathogenic bacteria such as *Legionella* or *Mycobacterium* [30, 31, 38].

The total length of the pipe lines in Seoul is 14,106 km (distribution pipe 71% and service line 25%). The material

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mainly used for distribution pipes is cement mortar ductile cast iron (53.4%), and for service lines is stainless steel pipes (28.9%). Until 1993, the service line was mainly composed of galvanized steel pipe (45%), stainless steel pipe (32%), copper pipe (11%), and PVC pipe (12%). As regulations banned the use of galvanized steel pipes in Korea since 1994, galvanized steel pipes decreased to 8% of service lines whereas stainless steel pipes increased to 80% in 2005 (Yearbook of Seoul Waterworks Office, 2006). Copper pipes have been steadily used (10%) for service lines owing to its easiness for construction and the social perception that copper pipes are hygienic. On the other hand, use of the PVC pipe has decreased to 2%.

In order to control biofilm in DWDS, it is very important to understand the relationship between pipe material and biofilm formation. Lehtola *et al*. [24] reported that biofilm formed slowly, as the copper ion decreased the bacterial concentration in water for the first 200 days, but there was no difference after another 100 days. Zacheus *et al*. [49] and Pedersen [33] reported that they could not observe any significant difference in bacterial concentrations with different pipe materials (*i.e*., stainless steel, PE, and PVC). van der Kooij *et al*. [46] observed high bacterial concentration with PEX (cross-linked polyethylene), but no significant difference was observed in bacterial concentrations of biofilm for copper and stainless steel pipe materials. When Schwartz *et al*. [40] studied the concentration of bacteria in biofilm on different pipe materials, the concentration in PE pipe was the highest, followed by stainless steel and PVC pipes, with the lowest in copper pipe. Unfortunately, most previous studies on biofilms of distribution system have used only culture-dependent methods to determine the bacteria present. Recently, Kalmbach *et al*. [16] studied attached bacterial community formed on PE and PVC pipes with molecular biological approaches including rRNA-targeted fluorescently labeled oligonucleotide probes. More recently developed molecular techniques, such as PCR–DGGE, offer some advantages in permitting determination of biofilm composition and dominant organisms, even if these cannot be isolated in culture [27]. Clearly, the pipe material has been known to affect the structural characteristics of bacterial communities [29]. However, most of the previous studies have not provided sufficient information to distinguish the best pipe material between STS and copper as well as plastic materials in plumbing systems.

In this study, using both culture-dependent and culture-independent methods, we have investigated the concentration, species diversity, and community structure of the bacteria in biofilm formed in four different pipe materials (steel, copper, stainless steel, and polyvinyl chloride), and analyzed the impact of bacteria on drinking water quality including metal dissolution and disinfection efficacy by pipe materials in the annular reactor.

Materials and Methods

Devices for the Experiment

Four annular reactors (BioSurface Technologies Co., USA) were installed to simulate the hydraulic conditions of a drinking water supplying system and the characteristics of the biofilm were investigated with different pipe materials (Fig. 1). Twenty sample coupons were installed in each reactor to compare the characteristics of biofilm by pipe materials (*i.e*., steel, copper, stainless steel, and PVC) which were dominantly used for home pipe systems for drinking water. Steel coupons were tested to simulate old corroded galvanized steel pipes, although galvanized steel pipes were banned for use as the material for drinking water pipes. The influent of the reactors was freshly produced drinking water from the water treatment plant. The reactor was operated under the similar hydraulic conditions as a drinking water pipe system in the home. The annular reactors were operated at a rational speed of 50 rpm, which can be translated into a shear stress of 0.25 N/m^2. A shear stress of 0.25 N/m^2 corresponds to a flow of approximately 0.3 m/s in a 100-mm-diameter smooth pipe, which is similar to shear conditions of other pilot and bench scale investigations of drinking water distribution systems [5, 11, 41]. The hydraulic residence time was determined by injection rate and the reactors were operated with the condition of 170 ml/min (40 l/day) for 15 months.

