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Factors Controlling Bacterial Growth in Drinking Water Distribution Pipes

YOONJIN LEE

Department of General Education, Konyang University, 119, Daehakro, Nonsan 320-711, South Korea

Corresponding author: E-mail: leeyj@konyang.ac.kr

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In this research, the multiplication of total coliform and heterotrophic count was observed with the use of phosphate-based inhibitor on copper and poly(vinyl chloride) pipes. This result was confirmed with the outcomes of adenosine triphosphate testing. Heterotrophic count growth was limited by inorganic substances on copper and poly(vinyl chloride) in this test; the growth of heterotrophic count with the added condition of phosphorous was higher than that of carbon and efficiency of both organic and inorganic nutrients should be evaluated in the existing unit process in water plants. The scanning electron microscope result shows a depression dug out on the poly(vinyl chloride) pipe and deposits on the surface of pipe. Thermal disinfection might not be effective to control the growth of attached microorganism in the plumbing system.

Key Words: Poly(vinyl chloride) pipe, Copper pipe, Heterotrophic count, Coliforms, Microbial regrowth, Tap water, Biofilm.

INTRODUCTION

The supply of biologically stable water after water treatment is an important process, because there is a lot of possibility for drinking water to be exposed to pollution before reaching the consumer's tap. In a water distribution system, it is crucial to understand the factors influencing the multiplication of microorganisms and the development of biofilm for a water distribution system to supply microbiologically stable water. Various types of information is required, *i.e.*, the pipe material, hydrological character, extent of corrosion and chemical and biological water condition contacting with the pipe, to appropriately manage the distribution system. Difficulties can occur such as coliform outbreaks, detection of high numbers of heterotrophic plate counts (HPCs), induction of bad taste and odor and increase in the corrosion rate as the water travels from the water plant to reach the consumer's tap.

The problems caused by water deterioration, such as regrowth and coliform detection in the distribution system, are related to various factors such as operational condition and water quality. A water distribution system is considered a huge bioreactor, wherein hygienically high-quality water should be produced[1]. Regrowth indicates the multiplication of microorganisms in treated water from a water plant and this arouses the concerns of many utilities and results in complaints by consumers.

The level of microorganisms is conventionally controlled by chemical disinfection with chlorine and hypochlorite. However, these chemicals have a possibility to split the high molecular weight of organic matters to simple ones; thus, potential of microbial growth is increased. Microbial inactivation is related strongly with dissolved organic carbon (DOC), because chlorine is consumed by dissolved organic carbon[2].

Treatment with higher chlorine to control microbiological problems enhances trihalomethane (THM) formation[3]. Therefore, elimination of limiting nutrients in tap water might be a good substitute for the chemical disinfection method if the crucial nutrients for microbiological growth can be determined. Carbon is essential for microbial growth and it is generally supposed to be a limiting source in tap water. The ratio of carbon, nitrogen and phosphorus for bacterial growth is known to be 100:10:1[4]. Assimilable organic carbon (AOC) has been found to be limited as a main nutrient in water distribution systems in North America[5]. Furthermore, Chandy and Angles reported that biofilm development in Sydney drinking water was limited by organic carbon[6]. The substance of phosphorous has been revealed to greatly enhance the number of bacteria in drinking water in Japan and Finland[7,8]. However, the addition of phosphate did not have any effect on HPC[9]. The reason for these different results was explained as the extent of corrosion, the microbiological availability of phosphate and the treatment applied in drinking water. Lehtola *et al.*[10] found that ultraviolet (UV) irradiation over 204 mWs/cm^2 could bring about the release of microbial available phosphate (MAP).

Present results related to biofilm development on pipe materials are as follows: Hallam *et al.*[11] found that the level of biofilm activity was affected by pipe materials. The highest ATP levels were shown with PVC pipe and then medium-density polyethylene (MDPE), cement and glass, in descending order, when the residual chlorine was 0.3 mg/L. Holden *et al.*[12] reported that cast iron pipe showed higher biofilm growth than MDPE pipe. The difference in biofilm growth was not large on pipes in chloramination systems. Lehtola *et al.*[13] reported that phosphorous was released from polyethylene (PE) pipe and that microbial growth in polyethylene pipe was higher than that in copper pipe, which was explained in that the copper concentration release was related with the growth of HPC on copper. Ozonation was not effective for the control of biofilm on poly(vinyl chloride) or stainless pipe[14]. Momba and Binda[15] found that the combination of chlorine and monochloramine was effective for the inhibition of biofilm on stainless steel and galvanized mild steel using free chlorine 2.5 mg/L followed by 1.5 mg/L monochloramine. The formation of biofilm on a smooth pipe surface was generally slower in terms of the initial rate than on a rough one, but smoothness did not greatly affect the total amount of biofilm[16].

