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Investigation of Nutrients Controlling Bacterial Regrowth in a PEX and Stainless Steel Plumbing Water System

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The growth of suspended microorganisms and biofilm development, as measured by heterotrophic plate count bacteria in PEX and stainless steel pipes, which are often used in the hot water system in Korea are investigated. There was no significant difference in the level of attached microorganisms from variations in water temperature on PEX and stainless steel pipes. However, the numbers of suspended microorganisms were higher at 55 ºC compared to 20 ºC in effluents from the PEX pipe. In order to understand the contribution of organic and inorganic nutrients to biofilm development, bacteria grown in tap water without supplements on PEX and stainless steel pipes was compared to bacteria grown with added acetate, phosphate or mixed inorganics and acetate. Bacterial multiplication in the PEX pipe was appreciably higher when the mixed inorganic cocktail and acetate were added. The density of attached microbes increased with the addition of phosphorus for both pipes. The influence of nutrients was comparatively higher in the PEX pipe with hot tap water. The application of chlorine was not found to be effective for controlling bacterial growth in high temperature tap water in PEX pipe and the level of UV-254 and dissolved organic carbon in the effluent increased after hot water stagnation.

Key Words: Biofilm, Drinking water, Plumbing system, Stainless steel, PEX, Bacterial regrowth, Limiting nutrient.

INTRODUCTION

The numbers of bacteria on a heterotrophic plate count (HPC) is regulated by the Korean standard for drinking water in order to supply sanitary tap water to the consumer[1], to protect the public health and to assess the operational process in a water plant or the microbial safety of water distribution systems. All HPC microbes are not pathogenic, but the HPC can be used as a good indicator for the evaluation of appropriate management for the water disinfection process and water supply system. Huck and Gagnon[2] suggested the concept of the HPC production index

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(HPI), which relates the HPC to various physical, chemical and water quality factors for management in distribution systems. They regarded the distribution system as a bioreactor in which managing HPC levels often means minimizing bioreactor efficiency[2]. The numbers of HPC may change over time after chlorination and on the point of devices or facilities in a distribution system[3]. Therefore, understanding of microbial behaviour in response to different influential factors is important for managing microbial water quality in distribution systems.

An increase in the HPC has been observed over time in relation to distance from the water treatment plant[3,4]. This microbial increase has been termed bacterial regrowth and is becoming a major problem in the water distribution system. An examination of HPCs from two network structures in Korea has shown counts to be relatively high nearer the end point of the water distribution systems[3]. An extended retention time was found in distribution system structures, such as the water tank in the home, resulting in the deterioration of water quality due to bacteria. However, Kerneïs *et al.*[4] in a survey of 99 water samples, did not find a correlation between HPC density and travel water in pipes. Mamba and Kaleni[5] reported that storage of drinking water for 48 h results in the regrowth of total coliform and emphasized the importance of hygienic management of household containers.

Pathogenic and opportunistic pathogens, such as *Pseudomonas, Klebsiella* and *Mycobacteria*, have been isolated in biofilms[6,7] and Kühn *et al.*[8] found that *Aeromonas* strains, which are regarded as potentially pathogenic, can persist for several months in drinking water. Pathogens entering through untreated water or through any point in the distribution system may survive and grow on the surface of pipes and other structures, such as water tanks. In addition, detachment from biofilms may contribute to water quality deterioration[9].

The growth of biofilms on pipes can cause the proliferation of organisms in finished water and cause tap water quality deterioration, from turbidity, taste or odour and biocorrosion of pipe materials. The attaching microorganisms on the pipe surface are generally known to have relatively high resistance against disinfectants. The development of biofilms has been related to various factors, such as water temperature, residual disinfectants, water velocity and pipe materials[10]. Volk and LeChevallier[11] reported biofilm formation is limited by nutritional level, corrosion and disinfection.

Many factors influencing bacterial regrowth in distribution systems have been evaluated, including the concentration of biodegradable organic matter, residual disinfectants, temperature conditions, pipe corrosion and pipe material[9,12-17]. Bacterial proliferation is promoted with favourable conditions of organic carbon and other nutrients, such as phosphorus and nitrogen. Conventionally, the concentration of biodegradable organic matter (BOM) entering the distribution system is reported to be limited microbial regrowth. In Finland and Japan bacterial growth has reported to be limited by phosphorus and Volk and LeChevallier[11] found that removing nutrients after biological filtration treatment results in lower biofilm densities. Lund and

Ormerod reported that 0.05 mg/L of chlorine application can control sludge production in a test system[18]. However, McFeters *et al.*[19] reported that coliform bacteria can survive with the standard chlorine residual in distribution systems. In addition, the attachment bacteria on pipe materials has been shown to increase bacterial resistance to chlorine, supporting the suggestion that disinfection alone is not sufficient to control biofilm development and reducing organic carbon in the distribution system is also necessary[20].