Water Quality

The concentration of residual chlorine was measured by the *N,N*-diethyl-*p*-phenylenediamine (DPD) method with a portable colorimeter (HACH Pocket Colorimeter). Turbidity was analyzed using a 2100P turbidimeter (Hach Co., Loveland, CO, USA). pH and total organic carbon (TOC) were analyzed with a pH meter (Metrohm 780) and TOC analyzer (Ionic, Sivers 820), respectively. Iron, copper, zinc, and manganese were measured with Inductively Coupled Plasma (Horiba Jobin Yvon/Activa M) according to a method in the standard methods [2]. Chlorine residual, turbidity, and pH were analyzed on a daily basis, while TOC and metals were analyzed at least bi-weekly.

Sampling and Bacterial Counts

Biofilm was scraped from the inside of the coupon with a sterilized scraper and sampled into the sterilized conical tube with 20 ml of phosphate buffer solution and 25 µl of sterile sodium thiosulfate [10% (W/V)]. The sampled specimen was treated with ultrasound for 30 s and dispersed completely by violent mixing, and this

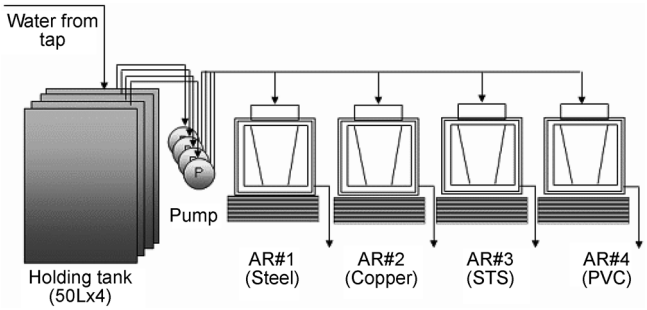

Fig. 1. Schematic diagram of the annular reactors.

procedure was repeated two times. The treated sample was diluted to an appropriate concentration by 10-step dilution, and spread on a plate of R2A agar. The sample was spread on 2 plates for each step with 0.1 ml of dilution solution. The inoculated plates were cultivated at 28°C for 7 days, and the plates with 30~300 colonies formed were selected. The numbers of bacteria per milliliter were averaged and converted into the number of bacteria per unit area (*i.e.*, CFU/cm^2).

DNA Extraction and PCR for 16S rDNA

In order to identify the species of bacteria, 176 bacterial colonies (approximately 24.3%) were selected from the R2A plate according to the colony morphology and cultivated at 28°C for 3~4 days. Total genomic DNA was extracted from each of the isolates with a Qiagen DNeasy Tissue Kit (QIAGEN, Hilden, Germany). PCR was carried out using the primers 27mf (5'-AGAGTTTGATCMTGGCT CAG-3') and 1492r (5'-GGTTACCTTGTTACGACT T-3'), which target the 16S rRNA genes of a wide range of members of the domain *Bacteria* at positions 28 through 1491 (*E. coli* 16S rRNA gene sequence numbering) [20]. PCR amplification of nearly full-length 16S rRNA genes was performed in 50-µl reaction mixtures containing 10× PCR buffer [160 mM $(NH_4)_2SO_4$, 670 mM Tris/HCl, pH 8.8, 25 mM $MgCl_2$, 0.1% Tween 20], 1 µl of template DNA, 25 pmol of each primer, 200 mM of each dNTP (GeneCraft, Munster, Germany), and 2.5 U of *Taq* polymerase (GeneCraft, Munster, Germany). The PCR cycle consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extention at 72°C for 7 min. After PCR amplification, 2-µl samples of the PCR products were checked by electrophoresis on 1.5% agarose gels. PCR products were purified by using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany).

Sequencing of 16S rDNA

Sequencing was performed with an ABI Prism BigDye Terminator Cycle Sequencing Ready Kit (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions with the sequencing primers 27f and 1492r [15]. Approximately 400 unambiguous nucleotide positions were used for comparison with the data in GenBank using the Basic Local Alignment Search Tool (BLAST) [1].

Denaturing Gradient Gel Electrophoresis (DGGE) Analysis for Biofilm Bacterial Community

Biofilm bacterial community DNA was extracted using a FastDNA Spin Kit (Qbiogene, Carlsbad, USA). PCR amplification of the 16S rRNA genes was performed with primers 1070f and 1392r (*E. coli* 16S rRNA gene sequence numbering) as described previously [8]. The PCR product contained a GC clamp of 40 bases, added to the 1392r reverse primer, and had a total length of 392 bp, including the highly variable V9 region. PCR cycles consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extention at 72°C for 7 min. PCR products were subjected to DGGE analysis with the Dcode Universal Mutation Detection System (Bio-RAD, Hercules, USA) as described previously [19]. After electrophoresis, the gels were stained with SYBR Green I for 15 min, rinsed for 25 min, and photographed with UV transillumination (302 nm).