Tap water sourced from surface water is often treated with conventional water treatment systems, chemical coagulation, sedimentation, filtration and disinfection with chlorine gas. Usually, home plumbing is constructed of plastics and copper. Growth of *Legionella pneumophila*, to which outbreaks of health risk from legionellosis is attributed, is a problem in hot water systems. Increased metal concentration and corrosion are problematic in house plumbing. Phosphate-based inhibitors are often used to control coloured water or corrosion inside buildings.

In this study, the effect of organic and inorganic nutrients on the contribution of bacterial regrowth and biofilm formation, as well as efficient methods for controlling bacterial colonization and regrowth in drinking water, were evaluated using common pipe materials: copper and PVC. This paper considers the potential of bacterial regrowth with the application of a phosphate-based inhibitor. The purpose of performing this research was to determine the limiting factors on microbial multiplication in water from house plumbing systems that it supplied through conventional treatment; the levels of carbon and inorganic matters were adjusted. The microbial quality management plan was evaluated to effectively manage water distribution systems and acquire microbiologically stable water in this research.

EXPERIMENTAL

This experiment was performed for microbial multiplication and biofilm growth in PVC and copper pipes. The sample used in this experiment was taken from a cold water tap in the Engineering Department Building at Konkuk University in South Korea. The samples were controlled for the purpose of the experiment, including adding chemicals in relation to the set of physical conditions. Tap water was drained during 2 or 3 min before sampling and checked immediately to ensure that the item was free of chlorine before use in the experiment. The sample for this experiment was treated by standard water treatment comprising coagulation with polyaluminium chloride (PAC), sedimentation, filtration and disinfection from G water plant. Water quality is presented in Table-1.

TABLE 1
TAP WATER QUALITY PARAMETERS USED IN THIS STUDY

Items	Condition
DOC (mg L^{-1})	1.3 ± 0.2
Turbidity (NTU)	< 0.3
pH	7.3 ± 0.05
Conductivity (μS cm^{-1})	180 ± 5
Residual chlorine (mg L^{-1})	< 0.05
DO (mg L^{-1})	10 ± 1.5

Nutrient sources of carbon, nitrogen and phosphorous were separately supplied for the biofilm test with different conditions of nutrient sources. Stock solution (500 mg/L) was initially produced by dissolving sodium acetate, ammonium chloride, potassium nitrate and inorganic nutrient cocktail. The composition of chemicals was dissolved in deionized water in separate brown bottles. Each nutrient bottle was autoclaved for 0.5 h at 121 ºC after being capped. The stock solutions were diluted with sterile deionized water to acquire the required concentration of nutrient solution set for the experiment. Twenty percent (as P_2O_5) of corrosion inhibitor for tap water (Check-phos DS, Handok TS Ltd.) was applied to test the contribution of phosphate-based inhibitor in microbial regrowth. The solution taken from the original chemical bottles was initially diluted to 200 mg/L with sterile deionized water before application with 1 mg/L in the pipe for the every test.

Copper and PVC pipes for the use of drinking water were selected in this experiment to test the plumbing material. The water temperature was controlled at 20 ºC in the equipment. Samples were changed after every 15 days. Pipe coupons were cut to 50 mm with a stainless steel cutter and cleaned with a sonificator before putting in the pipe units system. Eight pieces of pipe coupon was set in a pipe unit system. The internal diameters of the copper and PVC pipes were 20.0 and 16.5 mm for biofilm growth. The coupon and water samples were taken out for analysis when the reaction time was complete.

Microbial analysis was performed for total coliform and heterotrophic bacteria. Total coliform was spiked twice for reaction times of 0, 20 and 70 days to simulate the accidental coliform inlet in the water pipe system.

