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Impacts of Biodegradable Organic Matter and Pipe Materials on Bacterial Multiplication in Water Supply Systems

YOON-JIN LEE
Department of Environmental Engineering, Cheongju University, 36, Naedok-dong, Sangdang-gu, Cheongju, Chungbuk, 360-764, South Korea
E-mail: yjlee@cju.ac.kr

The present study investigated the influence of biodegradable organic matter on the multiplication of suspended and attached bacteria in pipe systems, and compared the bacterial growth for different pipe materials: stainless steel, PVC, galvanized iron and copper with biodegradable organic matter. Higher turbidity in effluent was seen with an increase in the chlorine dose to control bacterial regrowth for the galvanized and copper pipes. High multiplication of the heterotrophic plate count (HPC) was observed for the stainless steel and PVC pipes with a dose of 3 mg/L glucose while the galvanized iron and copper pipes were not comparatively sensitive under the condition of a dose of 3 mg/L glucose. Under the condition of a dose of 3mg/L glucose only and measured by adenosine triphosphate (ATP), the highest biofilm formation potential was observed for the stainless steel pipe, followed by the PVC, copper and galvanized iron pipes in descending order. The highest alkalinity value and lowest turbidity were seen for the PVC pipe in selected pipes in effluent. SEM observation of the PVC pipe showed a rough surface with holes due to substances released from the pipe.

Key Words: Biodegradable organic matter, Adenosine triphosphate, Biofilm, Heterotrophic plate count, Pipe material.

INTRODUCTION

Bacterial growth is reported to cause a number of hygienic and esthetical problems within the water supply system[1-4]. The formation of biofilm on distribution pipes contributes to microbial regrowth, disinfectant decay and corrosion tubercles[5-7]. Applying disinfection is conventionally regarded an effective method to suppress biofilm formation and prevent bacterial regrowth in water supply systems[6]. Farooq *et al.*[8] reported that the level of microorganisms was inversely proportional to residual chlorine in the water distribution network of Rawalpindi, Pakistan. Olivieri *et al.*[9] revealed that the occurrence frequency of microorganisms decreased with the presence of residual disinfectant. Yoon and Lee[10] showed that the level of the heterotrophic plate count (HPC) increased at the end point of distribution systems in which the level of residual chlorine was low in Seoul, South Korea.

However, application of high levels of chlorine can cause an unpleasant taste and odour and produce mutagenic substances such as trihalomethanes (THMs)[6,11]. Water authorities confront the need to provide a supply of high-quality tap water that balances the increasing consumer desire for excellent drinking water with the need to adhere to regulations for disinfectant by-products. Thus, the method of nutrient removal required for microbial growth should be evaluated for application for controlling bacterial regrowth in water supply systems.

The limiting factor for bacterial growth in water supply is considered to be organic carbon or phosphorous[2,7,12-16]. Microbial growth is generally regarded to be limited by organic carbon, especially by the parts called biodegradable dissolved organic carbon (BDOC) and assimilable organic carbon (AOC). Also, in Finland and Japan, microbial growth has been regulated by the availability of phosphorus[2,3,14]. Betté *et al.*[17] observed that phosphorous does not affect the levels of bacteria in biofilm development in carbon limiting water. Anticipating the availability of a limiting nutrient for multiplication of microorganisms in drinking water and removing the limiting nutrient would be an effective method for suppressing bacterial regrowth in a water supply system.

The influence of pipe material on biofilm density has been investigated by a number of researchers. Niquette *et al.*[18] reported that pipe material has a strong influence on bacterial regrowth in distribution systems and recommended PVC instead of iron or cement pipe to control bacterial regrowth and pipe corrosion. However, Hellam *et al.*[19] observed biofilm potential ranked in lower order for materials of glass, cement, MDPE and PVC when residual chlorine was less than 0.3 mg/L. Van der Kooij *et al.*[20] concluded that biofilm formation on copper surfaces was similar to that on stainless steel. Lehtola *et al.*[3] observed that biofilm formed more slowly on copper pipes than on plastic pipes.

The objective of this study was to investigate the contribution of biodegradable organic matter (BOM) to the multiplication of bacteria in pipe systems under controlled laboratory conditions, and determine the impact of the different pipe materials (stainless steel, copper, PVC and galvanized iron pipes) and water quality variation in the density of attached and suspended biomass under the addition of only 3 mg/L of glucose and surface river water.