Sequencing of DGGE DNA Bands

For sequencing, selected bands were excised from DGGE gels by using a sterile scalpel and placed in a sterile Eppendorf tube containing 40 µl of sterile water, and then DNA was eluted through five cycles of freeze-thawing (−70°C/37°C). Two µl of the solution was used as template in PCR with non-GC clamp primers as described previously [26]. The amplified products were purified from agarose gel slices using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The extracted products were cloned into the One shot TOP 10 Chemically competent *E. coli* using a par 2.1-TOPO vector according to the manufacturer's instruction (Invitrogen, Carlsbad, USA). Fifteen bands, which were not overlapped in the four pipe materials, were cut out. Then, three positive colonies were randomly selected from each DNA band and the 45 inserted regions were amplified with M13F and M13R primers. Sequencing was done as described above with an ABI 3730 DNA sequencer using the ABI Prism BigDye Terminator Cycle Sequencing Ready Kit (Applied Biosystems, Foster City, USA).

Nucleotide Sequence Accession Numbers

The partial 16S rRNA gene sequences determined in this study were deposited in the GenBank nucleotide sequence databases under accession numbers EU634723 through FJ535467.

Microscopy of Biofilm

An environmental scanning electron microscope (ESEM, XL-30 FEG, FEI Company) and energy dispersive X-ray spectrometer (EDX) were used to analyze the surface characteristics of the biofilm formed on the sample coupons.

RESULTS AND DISCUSSION

Water Quality

The water quality data of the influent of the reactors for 15 months are listed in Table 1. The average water temperature was 17°C (5.3~27.4°C). The water temperature of the winter season (November~March) was 9.4°C, whereas that of the summer season (July~September) was over 20°C, which was a favorable condition for microbial growth. The concentration of residual chlorine in the influent was 0.64 mg/l (0.36~0.83 mg/l) during the operation. The residual chlorine was consumed very rapidly by corrosion in steel coupons to 0.19 mg/l, which was lower by 0.31 mg/l than those with the other materials (Fig. 2). The concentrations of residual chlorine in the reactors with copper, stainless steel, and PVC coupons were lower by about 0.15 mg/l than the concentration in the influent (Fig. 2). Corrosion of steel was reported to increase when the chlorine concentration was over 0.4 mg/l [39] and residual chlorine was reduced by reactions with corrosion tubercles and biofilm. The decreasing rate of residual chlorine was higher with the pipe material, which was easier to be corroded [3, 13]. In this study, decreasing rates of residual chlorine in the effluent compared with that in the influent were 69% for steel coupons, 22% for copper coupons,

Table 1. Water quality of the influent.

Parameters (unit)	Mean	SD	Max.	Min.
Water temp. (°C)	17	6.4	27.4	5.3
Turbidity (NTU)	0.14	0.05	0.38	0.07
Chlorine residual (mg/l)	0.64	0.09	0.83	0.36
pH (U)	6.88	0.28	7.68	5.71
HPC (CFU/ml)	4	72.2	406	0
TOC (mg/l)	1.35	0.17	1.68	0.93
Fe (mg/l)	0.02	0.01	0.09	0.01
Cu (mg/l)	0.01	0.06	0.02	0
Zn (mg/l)	0.06	0.04	0.24	0.02
Mn (mg/l)	0.01	0.01	0.02	0.01
Al (mg/l)	0.03	0.02	0.09	0
NO_3-N (mg/l)	1.8	0.3	2.2	1.2
Alkalinity (mg/l as $CaCO_3$)	44.5	7.6	56.6	23.3
Hardness (mg/l as $CaCO_3$)	65	10.9	78	44
Cl^- (mg/l)	18	3.1	22	14
SO_4^{2-} (mg/l)	13	2.46	16	9
$KMnO_4$ (mg/l)	1.5	0.28	1.9	1
Total solids (mg/l)	114	11.8	130	89

24% for STS coupons, and 27% for PVC coupons. The relationship between residual chlorine in the effluent and in the influent was calculated (data not shown). The slope of copper coupons was steepest of all, followed by stainless steel and PVC coupons.