Microbiological analysis: The inoculums were obtained from a sewer adjacent to Konkuk University. Sewage was filtered through 11 μm membrane filters and filtrate (10 mL) was placed on M-endo medium in a Petri dish and incubated for 24 h at 35 ºC. Colonies formed were placed on a nutrient broth solution and incubated for 24 h at 25 ºC. Coliform bacteria were isolated using centrifugation at 5,000 g for 5 min, then washed three times and resuspended in the phosphate buffer at pH 7.0. The coliform bacteria isolated from the media were mixed using a vortex mixer. Fresh cell suspensions in the chlorine-demand-free solution were applied in all experiments.

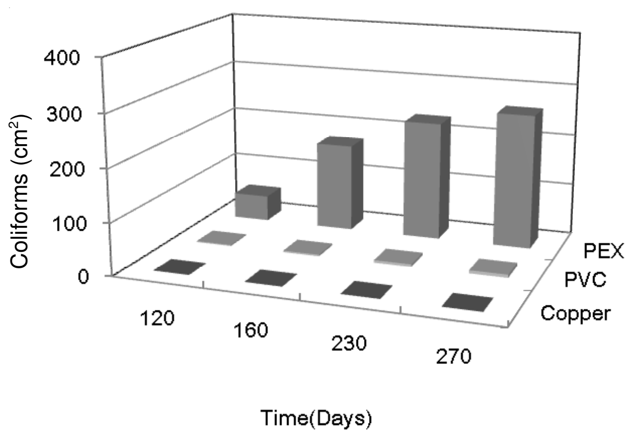
Heterotrophic bacteria were analyzed by the spread plate method with R_2A medium. Each sample (0.1 mL) was applied on the plates and incubated at 20 ºC for 7 days. All counts were achieved in triplicate. Attached microorganisms on pipes were enumerated using the following methods. Sterile deionized water (5 mL) was aliquoted into tubes. Biomass was removed from the pipe surface by sterilized swabs, which were then moved into the prepared test tubes and mixed with a vortex mixer for 2 min (Vision Scientific, KMC-1300). The removed biomass was inoculated on R_2A media. Colonies were counted and expressed as colony forming units per cm^2 and colony forming units per mL. The ATP level was determined by using an ATP analyzer (AMSA lite II). The PVC surface was observed with a scanning electron microscope (Leika stereo scan 440).

RESULTS AND DISCUSSION

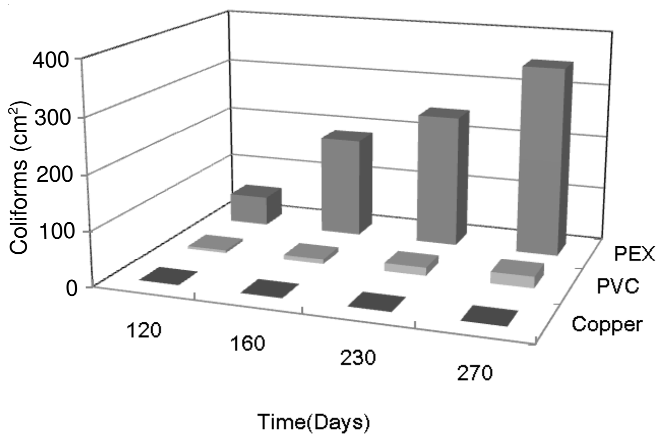
The removal efficiency of pollutants in water plants may affect microbial multiplication. It is expected that changes in microbial water quality will influence the microbial environment in pipe lines. Chemicals used during water treatment, such as pre- or post-chlorination at the inlet of the distribution system, or physical treatment such as UV treatment or hot water flushing, are often applied to control microbial regrowth, but can also contribute to microbial behaviour. In this experiment, the effect of such measures on microbial growth was evaluated with domestic water samples under various physicochemical conditions.