EXPERIMENTAL

Experimental equipment and sampling: Raw surface water was taken from the Han River (Seoul, Korea) by grab sampling and transported to the laboratory. Table-1 shows the microbiological and chemical characteristics of the Han River water used as the water source for this experiment. The feed sample for the biodegradable organic matter-only condition was prepared by the following method. First, 1,000 mg/L of stock solution of glucose was prepared by dissolving the glucose in de-ionized water. Next, the solution level was adjusted with deionized water to obtain a 3 mg/L level of glucose. Finally, 1 % of filtered raw surface river water with a Whatman membrane (pore size 2.5 μm) was inoculated in this glucose solution.

TABLE-1
WATER QUALITY CHARACTERISTICS OF HAN RIVER WATER

Parameter	Value
Heterotrophic plate count (CFU/mL)	7×10^3–5×10^4
Free chlorine (mg/L as Cl_2)	ND
pH	6.9-8.6
Dissolved organic carbon (mg/L as carbon)	1.4-2.8

For the study of bacterial growth on pipes and in effluent, copper, galvanized iron, stainless steel and PVC were chosen as the pipe materials. Each pipe was cut to a length of 30 cm for this experiment. The pipe was filled with the sample and then closed up with a rubber stopper and sealed with para-film. One set of pipe materials was exposed to raw surface water filtered through a membrane with 2.5 μm pores and the other set was exposed to the 3 mg/L glucose solution with micro-organisms brought from the Han river. These two pipe sets were placed in an incubator controlled at 20 ºC.

The samples for analyzing attached microorganisms on pipes were prepared by the following process. The four types of pipes were cut by a stainless steel cutter to the size of 1 cm. The amputated samples were treated by sonification for cleaning. The samples were next placed inside columns that were then exposed to the prepared samples. The pipe columns were located inside the incubator, and the sample was replaced every 3.5 days.

Analytical methods: Heterotrophic plate count (HPC) was determined by spreading 100 μL of the sample after proper decimal dilutions on the R2A medium (BD, USA). The colonies were counted after 7 days of incubation at 25 ºC. The analysis of the HPC attached on the pipes was determined as follows: Sterilized swabs (Fisher Scientific) were used to collect biofilm from the pipes at each reaction time. The swabs collecting biomass were placed in test tubes containing 5 mL of deionized water. After being mixed in a vortex stirrer (Vision Scientific, KMC-1300) for 1 min, the solution was inoculated on R2A medium to cultivate HPC bacteria.

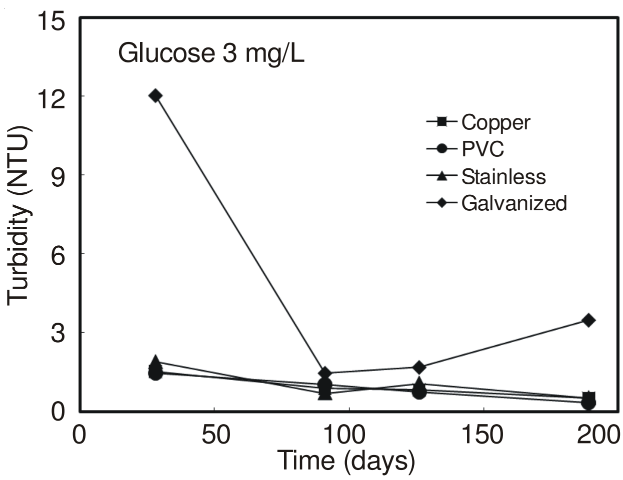
Reagent-grade sodium hypochlorite supplied by Junsei Chemical was used in this experiment. The initial level of sodium hypochlorite was determined immediately by the diethyl-*p*-phenylenediamine (DPD) procedure and stock solution as 1000 mg/L of sodium hypochlorite as free chlorine was produced. This was then diluted to lower concentrations that had been previously determined for these experiments. Residual chlorine concentrations were determined using the DPD procedure.