For the bacterial management in the water distribution system, methods for chlorine residual maintenance and elimination of limiting nutrients are currently being applied[20]. Organic carbon is regarded as the most important factor for controlling the activity of microorganisms[21-23]. But, all organic carbon existing in water is not used by microorganisms. Assimilable organic carbon (AOC) or biodegradable dissolved organic carbon (BDOC) is conventionally regarded as the most important limiting factor in water supply systems. The term assimilable organic carbon (AOC) refers to the carbonaceous compounds that are quickly utilized for microorganism growth[24] and AOC is regarded as an important factor for maintaining the stability of micro-organisms in distribution systems. The treatment with an oxidant is reported to affect the structure of natural organic matter (NOM) and area of low molecular weight (LMW) by breakdown from oxidation. Chien *et al.*[25] found a correlation between AOC and UV-254 and noted that AOC levels could increase by post chlorination. Reducing levels of AOC to under 50 μg/L for controlling coliform bacteria in drinking water has been suggested[10] and granular activated carbon (GAC) filtration was shown to be useful for decreasing the level of AOC in drinking water[26]. Assimilable organic carbon fluctuations are reported to be affected by seasonal variations[22]. The process of coagulation is effective for removing DOC and BDOC[11].

The availability of phosphorus has been found to limit bacterial regrowth in Japan, Norway and Finland[27,28]. Miettinen *et al.*[29] suggested that the application of phosphate to control corrosion can cause microbial problems in boreal regions. Chu *et al.*[30] found that the addition of 0.5 mg N/L of ammonium or above 0.01 mg P/L of phosphate resulted in significant effects to biofilm development. Lehtola *et al.*[31] concluded that microbial nutrition, such as microbially available phosphorus (MAP) and AOC, decreased after chemical coagulation treatments and GAC but increased by ozonation.

The objective of the present study is to improve present understanding of bacterial regrowth on different organic and inorganic compounds in a hot water supply system. The limiting factor was evaluated in hot tap water samples supplied from PEX and stainless steel pipes and the behaviour of suspended growth and fixed bacterial development on different pipe materials was compared with respect to temperature. The effectiveness of possible treatment regimes (UV irradiation and membrane filtration) was investigated for inhibiting bacterial regrowth as well as the availability of commonly used chlorine application.

EXPERIMENTAL

Feed water: The investigation to evaluate the variation in bacterial growth on PEX and stainless steel (SS) pipes was carried out in the engineering department of Konkuk University, Seoul, Korea. Warm tap water was allowed to run from the pipe for 2-3 min before sampling and was then transferred to the laboratory. The tap water had been conventionally treated by chemical coagulation with polyaluminum chloride (PAC), sedimentation, filtration and disinfection with free chlorine at the Guui water treatment plant. The tap water quality is shown in Table-1. Residual chlorine was almost depleted in the warm water and the initial adenosine triphosphate (ATP) value of the warm water was higher than that of the cold water.

TABLE-1
TAP WATER QUALITY USED IN THIS STUDY

Items	Cold water	Warm water
DOC (mg L^{-1})	1.5 ± 0.1	1.4 ± 0.1
Turbidity (NTU)	< 0.5	< 0.5
pH	7.1 ± 0.05	7.5 ± 0.05
Conductivity (μS cm^{-1})	183 ± 5	183 ± 5
Residual chlorine (mg L^{-1})	<0.3	ND
UV-254	0.009 ± 0.001	0.011 ± 0.001
ATP (RLU mL^{-1})	145	702
DO (mg L^{-1})	10 ± 2.5	10 ± 25

Preparation of nutrients: Sodium acetate and potassium dihydrogen phosphate were used as sources of C and P, respectively. Inorganic cocktails were mixed that contained[27] KNO_3, KH_2PO_4, Na_2SO_4, $CaCl_2·2H_2O$, $MgCl_2·2H_2O$, $FeCl_3·6H_2O$, $CoCl_2·6H_2O$, $CuCl_2·2H_2O$, $MnSO_4·5H_2O$, $FeCl_3·6H_2O$, $CoCl_2·6H_2O$, $MnSO_4·5H_2O$, $ZnCl_2$, $(NH_4)_6Mo_7O_{24}H_2O$. A stock solution (500 mg/L) was initially prepared by dissolving sodium acetate, potassium dihydrogen phosphate and inorganic nutrient cocktail in deionized water in glass bottles. Each solution was autoclaved for 0.5 h at 121 °C after being capped with a plastic stopper. The solution was diluted with sterile deionzed water to acquire the required concentration of acetate, phosphate and inorganic nutrient cocktail.

Pipe system and experimental design: PEX and SS were selected in this experiment for the pipe material because these are used in hot water plumbing. The pipe coupons for the analysis of biofilm were amputated to 71 mm with a stainless steel cutter and washed with a sonificator. The pipe units consisted of eight pieces of coupons, with an internal diameter of 16 mm (PEX) and 17 mm (SS). Each pipe section was used for sampling biofilm accumulation. The water temperature was set at 20 and 55 ºC in equipment with constant temperature. The samples were changed every 7 days. After the reaction time was reached, water samples and pipe coupons were withdrawn for microbial and chemical analysis.