High level of coliforms with the approximate level of 10^6 numbers/100 mL were spiked twice during this experiment to simulate accidental invasion by coliforms in a water distribution system. Attached coliforms were not detected on copper pipe in this case. However, they were found on PVC and PEX (Cross linked polyethylene) pipes (Fig. 1). Plastics pipes seemed comparatively vulnerable for the growth of coliform in this test. Domek *et al.*[17] found that a low level of copper brought injury to coliform bacteria and the respiratory chain was damaged in injured *Escherichia coli* cells. Copper released from copper pipes could hinder the growth of coliform in pipes. A similar result was reported by Lehtola *et al.*[13], who found that the number of virus-like particles in copper pipes was lower than that in plastic pipes owing to the effect of released copper in water. Coliform was not even detected with a dose of phosphate-based inhibitor in copper pipe (data not shown). Yu *et al.*[18] also reported that the pipe material was the main factor relating to the formation of biofilm and microbial diversity and that the number of *E. coli* was lowest on copper pipe among different pipe materials, specifically copper, chlorinated PVC, polybutylene, polyethylene, stainless steel and steel coated with zinc.

Dosing with phosphate-based inhibitor affects the growth of coliform on PVC pipes. A similar result can be expected for components of the same PVC material with the nutrient



(a) Control



(b) With phosphate inhibitor

Fig. 1. Coliform detection on the different kinds of pipes

source of phosphorus used in home plumbing. Regrowth of coliform was not detected on biofilm on copper pipe during this test. Higher numbers of coliform were detected on biofilm on PEX. This result indicated that coliform multiplication could suddenly occur through an accidental inlet of coliform and a change in the nutrient availability condition in different environments during distribution. Previous research results showed that the number of coliform were higher in samples from water distribution systems compared to samples from water plants[19].

The growth of HPC on biofilm did not show an evident tendency to increase with the use of phosphate-based inhibitors in copper pipe (Fig. 2). However, previous reports have produced results that conflict with this finding. Zhao[20] reported that bacterial densities measured with HPC were higher after the addition of a phosphate-based inhibitor such as orthophosphorus, zinc orthophosphate in PVC, lined cast iron, unlined cast iron and galvanized steel pipe[21]. Appenzeller *et al.*[21] reported that the cases of limitation by phosphorous nutrients were very specific; in other words, a high ratio of C/P and low P level was typical and the bacterial blooming brought about by phosphate inhibitor is possibly limited to the specific case, although the researchers also found a low level of microbial growth in slightly corroded stainless steel[21]. The bacterial regrowth in water distribution systems could be affected by various environmental factors, such as the surface condition of pipes, the bioavailability of phosphate, *etc*. However, because phosphate inhibitors are currently applied in house plumbing systems to control corrosion of the system or solve the problem of coloured water, such as blue water, in copper pipes, effect of phosphate-based inhibitor on microbial regrowth and the behaviour of microbes in specific environmental conditions should be consistently investigated. In this experiment, the pipe was not corroded; an initial released metal concentration might affect to microbial growth on copper pipes and the initial surface roughness of copper is also greater than that of plastics.

In PVC pipe, the effect of the phosphate inhibitor was clear and the HPC multiplication on biofilm was 2.5 times greater than in the case where the phosphate inhibitor was not dosed. The amount of HPC on biofilm was higher in PVC compared to copper pipe. The number of microorganisms was higher in PVC pipe than in copper for suspended HPC.