Adenosine triphosphate (ATP) was measured by AMSA lite III. The concentration of ATP is expressed by the relative light unit (RLU). Dissolved organic carbon (DOC) was measured by the TOC analyzer (TOC 5000, Shimadzu) with a mode of non-purgeable organic carbon after filtering through 0.45 μm membranes. The corrosion rate was analyzed by measuring the weight loss of the pipe samples between the initial weight and the weight at the reaction time. The samples were exposed for 280 days with the 3 mg/L of glucose solution after the measurement of the initial weight of the coupons and the surface area. The samples were separated at 280 days from the columns, deposits on the pipes eliminated by sterilized swabs, and the samples were washed by sonification for 0.5 h at 20 ºC. They were washed three times with distilled water, dried and the weight measured. The corrosion rate was presented as (mdd); (mg/dm^2)/d. The surface of the PVC pipe was observed with a scanning electron microscope (Leika stereo scan 440). Metals such as copper, zinc and iron were determined by inductively coupled plasma spectroscopy (Labtam 8440). Turbidity was measured by a Hach 2100 turbidity meter and was presented as NTU.

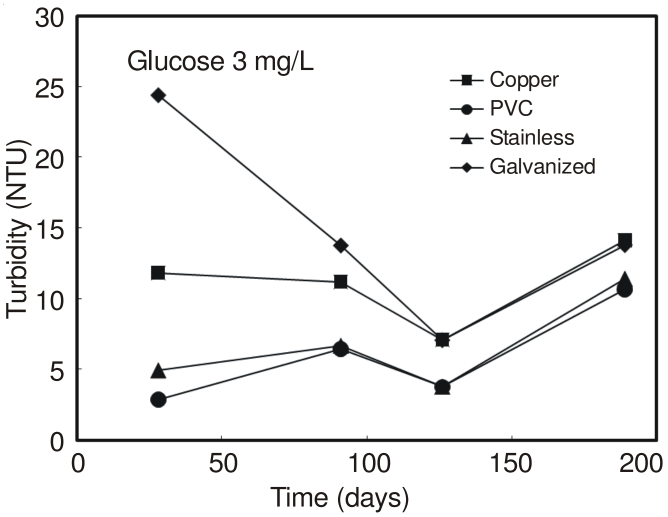
RESULTS AND DISCUSSION

Water quality variation: The variation in turbidity is presented in Fig. 1a and 1b for the biodegradable organic matter-supplemented and surface river water, respectively. More than 1 NTU of turbidity was detected at 28 days for the PVC, copper and stainless steel pipes. However, those levels decreased over time and the turbidity for the copper, PVC and stainless steel pipes was 0.50, 0.35 and 0.52 NTU at 189 days. The PVC pipe showed the lowest level of turbidity. Burying galvanized iron pipe for drinking water has been prohibited since 1994 in Korea. But pipes already exist and the ratio of galvanized pipes over service life is reported to be 63.5 %[21]. Kim *et al.*[22] reported that high levels of turbidity have been found in the initial operation time in the galvanized iron pipe and the cause was explained by zinc leaching from the pipe. This result indicates that old galvanized pipes in the service lines could arouse to heavy metal leaching and turbid water problems.

During present experiment, the highest amount of turbidity was released from the galvanized pipes at 28 days with surface river water and gradually decreased over time. A similar level of turbidity was seen from the galvanized iron and copper pipes with surface river water at 189 days.



(a) Glucose 3 mg/L



(b) Surface river water

Fig. 1. Turbidity in effluents for the supplied 3 mg/L glucose and surface river water

The variations in alkalinity are presented in Table-2 during the contact of two series of pipes with surface river water and the 3 mg/L glucose solution. The highest level of alkalinity was observed with the PVC pipe. The alkalinity levels for the copper, stainless steel and galvanized iron pipes were same with the BOM-supplimented water at 189 days. The alkalinity levels for the surface river water were observed to be highest for the PVC pipe, followed by the stainless steel, copper and galvanized iron pipes, in descending order of alkalinity. The alkalinity level for the galvanized pipe was the lowest of the four pipe types before 91 days with surface river water. However, the value increased after 91 days.