Although the residual chlorine concentration in the annular reactor with copper coupons was highest at the early stage of operation, the decreasing rate of residual chlorine increased with corrosion after 120 days (Fig. 2). As for the copper pipe, residual chlorine consumption by corrosion tubercles may affect the disinfection capacity [12, 23]. The bacterial concentrations in the effluent from the reactors increased in the reactors with steel and copper

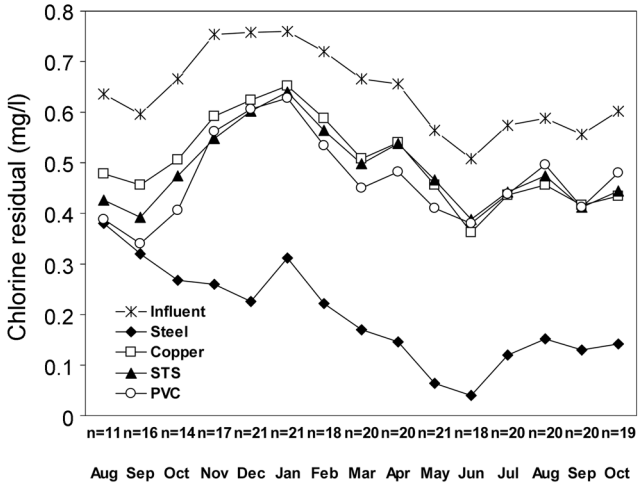

Fig. 2. Change of residual chlorine by pipe material during the experiment.

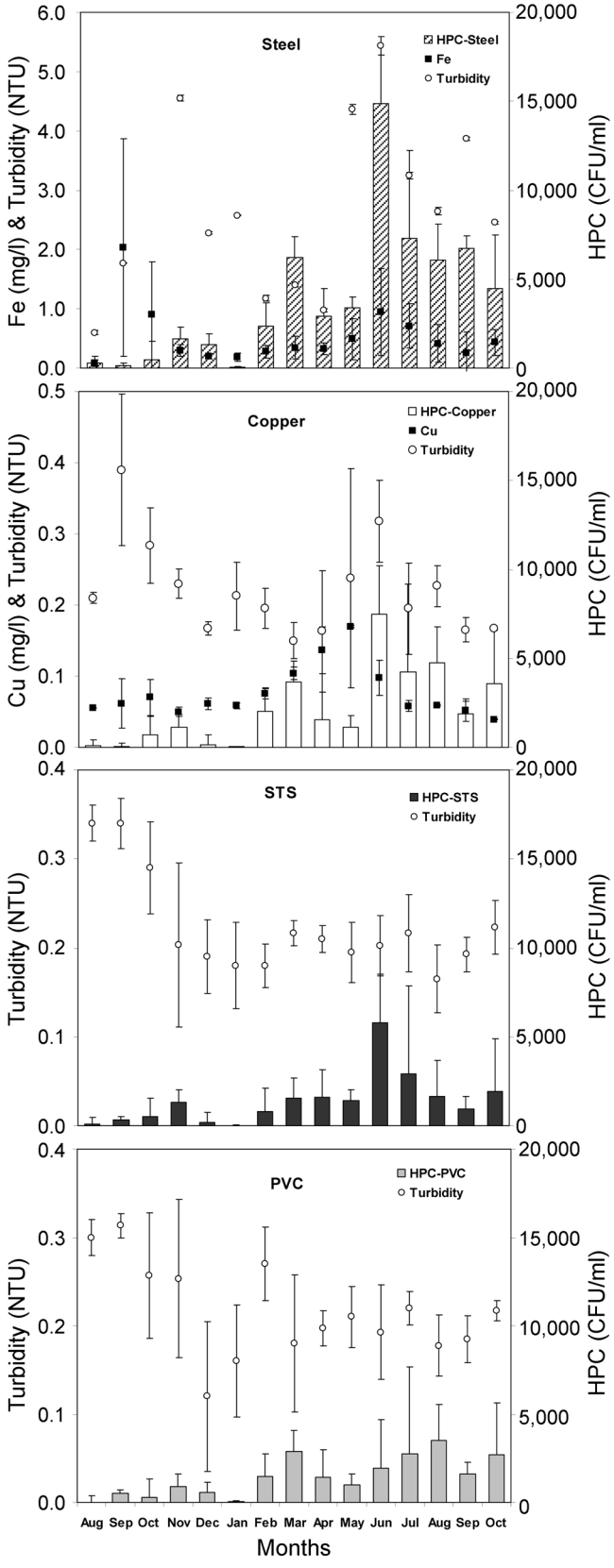

Fig. 3. Monthly changes of suspended bacterial concentration, dissolved ion, and water turbidity during the experiment.