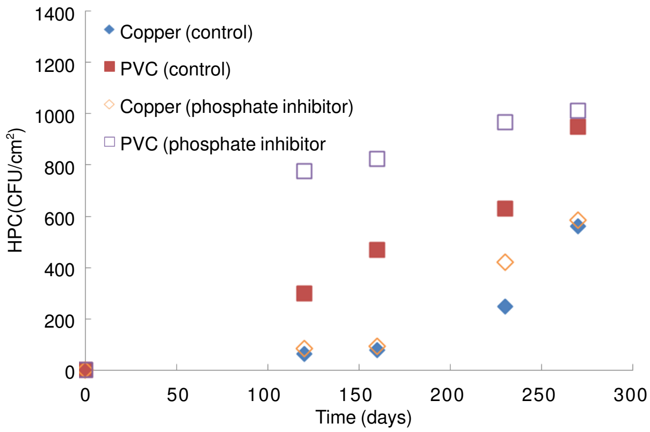


Fig. 2. Distribution of attached HPC in the different kinds of pipes

The level of ATP on biofilm showed a similar tendency to the result of microbial test (Fig. 3). The ATP values were not shown to increase after the dose of phosphate-based inhibitor in the copper pipe. However, the level of HPC was higher with the added condition of phosphate-based corrosion inhibitor compared to the amount of control on the PVC pipe. The level of ATP in the copper pipe was low compared to that of PVC and the specific tendency of each nutrient of the attached microbes for ATP was clearer than that of suspended microbes. Domek *et al.*[17] found that copper with 0.025 mg/L and 0.05 mg/L brought over 90 % injury during 6 and 2 days and the metabolism of injured *Escherichia coli* cells was determined from the damage to the respiratory chain. Lee[22] reported that metal leaching from pipe material influenced the multiplication of microorganisms. Health effects such as toxicity generated by metals and turbidity in water should be considered; an alkalinity difference was also found on pipe materials. This variation in water quality may affect the growth of microorganisms on pipes.

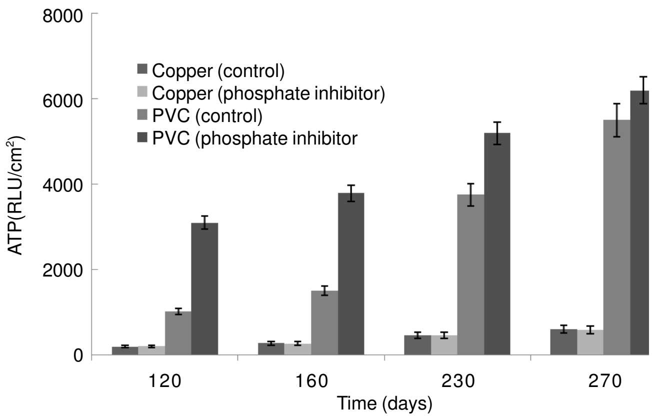


Fig. 3. Variation of ATP in the different kinds of pipes

HPC multiplication in copper and PVC according to the different nutrients is presented in Figs. 4 and 5. The limitation of inorganic compounds is clearly shown in the copper pipe for the HPC bacteria. The level of HPC with the addition of phosphorus was higher than that of carbon. Microbial growth increased with the addition of nitrogen and phosphorous. Chu *et al.*[23] found that the effects of nutrients limited to under 0.1 mg/L on microbial regrowth are less important for nitrogen

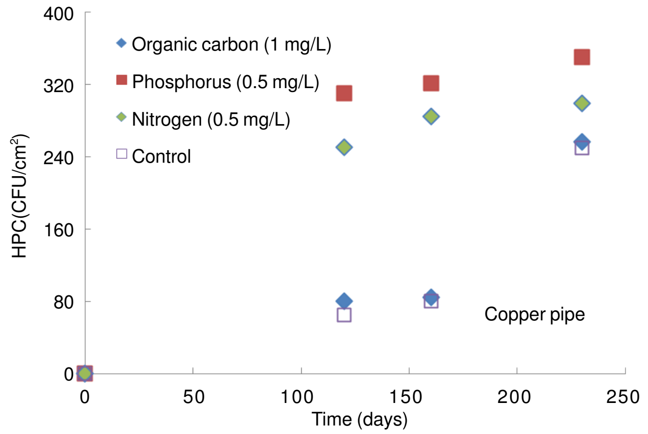


Fig. 4. Variation of attached HPC in relation to the different sources of nutrients on copper pipes

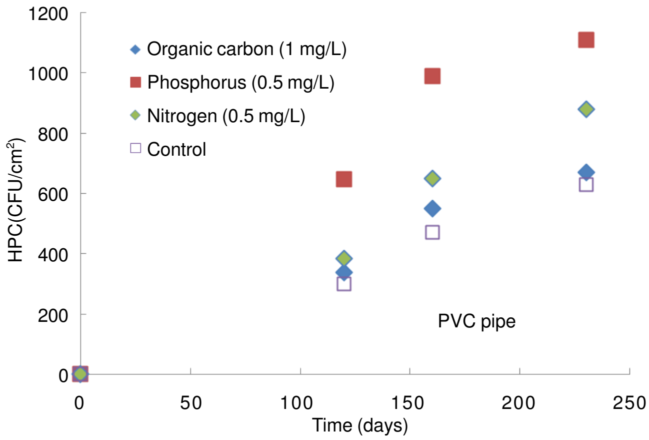


Fig. 5. Variation of attached HPC according to the different sources of nutrients in PVC pipes

and phosphate in the Taiwanese water distribution system. These sources were injected with 0.5 mg/L in this experiment and microbial multiplication on the source of nitrogen and phosphate was clearly shown. This result indicates the extent of nutrient removal in water plants or accidental inlet to the water distribution system could be important causes of microbial regrowth and might influence the quality of tap water.