TABLE-2
VARIATIONS IN ALKALINITY FOR BOM ADDED WITH 3mg/L GLUCOSE AND SURFACE RIVER WATER (mg/L as $CaCO_3$)

Reaction time	Glucose 3 mg/L				Surface river water			
	Copper	PVC	Stainless steel	Galvanized	Copper	PVC	Stainless steel	Galvanized
28	8.0	15.0	6.0	12.0	51.0	60.0	58.0	41.0
56	6.3	15.0	4.3	7.5	60.0	63.0	60.0	46.3
91	7.5	8.8	6.3	6.3	50.0	56.3	53.8	45.0
126	11.3	10.0	10.0	9.4	56.3	65.0	61.3	48.8
189	5.0	6.3	5.0	5.0	48.8	53.8	52.5	53.8

Corrosion rate and metal release: The corrosion rate is shown in Fig. 2 for the different pipe materials with the glucose dose of 3 mg/L at 280 days. The segment of PVC pipe that was measured for the corrosion rate did not show significant weight difference compared to the initial weight. So, this pipe material is not discussed

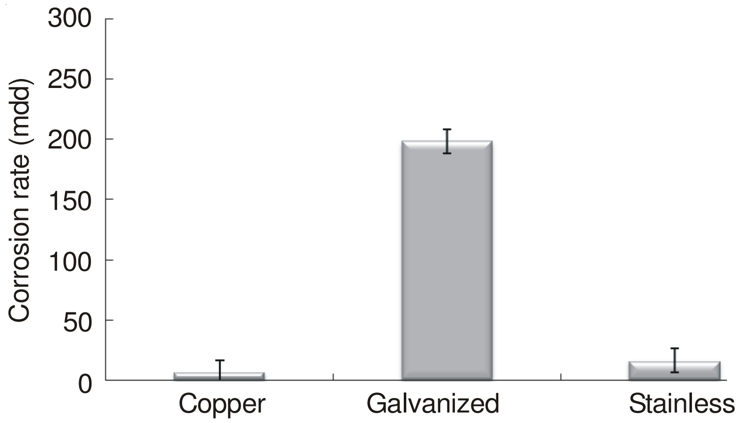


Fig. 2. Corrosion rate for copper, galvanized iron and stainless steel pipe with the glucose dose of 3 mg/L

here. The corrosion rate for stainless steel has generally been reported to be low compared with the other metal material pipes. However, the corrosion rate of the stainless steel pipe in the 3 mg/L glucose condition was 2.5 times higher than that of the copper pipe. Park *et al.*[23] reported that acetate was a carbon source that could increase the activity of microorganisms and glucose was a more influential substance than humic acid for the corrosion rate of iron. The corrosion rate for the galvanized iron pipe was 31 times higher than that of the copper pipe and was the highest of the selected pipes.

The variation in metals leaching from the pipes in effluent was evaluated for the galvanized iron, copper and stainless steel pipes and the results are shown in Table-2. About 0.10 mg/L concentration of iron was released during this experiment period from the galvanized iron pipe with the surface river water and a high concentration of zinc was released during the initial 56 days. The level of iron was found to be *ca.* 0.16 mg/L during this experiment period in the galvanized iron pipe with the 3 mg/L glucose solution. The largest amount of zinc was released at 28 days and the mean value of zinc after 28 days was 2.6 mg/L.

TABLE-3
METAL LEACHING IN PIPES FOR BOM ADDED WITH 3 mg/L GLUCOSE AND SURFACE RIVER WATER (mg/L)

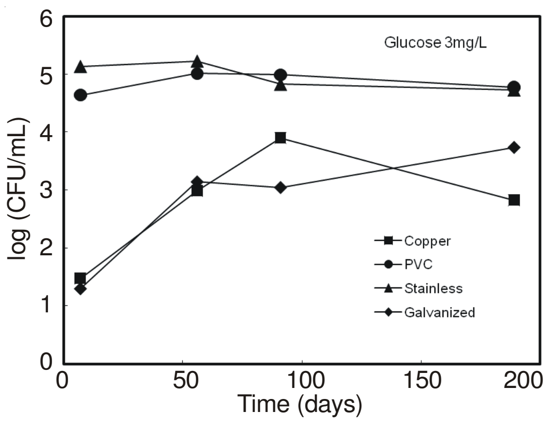
Reaction time	Glucose 3 mg/L				Surface river water			
	Galvanized pipe		Copper pipe		Galvanized pipe		Copper pipe	
	Zn	Fe	Cu	Fe	Zn	Fe	Cu	Fe
28	28.9	0.20	1.54	0.15	40.3	0.20	2.03	0.28
56	3.06	0.11	1.02	0.23	18.7	0.10	1.41	0.25
91	2.34	0.24	0.30	0.04	5.83	0.07	1.88	0.09
126	2.23	0.21	0.97	0.11	0.73	0.09	2.14	0.24
189	2.92	0.05	0.68	0.07	5.01	0.11	2.42	0.37

The mean level of copper was 0.9 mg/L with 3 mg/L glucose. An insignificant level of metal substances was observed from the stainless steel and PVC pipes during this experiment (data not shown). Stabilizer substances such as lead, calcium, cadmium and organotin are used to control the decomposition of unplasticized PVC[24]. Some research has revealed concerns about the migration of these materials from PVC pipes into water[24-26]. So, there is a possibility of detecting significant levels of metals under extended experimental terms, according to these reports and future research on old PVC service lines is required.