coupons during the rainy season when corrosion was promoted by high organic materials and low pH. The bacterial concentrations with STS and PVC were affected mainly by water temperature (Fig. 3). There are various parameters that affect the corrosion reaction, including pH, alkalinity, water temperature, dissolved oxygen, suspended particles, residual chlorine, organic materials, and bioactivity [42]. The corrosion reaction was promoted owing to increased concentration of organic materials and low pH during the rainy season, and this observation could be related to a rapid increase in the number of suspended bacteria as a result of decreased disinfection capability. The annual average of pH was 6.9 (5.7~7.7), and the pH during dry season (January~March) increased by algal bloom while decreased in rainy season (May~July) because of precipitation. The average pH values in the dry and rainy seasons were 7.1 and 6.6, respectively. The concentration of the TOC in the influent was about 1.36 mg/l (0.8~2.0 mg/l). The average TOC of the dry season was 1.38 mg/l, whereas that of the rainy season was 1.51 mg/l. In Seoul, during the rainy season in late summer with lots of heavy rain, TOC increases because heavy rain washes large amounts of organic carbon from the watershed into the river, while during the dry season, TOC increases because of algal biomass. The concentrations of TOC in the reactors were similar (average 1.35 mg/l; paired *t*-test, $p>0.05$) for copper, STS, and PVC coupons, whereas TOC in the effluent of the reactor with steel coupons was lower (paired *t*-test, $p<0.01$) by 0.06 mg/l than that in the influent. Organic carbon acts as the nutrients and is essential for bacterial growth. Adsorption of organic carbon by iron oxide containing corrosion product can promote biofilm growth. Thus, more organic carbon might be consumed in steel pipes containing corrosion product with more biofilm than other pipe materials [21, 25, 44]. The average concentrations of iron and zinc in the effluent of the annular reactor with steel coupons increased by 0.58 and 0.43 mg/l, respectively, compared with those in the influents. The average increase in copper concentration in the effluent of the reactor with copper coupons was 0.07 mg/l (max. 0.15 mg/l). Although iron ions from steel coupons dissolved because of rapid corrosion from the early stage of the experiment, copper ions did not dissolve in water owing to the relatively slow corrosion at the early stage. The copper ions in water increased later during the rainy season, as well as other water quality parameters such as turbidity. In the reactor with steel coupons, dissolved zinc ions increased in the rainy season when organic materials increased and pH was relatively low.

Concentration of Bacteria in Biofilm

The concentrations of bacteria in biofims formed on the coupons by season are illustrated in Fig. 4. The concentration of suspended bacteria in the effluent was

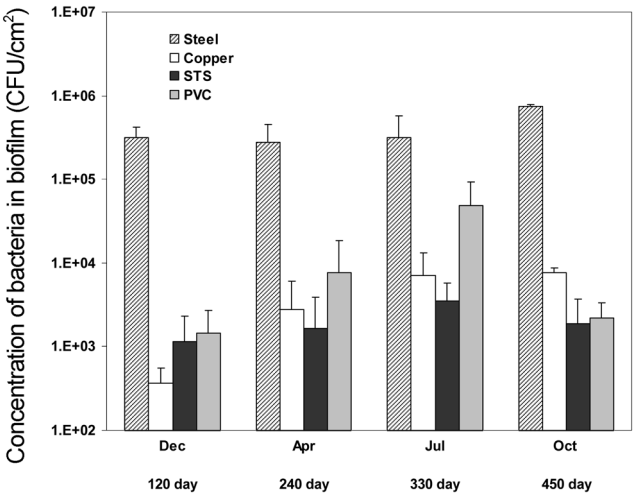

Fig. 4. Seasonal changes of bacterial concentration in biofim formed on the reactor coupons.

high when biofilm formation increased. The bacterial count in biofilm formed on the steel coupons was about 100 times higher (*i.e.*, about 10^5 CFU/cm^2) than those formed on the other metal coupons. Although the concentration of bacteria in biofilms was lowest in the reactor with copper coupons during the first 120 days of the early stage, the bacterial concentration on the copper coupons increased with corrosion, and the lowest bacterial concentration was found on STS coupons at the end of the experiment. The suspended bacteria in the effluent of the reactor with copper coupons were not high, whereas the concentrations of bacteria in the biofilm increased significantly during the spring and summer season (*i.e.*, April and May) when copper dissolution increased.