The first experiment for evaluating the effect of temperature on HPC growth consisted of four pipes with a combination of two temperatures (20, 55 ºC), with no supplements and two pipe materials (PEX, SS). Suspended and attached microorganisms were determined at 15, 30, 60 and 90 days. In the second experiment, biofilm development was studied by adding different nutrient sources into pipes. Bacterial growth was monitored at the chosen nutrients conditions which fed sodium acetate, potassium dihydrogen phosphate and a mixture of inorganics in PEX and stainless steel pipes. In the third experiment, bacterial stability in the pipes was monitored with chlorination, UV irradiation and membrane filtration (Fig. 1). Chlorinated water was produced with sodium hypochlorite; UV disinfected water was produced by irradiation with a UV lamp (Sankyo Denki, model G3078), which was cleaned prior to the startup of each trial with 99 % ethanol solution. Filtration was performed through a 0.45-μm membrane (Millipore).

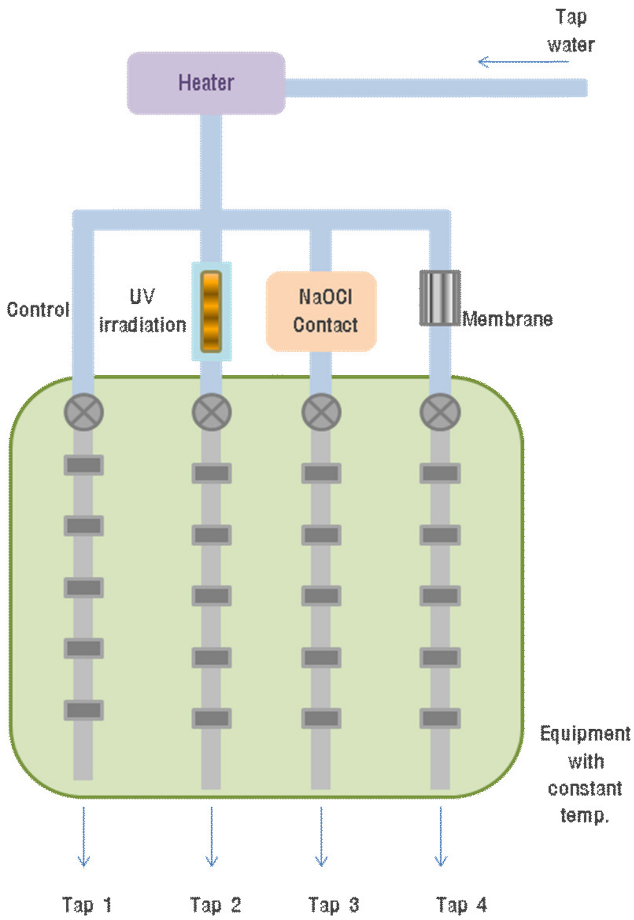

Fig. 1. Schematic diagram of the experimental system. Tap 1: control, Tap 2 : UV irradiation, Tap 3: chlorination, Tap 4: membrane filtration

Analytical method: Chemical analysis: All analyses were based on standard methods for the examination of water wastewater[32] and standard methods for drinking water in Korea[33]. Dissolved organic carbon (DOC) was analyzed using a TOC analyzer (GE Sievers, 5310 C laboratory) with a Sivers 900 autosampler after filtering with a 0.45 μm pore size. Residual chlorine was determined at the time of collection, using the N,N-diethyl-*p*-phenylenediamine (DPD) procedure. Turbidity was measured with a Hach turbidity meter (Hach 2100A). UV-254 was determined by using a UV visible spectrophotometer (Shimadzu, UV-1601) with a 10 mm cell at 254 nm.

Microbiological analysis: Heterotrophic bacteria were enumerated by the spread plate method[32] with R_2A agar. Plates were inoculated with 0.1 mL of each sample and were incubated at 20 ºC for 7 days. All bacterial counts were performed in triplicate. Attached microorganisms on pipes were determined as follows: Deionized water was sterilized in the autoclave and 5 mL aliquoted into tubes. Biomass was removed from the pipe surface by sterilized swabs, which were then placed into the prepared test tubes and mixed with a vortex mixer for 2 min (Vision Scientific, KMC-1300). The prepared samples were then inoculated on R2A media. Colonies were counted and expressed as colony forming units per cm^2 and colony forming units per mL. Adenosine triphosphate (ATP) level was analyzed by using an ATP analyzer (AMSA lite II).

RESULTS AND DISCUSSION

Effect of temperature on microbial growth: PEX pipe is often used domestically in hot plumbing systems because of its good flexibility and relatively inexpensive price. Investigating variations in microbial characteristics of pipes with a high water temperature is necessary to ensure biologically stable conditions in water distribution systems.