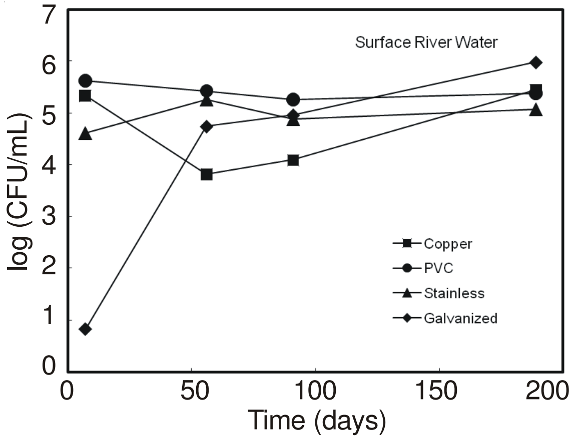
Evaluation of microbial regrowth: The variation in the microorganism level for surface river water and biodegradable organic matter-only conditions was monitored to investigate the influence of biodegradable organic matter as a factor limiting bacterial growth in pipe systems. The behaviour of suspended microorganisms is shown in Fig. 3 for the copper, stainless, PVC and galvanized iron pipes. The level of heterotrophic plate count during the initial 7 days was low in the galvanized steel pipe compared with the other types of pipe. The level of heterotrophic plate count for river water was shown to have the highest value at 189 days.

The number of heterotrophic plate count in effluents in the copper pipe reached the highest level at 91 days for the condition of the supply of glucose water only and then declined at 189 days. The highest heterotrophic plate count level was 2.8 $\times 10^5$ and 8.0×10^3 CFU/mL for the condition of surface river water and the addition of 3 mg/L glucose in the copper pipes.

The growth of suspended heterotrophic plate count reached the highest value at 56 days for the river water in the stainless steel pipe. In regard to the 3 mg/L of glucose dissolved in water, the highest level of heterotrophic plate count was similar to that of the surface water. The initial growth rate was fast for the PVC pipe. The HPC variation with time in the PVC pipe is shown to be similar for both conditions. Lu and Chu[27] reported that assimilable organic carbon content increased due to the release of assimilable organic carbon from plasticized PVC pipe in the distribution system.



(a) Glucose 3 mg/L



(b) Surface river water

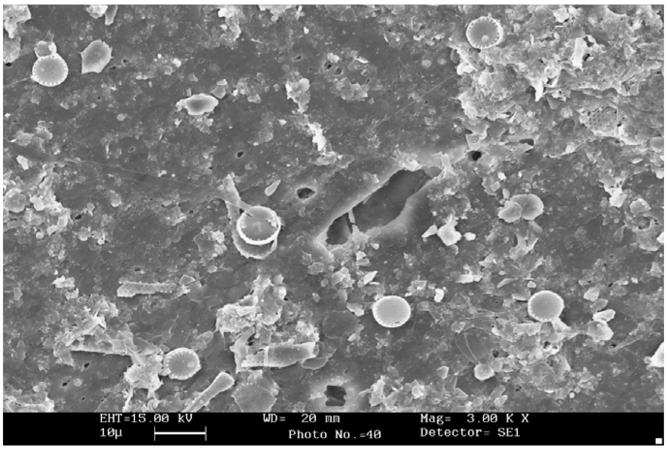
Fig. 3. Changes in the HPC level in the 3 mg/L glucose solution and Han river water from copper, PVC, stainless steel, and galvanized iron pipes

The increase in heterotrophic plate count was observed over time until 100 days for the copper and galvanized iron pipes, but the stainless steel and PVC pipes did not show a big change with the passage of time after 7 days under the 3 mg/L glucose condition. The highest number of microorganisms was observed with the PVC pipe followed by the stainless steel, galvanized, and copper pipes in descending order and the microbial number difference between the PVC and stainless steel pipes and between the copper and galvanized iron pipes was not immense. The low microbial multiplication observed initially for the galvanized iron and copper pipes was thought to be caused by metal leaching from pipes[22].