Being different from suspended bacteria, bacteria in biofilm formed on the surface of copper pipes were wrapped by EPS to form a stable slime layer, which could protect the biofilm from toxic compounds like copper [18, 36]. At the same time, corrosion of copper was promoted by bacteria attached on the surface of the copper coupons, and the concentration of bacteria in the biofilm was higher than that on the STS coupons as the copper surface reacting with disinfectant increased to consume more chlorine [6, 24, 43]. The surface of STS is moderately hydrophilic, with a slightly negative surface charge under the pH conditions used for the experiment. The negative electrostatic force on the surface hindered bacterial adhesion, as the surface of bacteria was also slightly negatively charged [10]. The concentration of bacteria in biofilm formed on the surface of PVC coupons was 3 times higher than that on STS coupons. Bacterial growth in the reactor with PVC coupons could be promoted, as the dissolved compounds from the PVC coupons included organic materials that were advantageous for bacterial growth [4, 47].

Identification of Bacteria in Biofilm

16S rRNA gene sequences of 176 bacterial colonies cultivated in R2A agar were analyzed to identify the species of bacteria in the biofilm. The dominant class was *Proteobacteria* (66%), followed by unclassified bacteria (20%), *Actinobacteria* (6%), *Bacilli* (2%), and unknown (6%) (Fig. 5). α-Subclass *Proteobacteria* was 98.2% and β-subclass and γ-subclass were found only in the PVC pipe. *Sphingomonas*, which is an α-*Proteobacteria*, was dominant regardless of pipe material, and *Methylobacterium* was the next dominant species. When analyzing the biofilm formed on the surface of plastic shower curtains, Kelly *et al.* [17] reported that *Sphingomonas*, which was a frontier at the early stage of biofilm formation, was predominant, and *Methylobacterium*, which could use various carbon resources as its nutrient and showed pinkish color in water-contacting conditions, was one of the main species. They also detected *Norcardia*, *Gordonia*, *Afipia*, and *Moraxella*.

On the other hand, aerobic Gram-negative bacteria such as *Pseudomonas* and *Alcaligenes* were reported as the dominant species when biofilm in the stainless steel pipe was studied [34]. Critchley and Fallowfield [7] reported *Acidovorax delafieldii*, *Cytophaga johnsonae*, and *Micrococcus kristinae* as the dominant bacteria in copper pipes and suggested that the bacteria increased the copper concentration in the tap water. In this study, *Methylobacterium* was dominant on steel coupons and copper coupons, which formed metal oxides layers by corrosion. All the bacteria belonging to *Bacilli* class, which forms endospore resistive to environmental change, were found only in pipe materials susceptible to corrosion. Drinking water bacterium, which belongs to unclassified bacteria, was the dominant species in the reactors with corrosion-resisting STS coupons and PVC coupons. It was found that metal corrosion could affect bacterial diversity as well as bacterial concentration. The bacterial species in the reactor with PVC coupons were most diverse at the early stage of the experiment, whereas the reactors with steel coupons and copper coupons had the most variety of bacterial species in April as corrosion was promoted. Overall, the diversity of bacterial species and the difference by pipe materials in biofilm decreased with time.