Growth of suspended microorganisms in effluents at 20 and 55 ºC over time was shown in Fig. 2. After the first 15 days, the number of suspended microorganisms at 55 ºC was 61.8 times higher than that at 20 ºC in the PEX pipe and the highest count was 6.20×10^4 CFU/mL, which is 620 times the drinking water standard in Korea. Even though hot water is not normally used for drinking, it can still affect the public by contact through oral means, skin or inhalation during daily tap water usage. Temperature is known to be a crucial factor in the growth of microorganisms in treated water. Hot tap water might have different microbial and chemical characteristics from cold water because hot water passes through additional facilities and/ or processes, such as hot water tanks or heating systems, before reaching the consumer's tap. Hot water may also have higher bacterial levels because of the longer stagnation time and various pollution sources in the hot water distribution system. Higher bacterial levels result a higher risk to unspecified individuals from possible contamination, such as outbreaks of *Legionellosis* from the central supply. This can also affect the durability of the water facility and sanitary conditions of the hot water supply in the long term.

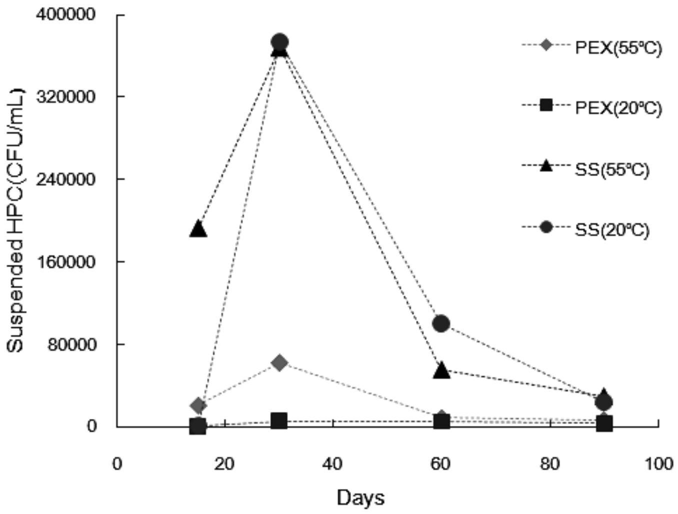

Fig. 2. Effect of temperature on suspended bacterial growth in stainless steel and PEX pipes

The suspended HPC at 55 ºC was still higher than that at 20 ºC after 60 days for the PEX pipe, but the difference between two temperatures was no greater than at 30 days. Hot tap water is expected to be stagnant in the hot water supply system and show a variation in temperature between the hot water tank and the tap. Bagh *et al.*[34] reported that a high level of bacteria is found on the surfaces of the sacrificial aluminum anodes and the heating coils in the hot water tank and the hot water tank supports a great diversity of microorganism because of temperature stratifications, sedimentation of aluminum flocks and organic matter. User guidelines for sanitary management of hot water pipes that are different from those for cold water pipes should be supplied to the consumer. In addition a safety investigation of the use of plastics pipes plumbed for hot water supply should be provided.

It is already found that the level of BDOC increased after treatment at a high temperature (121 ºC) and high pressure (1.50 kg/cm^2) for 20 min[35]. Herson *et al.*[36] reported the level of *Enterobacter cloacae* increased in autoclaved filtrates of particle water and noted that autoclaving should serve the dual purpose of releasing absorbed nutrients from the particles and lysing attached indigenous cells. Hot water also goes through a similar process because it is subjected to high temperature and pressure in the hot water system during heating. This suggests that biological availability might increase during hot water distribution.

The growth of microorganisms in effluents at 20 ºC was shown to be stable compared to 55 ºC for the PEX pipes. Niquette *et al.*[37] showed that the water source is an important factor at water temperatures over 15 ºC and microbial growth was higher in surface water than in ground water or mixed surface and groundwater.

The number of HPC at 55 ºC was measured to be 568 times higher than at 20 ºC during the initial 15 days in SS pipe. The level of HPC registered 2.35×10^4 and 2.95×10^4 CFU/mL for 20 and 55 ºC, respectively, at 90 days. Previous research reported bacterial growth to be similar at 30 and 40 ºC for SS pipe[38]. van der Kooij *et al.*[39] found the HPC was 1.6×10^3 and 4.9×10^3 CFU/mL for SS and PEX pipes, respectively, in model warm water systems during recirculation of tap water at 25-35 ºC. However, a higher number of HPC in effluents from SS pipes was detected compared to PEX pipes in this experiment.