It was found that when nutrients such as nitrogen and phosphorus enter the water distribution system by accident or through inappropriate management, owing to a broken joint into distribution system or the unsuitable elimination of nutrients during water treatment, the removal of those nutrients in tap water management should be a crucial assignment to control microbial multiplication. Domestically, many water plants adopt a conventional water treatment system normally targeted for turbidity. However, there are some cases where a high level of inorganic nutrients can be detected in the distribution system, such as in the case of eutrophication of lake water, which is an important source of raw water in Korea, or when they are inlet into the distribution system by various accidents such as broken pipes and joints. The growth of attached microorganisms might be directly related to the pipe materials and nutrients in water.

Conventionally, carbon was regarded as a main source for limiting bacterial growth in water distribution systems. However, HPC bacteria on biofilm did not increase when additional organic carbon was injected into tap water in this experiment. This result points toward the important contribution of inorganic nutrients in HPC bacterial growth. These results show that organic carbon is not the only limiting source for microbial regrowth and HPC multiplication; rather, the distribution system was affected by the ratio of carbon: nitrogen: phosphorous. The removal target and efficiency in the existing process should also be checked for inorganic nutrients, along with the disinfection process in the water plant, to set a suitable strategic plan to control microbial regrowth.

Microbes increased with 2.4 and 2.7 log in the addition condition of nitrogen and phosphorus at 270 days in PVC. Attached microorganisms were noticeably affected with the source of phosphorus on PVC; a higher growth level was shown compared to control and the addition of nitrogen and carbon. On the other hand, HPC multiplication by organic carbon was not noticeable and the contribution of the carbon level increase might be, respectively low on the formation of biofilm on PVC pipes in this case. The PVC surface of is shown in Fig. 6. It exhibits a depression dug by washing away from the water passage; furthermore, deposits were found on the surface. This indicates the potential that the pipe material leached into the tap water and the intensity of the pipe year may have been weakened by pipe substances flowing out. In addition, a health risk could occur and these points need to be considered in future work. The contribution of chemicals such as phosphate inhibitor or residuals during water supply could enhance the growth of microorganisms and nutrient management would be a crucial work in water distribution systems for application in water reuse at swimming pools and public baths.

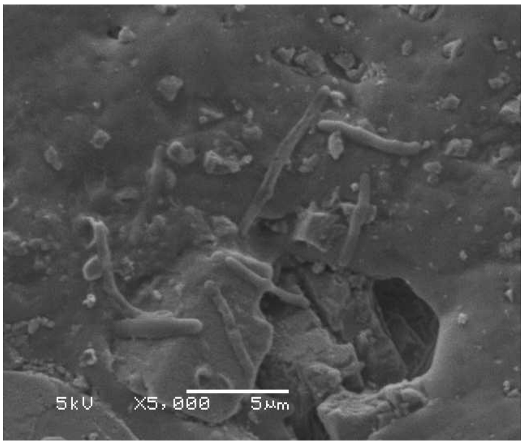


Fig. 6. SEM photo of PVC pipe after 270 days of operation

Knowing the limitation source in a distribution system and the water quality variation of treated tap water from water plants should be an important process; a suitable treatment approach should be followed to control microbial regrowth in distribution systems. Furthermore, the removal efficiency of unit process in water treatment should be estimated to ensure that it can affect bacterial multiplication. Moreover, the condition of attached and growing microbes in water pipe system can differ due to the condition and/or extent of system corrosion, disinfection method and efficiency.

Microbial growth is different according to the temperature of the tap water supplied[24] and temperature can affect the availability of nutrient substances. In this experiment, the growth of attached microorganisms for thermal treatment on PVC pipes was higher than that of the control (Fig. 7). There is a possibility that various pollution factors exist in hot water supply.