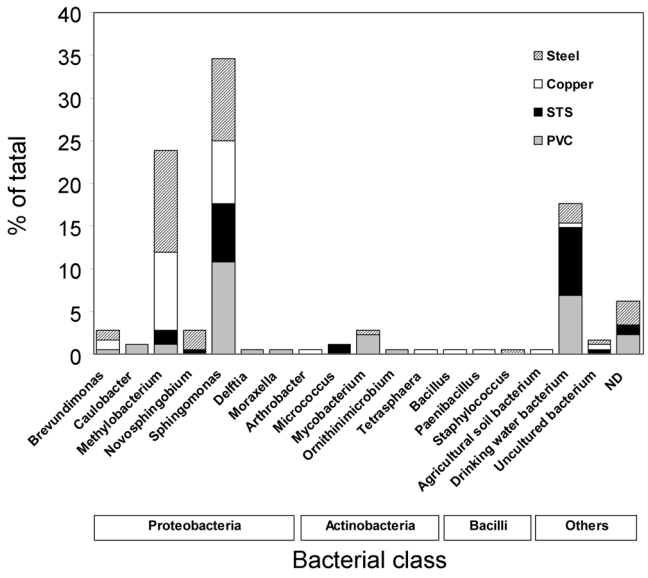

Fig. 5. Distribution of bacterial genera in biofilm by pipe material.

DGGE Analysis of Biofilm Bacterial Community

PCR–DGGE was performed to investigate the phylogenetic diversity of the biofilm bacterial communities formed at the late stage in the reactor (Fig. 6). When the general banding patterns of DGGE gel were investigated, it was found that the bacterial community in the reactor with steel coupons was the most diverse among the metallic coupons, followed by copper and STS coupons. As mentioned before, this was due to corrosion of the metals. Corroded metal oxides provide bacteria with advantageous ecological environments and increase the bacterial concentration. In PVC coupons, the DGGE profile of biofilm showed three additional bands that were not detected in the profiles of the metallic materials.

The prevalent DNA band was cut out from the DGGE gel, and the DNA sequence was analyzed for identification of the dominant bacterial species through the process of

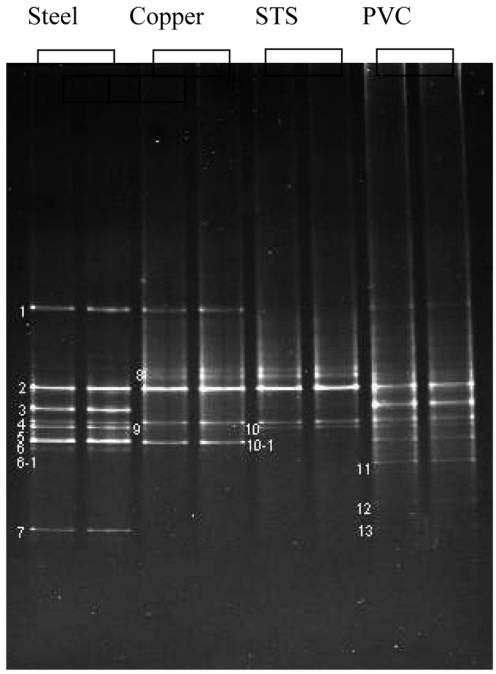

Fig. 6. DGGE profiles of bacterial community in biofilms.
Each DNA band represented by a number was isolated, cloned, and sequenced for identification of bacterial species.

reamplification, cloning, and sequencing. Fifteen bands, which were not overlapped in the 4 pipe materials, were cut. Three colonies were selected from the bacterial colonies acquired from cloning of each DNA band. Thirty-eight out of the 45 DNA sequences showed sequence similarity of more than 97% when compared with the GenBank database, and most of the bacteria were Gram-negative bacteria. As in the culture method, *Proteobacteria* was the predominant class (27 out of 38 sequences) in all the samples, and all of them were α-subclass except for one *Acidovorax*. The next dominant class was *Actinobacteria* (11 out of 38 sequences). The most commonly dominant DNA bands in all pipe materials were closely related to *Sphingomonas*, *Novosphingobium* (No. 5, 9, 10 and 10-1), and *Erwinia* (No. 2). DNA bands of No. 6 and 6-1 were closely related to *Methylobacterium, Afipia, Bradyrhizobium*, and *Bosea.* The DNA band of No. 7, which was only found in steel pipe, was closely related to *Rhodococcus*. DNA bands of No. 11, 12, and 13 found only from the reactor with PVC coupons were closely related to *Propionibacterium* and *Roseomonas*, which were not detected by the culture method.