The attached HPC did not exceed 3.5×10^4 on PEX and SS pipes during this experiment and the growth of attached bacteria was similar on the PEX and SS pipes at 20 and 55 ºC (Fig. 3). The counts of attached microorganisms at 90 days were 5.0×10^3 and 3×10^3 CFU/cm^2 for 20 and 55 ºC, respectively, on PEX pipes and 4.0×10^3 and 2×10^3 CFU/cm^2 for 20 and 55 ºC, respectively, on SS pipes. In ozonated water, biofilm formation on PE and SS were found to be quite similar[40]. Water temperature is reported to be strongly related to the attached microbial population density for the service area of the Philadelphia Suburban Water Company (PSWA)[41]. Different pipe materials may affect the formation of biofilm on water distribution pipes. Niquette *et al.*[42] reported that less fixed biomass was detected on plastic-based materials, such as PE and PVC, than iron and cement-based pipe in a distribution system.

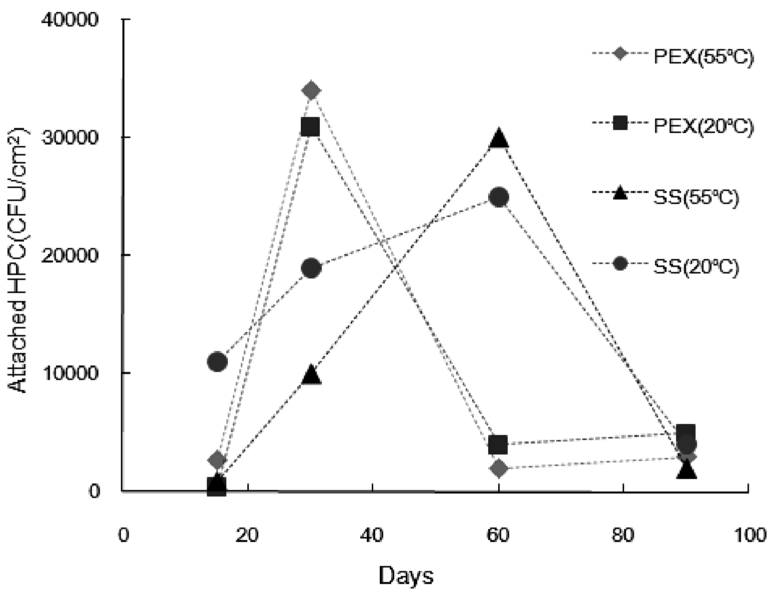

Fig. 3. Effect of temperature on attached bacterial growth in stainless steel and PEX pipes

Variation of bacterial growth on pipes with nutrient additive: The growth of attached microorganism on PEX pipes as a result of nutrient variations in tap water is presented in Fig. 4. The number of attached microorganisms on PEX pipes was similar at 30 days for the addition of 1 mg/L acetate and control (tap water without any addition). However, the level of attached microorganisms with 1 mg/L

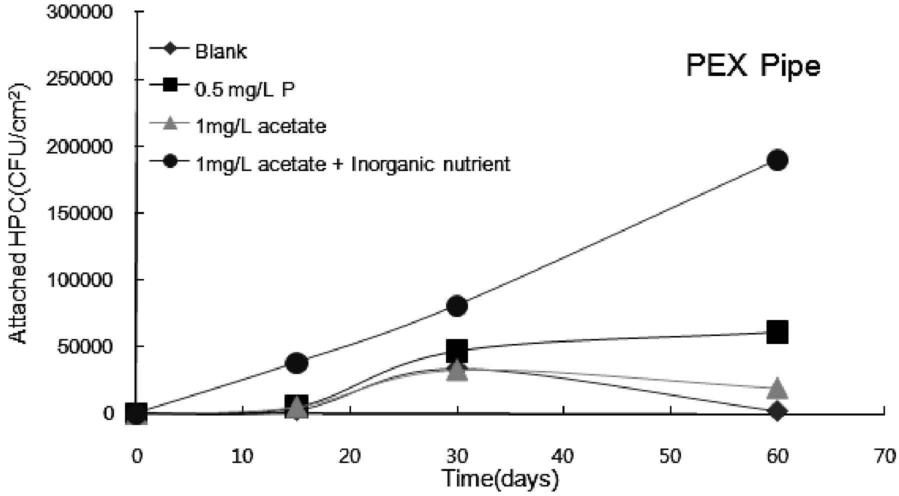

Fig. 4. Variations in attached microorganisms for the different combinations of nutrients in PEX pipe

acetate was 9.8 times that of the control at 60 days. Lu and Chu[21] showed that the AOC concentration in water samples contributes to bacterial regrowth, with a maximum biofilm HPC of 2.9×10^7 CFU/cm^2 for a 50 μg/L acetate-supplemented condition in a PVC pipe system.

In this experiment, the attached bacteria number was higher with the addition of phosphorus and acetate than that of the control in PEX pipes. But, a remarkable multiplication of attached microorganisms was shown with the addition of 1 mg/L acetate and inorganic nutrient cocktail. This bacterial growth in the tap water sample from the PEX pipes was stimulated by inorganic nutrients. The multiplication of HPC was linearly related with time for the addition of 1 mg/L acetate and inorganic nutrient cocktail.