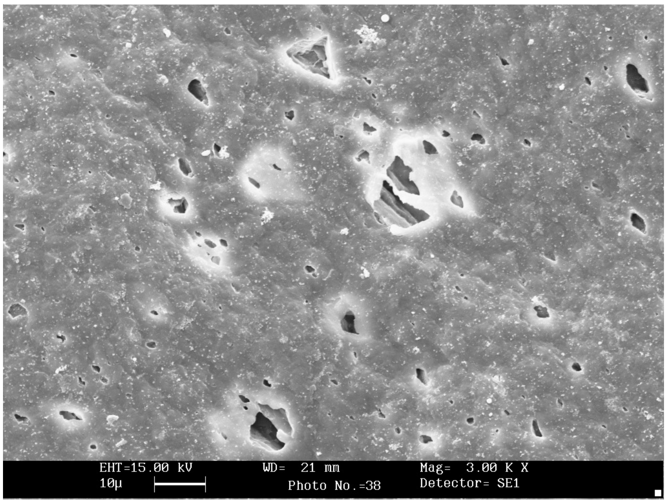
The glucose-only supplemented condition was shown to promote high multiplication in the PVC and stainless steel pipes, while microbial multiplication was not sensitive to the increase in biodegradable organic matter for the metal-based pipes, the copper and galvanized pipes. From this result, it appeared that the pipe metal concentration released from pipes might significantly affect microbial multiplication in pipe systems. Bacterial limitation might be related to the heavy metal substances discharged from pipes for the case of metal-based pipes such as copper and galvanized iron.

SEM photos of surfaces on PVC pipes in contact with surface river water and tap water with 3 mg/L of chlorine after 322 days of exposure are shown in Fig. 4. The surface of the PVC pipes was detected to be hollow in various places. This might be caused by the release of plastic substances with the contact of the samples.

Most often, application methods for controlling microbial regrowth in pipes are regarded as methods that maintain residual chlorine in the water distribution system. The concentration of chlorine is regulated to be less than 4 mg/L in Korea. Previous research reported that control of preformed attached microorganisms was difficult due to the chlorine-only application[22]. The variation of turbidity in effluents



(a)



(b)

Fig. 4. SEM photographs for PVC samples with surface river (a) and tap water after treatment of 3 mg/L chlorine (b)

was observed with tap water for the application of chlorine to suppress microbial water pollution (Fig. 5). Turbidity in the galvanized iron and copper pipes increased as the dose of chlorine increased. Turbidity for the 3 mg/L dose was 1.9 times higher than that of the 1 mg/L dose in galvanized pipe. Turbidity for the galvanized iron pipe was more than 20 NTU, which was the highest value during the operation time with the 3 mg/L chlorine at 56 days. Turbidity was increased to a small extent with the dose of chlorine for the PVC. This result indicated the possibility that tap water could contain high turbidity with long term doses of chlorine to control microbial regrowth in distribution systems and especially, the symptoms would be prominent for the galvanized pipe.

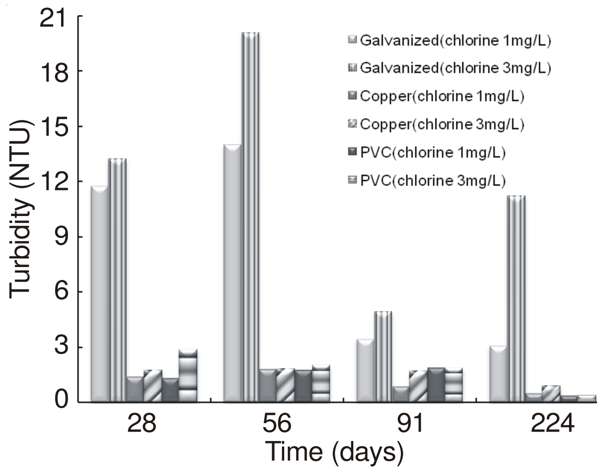
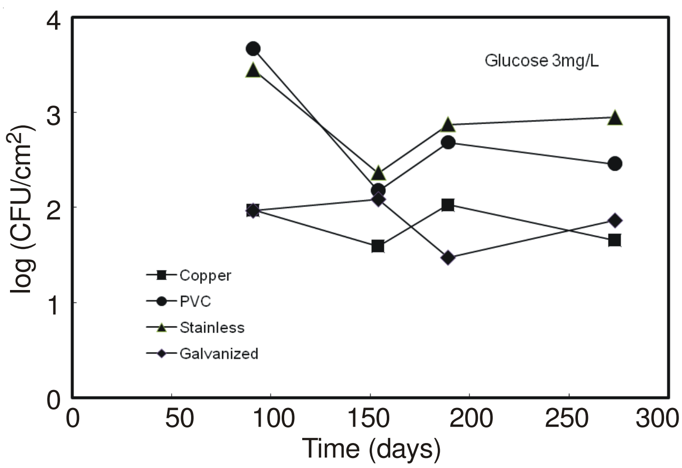