Electron Microscopy and Surface Characteristics

The coupons, which contacted and reacted with the tap water in the reactors for 15 months, were investigated with the environmental scanning electron microscope and the compositions on the surface were analyzed by EDX (Fig. 7). A thick biofilm wrapped by EPS, turbidity materials, and corroded oxide was observed on the surface of the steel coupon (Fig. 7A). On the surface of the STS coupon, little biofilm was formed (Fig. 7C). In the case of the copper coupon and PVC coupon, the structure and thickness of the biofilm were halfway between those two cases (Fig. 7B and 7D). According to the results of EDX analyses on the biofilm formed on the surface of the copper coupon, copper and copper oxides took 64%, and a small amount of silicon, aluminum, and sulfur were detected (Fig. 7C). The level of carbon in the case with the copper coupon (20.6%) was midway between that with STS (4.8%) and PVC (32.6%). In the case of the PVC coupon, the main components included carbon (32.6%) and chlorine (25.1%), and oxygen (23.8%) was mid-level between the cases of copper coupon and STS coupon. Silicon (13.4%) derived from outside of the pipe was the highest, and other trace amounts such as lead (2.0%), calcium (1.3%), iron (1.0%), and aluminum (0.9%) were detected. Stainless steel 304 (STS 304) used for drinking water pipes was composed of mainly iron and included crominum (18%), nickel (8~10.5%), manganese (2%), and silicon (1%). According to the results of EDX analyses on the STS coupon, iron (65%), chromium (16%), nickel (8.5%), and silicon (1.7%) were detected and the percentages were similar with the matrix metal. The contents of oxides and carbon on the

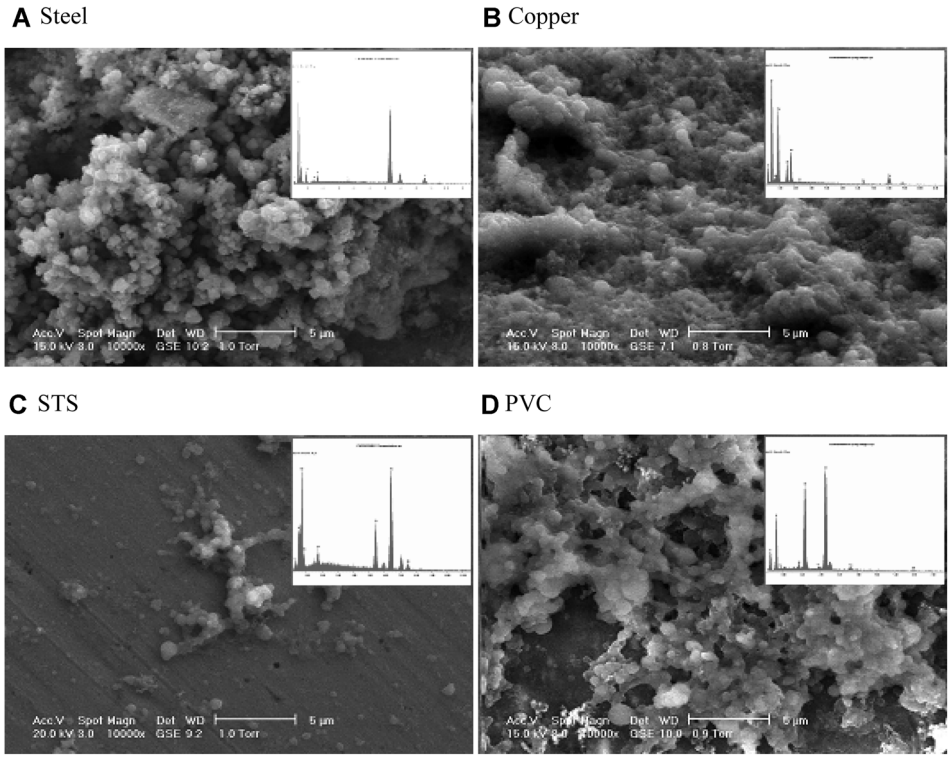

Fig. 7. Environmental scanning electron micrographs of biofilms.

STS coupon were significantly low compared with the other coupons.

In conclusion, biofilm formation and water quality were substantially affected by the pipe materials of the annular reactor in this study. The bacterial concentration and species diversity in the biofilms were much increased with corrosion of the pipe, which consequently deteriorated the water quality. The lower bacterial concentration, lower corrosion level, and better water quality in the STS coupon observed through this study suggests that stainless steel is the best material among the tested materials for drinking water pipes for the home in terms of sanitation and hygiene safety.

Acknowledgment

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