The variation in the level of attached microorganisms on SS pipes is shown with the addition of different nutrients in Fig. 5. The HPC did not appreciably vary after addition of organic carbon, phosphorus and inorganic nutrients in SS pipes, which is in contrast to PEX pipes in which microbial growth was high with inorganic nutrients. In the Feug-Yuan drinking water distribution system in Taiwan, bacterial growth was stimulated in the treatment plant and distributed water of the urban area but not in the suburban area water by the addition of ammonium, phosphate and nitrate. Similar results were found for the addition of acetate[43].

Previous reports have suggested a limiting nutrient in certain types of water. In central Europe and North America bacterial growth in drinking water has been found to be limited by organic carbon[16,23]; in Finland and Japan, it was limited by phosphorus[27,29]. However, research has indicated that the limiting factor for bacterial regrowth can change due to variations in local environmental conditions, even within the same area of water distribution.

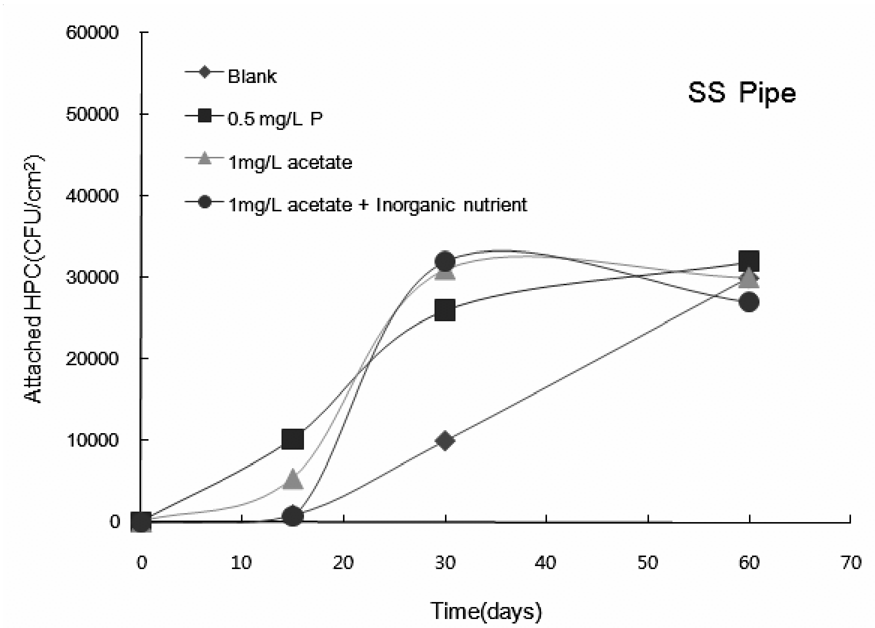

Fig. 5. Variations in attached microorganisms for the different combinations of nutrients in stainless steel pipe

Bacterial growth is higher in SS pipe than galvanized iron and copper pipe with a supplement of 3 mg/L glucose[38]. In this experiment, the level of attached bacteria with the acetate and phosphate supplement increased for both pipes, although the increase was not as dramatic in the SS pipe compared to the PEX pipe. The results suppose the possibility for the existence of a specific composition ratio for the main nutrient, such as organic carbon, nitrogen and phosphorus, to stimulate bacterial growth. Because chlorine residual depletion, extended stagnation time in a house water tank and a decrease in water velocity in a house plumbing system is expected, the growth of biofilms might be affected more by limiting the nutrient source rather than other environmental factors. Thomson *et al.*[43] found C:N:P ratios influence the maximizing of biofilm formation.

Availability of nutrients is a crucial factor that stimulates bacterial regrowth can be altered by varying the water source for producing tap water, treatment applications at the water plant and chemical usage to control bacterial regrowth in distribution systems. Full-scale conventional treatment process can eliminate phosphorus to the level of 5 μg P/L from approximately 80 μg-P/L[27]. The AOC potential increases after ozonation and disinfection with liming[32]. UV irradiation decreases the level of AOC potential and the sum of molecular size fractions[44]. Lehtola *et al.*[45] found faster biofilm formation compared to that of copper pipes and phosphrous release from new PE pipes. Dead microorganisms detached from biofilms can cause to increase the level of AOC. Granular activated carbon was able to diminish the level of MAP[46].

Domestically, water through the hot water system is provided to a number of public central supplies, including apartment complexes, public bathing facilities, hotels, hospitals and swimming pools and disinfection and cleaning can be problematic at some of these facilities[47,48]. Phosphate-based corrosion inhibitor is applied to control corrosion in pipes and facilities and there is a possibility that this phosphate-based product enhances bacterial regrowth. Park *et al.*[49] reported that microbial growth with the presence of 1.2 mg/L biodegradable organic carbon resulting from the addition of orthophosphate for corrosion control was higher than that resulting from orthophosphate without BDOC in PVC systems. This is consistent with present investigation results (Fig. 4) that bacterial growth was stimulated by the addition of inorganic nutrients with a high concentration of acetate.