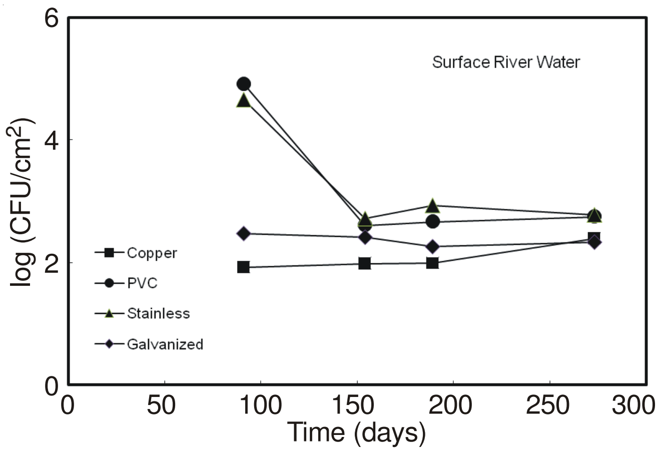
Fig. 5. Turbidity variation in effluents after application of 1 and 3 mg/L of chlorine in copper, galvanized iron and PVC pipes

Attached microorganisms reached the maximum level at 91 days for the stainless steel and PVC pipes (Fig. 6). A high density of initial biofilm development was observed in the PVC and stainless steel pipes under the 3 mg/L glucose condition. Colonization in copper and galvanized pipes for the biodegradable organic matter-only condition was lower than that of the PVC and stainless steel pipes.

The level of heterotrophic plate count attached on the galvanized iron pipe was at the highest value at 91 days with the surface river water and did not change remarkably with time. The growth of heterotrophic plate count attached on the copper pipe with the surface water was low initially but increased with the passage of time. The biofilm densities on the PVC and stainless steel pipes after 154 days did not vary much and remained at similar levels.



(a) Glucose 3 mg/L

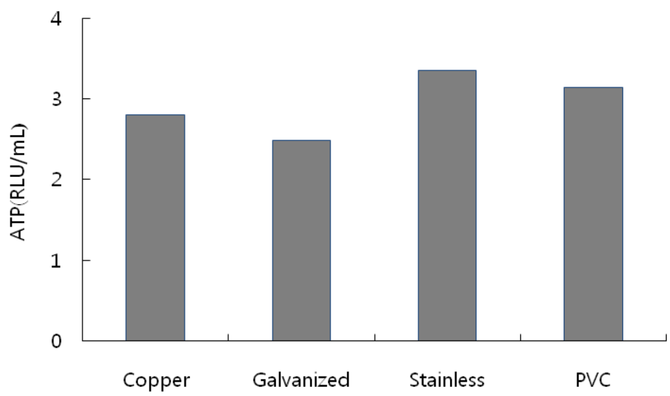


(b) Surface river water

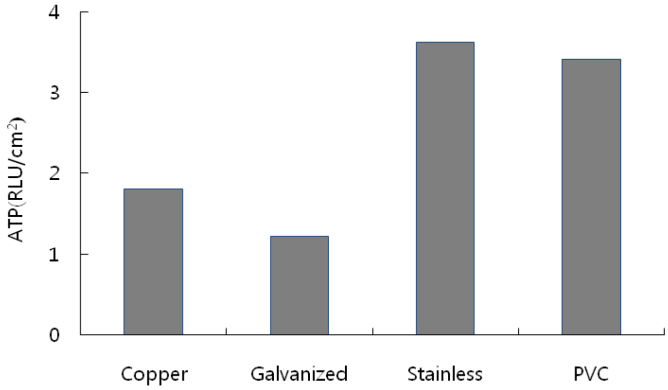
Fig. 6. Level variations of attached heterotrophic plate count in galvanized iron, copper, stainless steel and PVC pipe over time