The level of DOC and UV-254 in PEX pipes was higher than in SS pipes (Fig. 6). The DOC levels increased after coming into contact with water in both pipes. The value of UV-254 in PEX was two times higher than that in SS at 60 days. Assimilable organic carbon (AOC) release was found from PVC pipes[50]. Skjevrak *et al.*[51] found that the organic component measured by COD-Mn and TOC migrates significantly from PEX pipes and the identified volatile organic components (VOC) were mainly constituted by oxygenates with methyl-*tert*-butyl ether (MTBE) as the major individual VOC. Brocca *et al.*[52] reported a diffusion of organic compounds, which typically present a phenolic ring, from PE pipes, including cross-linked polyethylene (PEX), medium-density polyethylene (PEM) and low-density polyethylene (PEL). This result indicates that leaching of organic materials can cause taste and odour problems and production of harmful byproducts in tap water. In addition, these reactions can be accelerated with the higher temperatures of a hot water supply line.

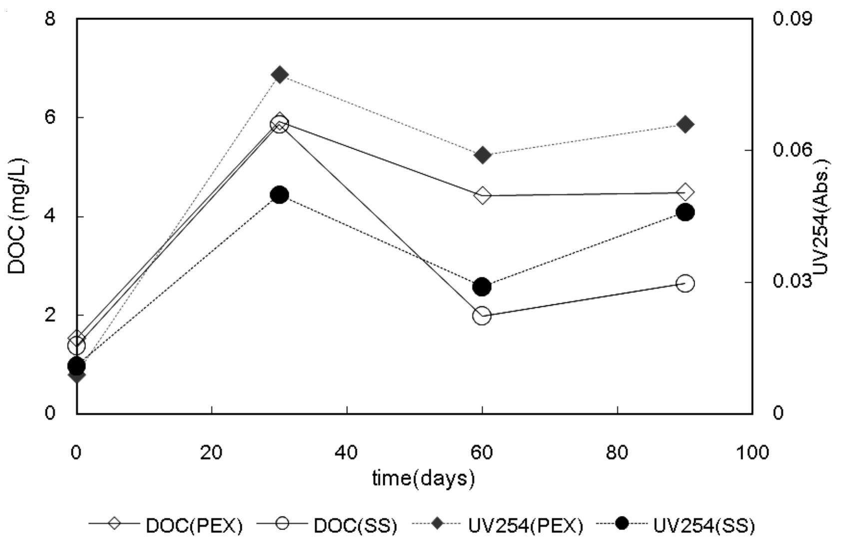

Fig. 6. Variation of UV-254 and DOC in effluents from PEX and stainless steel pipes

The control of bacterial regrowth by membrane filtration, chlorine dosing and UV irradiation for attached and suspended microorganisms in PEX pipes were evaluated shown in Figs. 7 and 8. The level of suspended bacteria from dosing with chlorine was higher than from treatment with membrane filtration or UV irradiation. Generally, chlorine is added in an amount that maintains residual chlorine in the water distribution system to control microbial regrowth. However, the application of chlorine is difficult because high temperature accelerates chlorine decay in hot water system. Therefore, a large amount of chlorine is required to maintain the required chlorine residual in hot water. This causes odour problems, corrosion of metal based pipes and disinfection by products (DBPs) formation, resulting in consumer's aversion for tap water. It is already reported that the use of chlorine for SS and galvanized iron pipe is not efficient in controlling attached microorganism on pipes[53]. Miettinen *et al.*[54] reported that chlorine can increase the AOC level. Kim and Lee[3] reported that the predominant genera from two distribution systems was *Acinetobacter*, *Sphingomonas*, *Micrococcus*, *Bacillus* and *Staphylococcus*. Most of them are either encapsulated cell structure or gram positive *cocci* which have a strong tolerance level to the disinfectant.

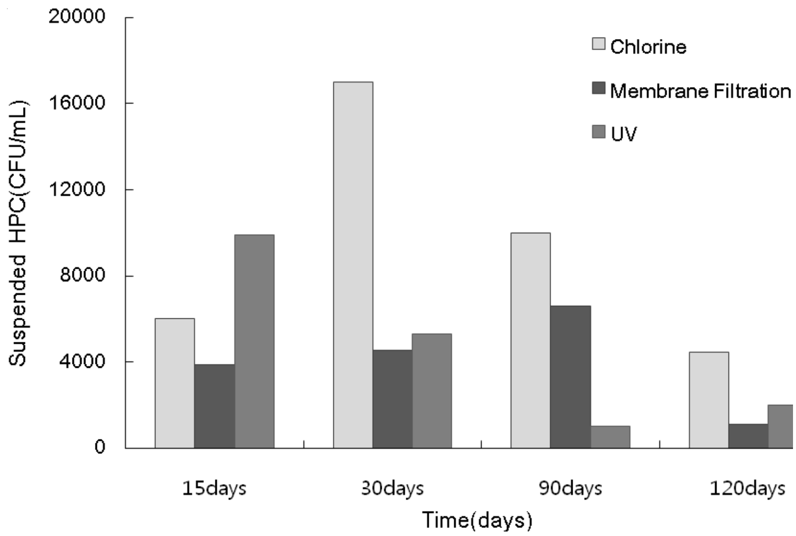

Fig. 7. Comparative efficiency of chlorine, membrane filtration and UV irradiation in controlling suspended HPC regrowth in effluents from PEX pipe

Microfiltration has various benefits, including the elimination of DBPs, turbidity and BOM. However, the control of biofilm development by membrane filtration was less than by the other processes. Additional physical operations, such as heat flushing and reservoir cleaning, would be helpful because maintaining a chlorine residual is not easy in hot water and managing home water supply facilities to ensure sanitary conditions of tap water is important.

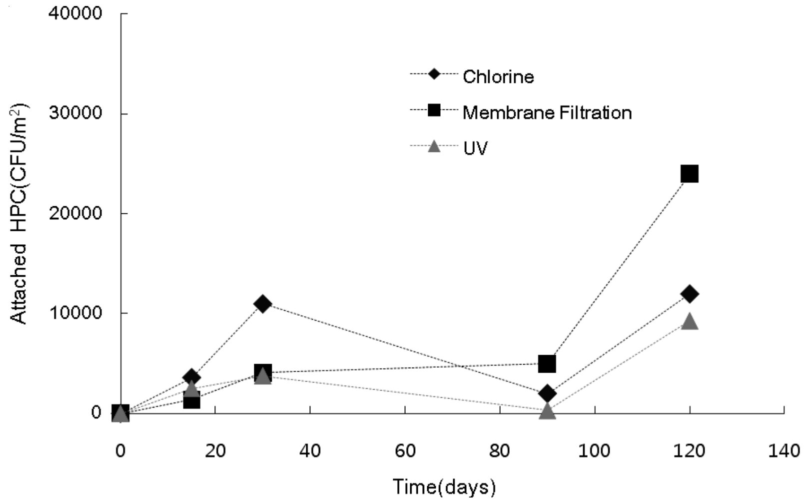

Fig. 8. Comparative efficiency of chlorine, membrane filtration and UV irradiation in controlling attached HPC on PEX pipe

The disinfection efficiency of UV irradiation was higher than chlorine dosing in this experiment. This is because treated water in PEX pipes, which do not release metals, has low turbidity and so UV irradiation is less affected by the water temperature. Lund and Ormerod[18] reported that UV irradiated water shows considerably less production of biofilm. Murphy *et al.*[55] reported that UV irradiation with chlorine-based disinfectants ameliorates the log elimination of *Escherichia coli* K12 from effluent and biofilm samples. UV irradiation decreased the level of AOC potential[44]. Because we used tap water, which had added chlorine from the water treatment plant, for this UV irradiation condition. This condition can strictly designate for UV irradiation after chlorination. Therefore, UV irradiation could be effectively applied to chlorine-treated water, which has low turbidity, when supply lines are not long. Future work will entail additional full-scale trials in the water supply system for controlling bacterial regrowth based on previous examined results.

Conclusion

This study investigated variations in suspended and attached bacterial growth in relation to different nutrients in SS and PEX pipes, which are often used in hot water plumbing systems and evaluated treatment and management strategies to control bacterial regrowth in water supply systems. The following conclusions can be drawn from present study:

The level of suspended HPC bacteria was higher in SS pipe than in PEX pipe. There was no difference in HPC biofilm densities at 20 or 55 ºC for both types of pipe. However, a high HPC was obtained from the PEX pipe effluent at 55 ºC compared to 20 ºC. The nutrient contribution to biofilm development differed according to pipe type, being less for SS pipe.

The bacterial growth on PEX pipe was higher with the addition of 0.5 mg/L phosphorus than with the addition of 1 mg/L acetate. Biofilm development was promoted with the addition of mixed inorganics and 1 mg/L acetate on PEX pipes. The results indicate that inorganic nutrients could play an important role in bacterial regrowth in hot tap water. An application with chemicals, such as phosphate base inhibitor, could result bacterial regrowth in water plumbing system.

The application of only free chlorine was not effective for controlling bacterial regrowth in hot water effluents from the PEX pipes. Chlorination in hot water would require a higher chemical dose to ensure an adequate disinfection effect and the level of UV-254 and DOC increased during tap water stagnation in PEX pipes. This indicates that there is a potential risk associated with the application of high concentration of chlorine to regulate bacterial regrowth in hot water from PEX pipe, such as migration of harmful organic chemicals and DBP formation. Therefore, incorporating system management strategies that consider the pipe material and limiting nutrients in a hot water supply system should be required instead of relying solely on chlorine application to restrict microbial growth.

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