The highest level of attached heterotrophic plate count during the contact time was 2.5×10^2, 3.0×10^2, 4.6×10^4 and 8.2×10^4 CFU/cm^2 for copper, galvanized iron, stainless steel, and PVC pipes, respectively, with surface river water. The highest number of heterotrophic plate count attached on the copper, galvanized iron, stainless steel and PVC pipes was 1.1×10^2, 1.2×10^2, 2.8×10^3 and 4.7×10^3 CFU/cm^2, respectively, with 3 mg/L of glucose.

The microbial regrowth potential for different kinds of pipes was evaluated by ATP level at 322 days for the 3 mg/L glucose condition, as shown in Fig. 7. The microbial regrowth potential measured by ATP in effluent did not show a big difference for the stainless steel and PVC pipes with the biodegradable organic matter-only supplemented. The highest level of ATP was observed in the stainless steel pipe and the lowest was for the galvanized iron pipes with 3 mg/L of glucose.



(a)



(b)

Fig. 7. Level of ATP in effluents from each pipe (a) and from biofilm on each pipe (b) with the glucose dose of 3 mg/L

Biofilm growth potential on the pipe was registered at the higher order for stainless steel, PVC, copper and galvanized iron with the 3 mg/L glucose condition, as observed by ATP measurement. The ATP level on the stainless steel and PVC pipes was 3.0 and 2.8 times higher than that of the galvanized pipe with contact with glucose-only supplemented water. This result indicated the high density of biofilm formation potential for stainless steel and PVC pipes in water with high levels of biodegradable organic matter.

Carbon is generally considered the limitation factor for bacterial growth in water distribution systems. O'Connor *et al.*[28] reported that bacterial growth was correlated with the level of dissolved organic carbon in water. In this experiment, the mean value of dissolved organic carbon was 2.74, 2.39, 2.28 and 2.57 mg/L with the Han river water during the experiment for the copper, galvanized, stainless steel and PVC pipes, respectively. The dissolved organic carbon value did not closely correspond to the level of heterotrophic plate count attached on pipes during the experiment. Piriou *et al.*[29] reported the actual contribution of bacterial growth was by the portion of biodegradable matter represented as AOC and biodegradable dissolved organic carbon. Previous research showed that the level of biodegradable dissolved organic carbon increased in tap water after it was treated at a high pressure and temperature[30]. The level of heterotrophic plate count on the copper and galvanized iron pipes was comparatively lower than the other two kinds of pipe. The multiplication of microorganisms seemed to be more affected by the release of metals rather than that of organic matter for the copper and galvanized pipes.

Conclusion

This research was performed to evaluate the contribution of organic nutrients to the multiplication of suspended bacteria and development of biofilm and to compare the level of fixed and suspended bacteria on different pipe materials. The following conclusions can be drawn from this study:

The multiplication of heterotrophic plate count in PVC and stainless steel pipe was 89 and 80 times higher, respectively, in effluent compared to that of copper pipe with 3 mg/L glucose at 189 days. Stainless steel pipe had the highest biofilm potential, followed by PVC, copper and galvanized pipe, in descending order, with a supply of 3 mg/L glucose. The biofilm development in stainless steel and PVC pipe might be affected by the condition of water containing 3 mg/L glucose in contrast to those of copper and galvanized iron pipe, which seemed to be more affected by the leaching of metals. A higher level of turbidity was detected with the increase in the chlorine dose for the galvanized iron and copper pipes. Therefore, strategies for controlling microbial regrowth effectively have to prepare for the consideration of pipe materials.

The highest alkalinity value and lowest turbidity was seen for the PVC pipe in selected pipes in effluent. Rough surfaces and holes thought to be caused by the migration of pipe material were observed on the PVC pipe in the SEM image. Migration of pipe substance and heat stabilizer and high multiplication of micro-organisms from real unplasticized PVC service lines during long-term periods should be monitored in future research. In addition, the possibility was seen for a high rate of microbial growth for facilities made from PVC such as water tanks for the high multiplication of heterotrophic plate count in PVC pipe in this experiment. Therefore, that should also be considered while managing the water supply.

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