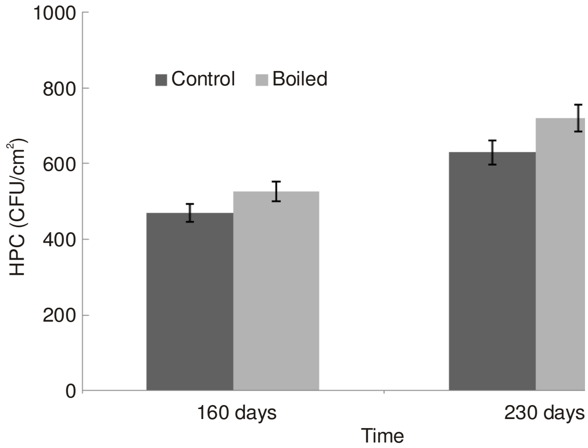


Fig. 7. Control of attached microorganisms on copper pipe using thermal-disinfected tap water. (Data for the control condition are the same as in Fig. 2)

It could be expected that the pipe used for the hot water supply undergoes expansion and shrinkage according to the material in relation to variations in temperature; hot water sometimes stagnates and moves in the water supply system. Hot water is not always supplied during the summer time in some buildings. The level of attached microorganisms increases with contact time after heating up the supplied water on PVC pipes. Nutrients might be changed favourably with the growth of microbes after heating up the water. This was explained in Herson *et al.*[25] report; these researcher have found an enhanced microbial level after treatment with high pressure and temperature. This result indicates the availability of microorganisms and concentrations of organic and inorganic nutrients could affect microorganisms in water. The attached HPC level increased with 2 log after thermal treatment compared to control. Thermal disinfection might not be effective in this plumbing system and can bring about the multiplication of microbes on pipes by affecting the microbial availability of nutrients.

Sometimes, thermal disinfection is used to control the multiplication of specific microbes, such as Legionellae in hotels and hospitals. In this experiment, microbial growth is stimulating in a PVC pipe after thermal treatment. Mouchtouri *et al.*[26] reported that applying thermal disinfection was not useful to control Legionellae. However, they also added that two methods-chlorination and heat flushing-were effective in controlling Legionellae. A high level of HPC and the detection of coliforms and Legionellae were reported in pubic baths in Korea[27]. In this experiment, UV irradiation and chlorine dosage were shown to have a similar effect in controlling attached microbes on copper pipes (Fig. 8). Not only reliance on the application of chlorination, but also nutrient controlling and other disinfection methods such as UV and physical treatment such as flushing should be considered for the appropriate management for water plumbing systems. In addition, various facilities such as valves, connectors and reservoirs, as well as

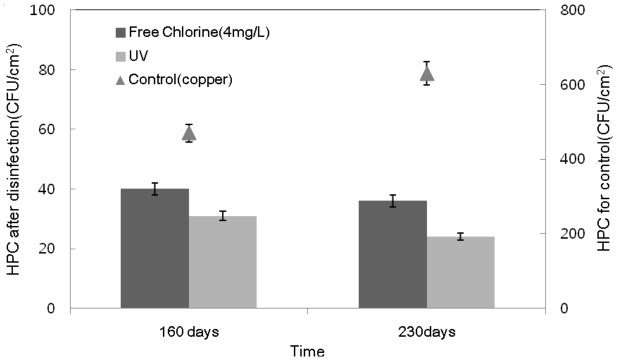


Fig. 8. Control of attached microorganisms on copper pipes using UV and chlorine dose. (Data for the control condition are the same as in Fig. 2)

the pipe system, should be evaluated in terms of the material used, nutrient level, water stagnation time and disinfectant residuals, *etc*.

Conclusion

This research was performed to evaluate the contribution of inorganic substances to bacterial multiplication in home plumbing systems and the points to consider in preparing a strategic management plan were presented in terms of controlling microbial regrowth, including the use of chemicals such as corrosion inhibitor and disinfectants. The following conclusions were drawn: The multiplication of total coliform was higher with a dose of phosphate-based inhibitor. In copper pipes, the growth of coliforms was not shown. This result is consistent with a previous report, which found that copper leached from copper pipes and there was lower HPC multiplication in copper pipes. Coliform growth on biofilm was increased to a small extent with the addition of phosphate inhibitor. The growth of HPC on PVC pipes using the phosphate-based inhibitor was higher than that on copper pipes and the influence of the inhibitor was clear. The multiplication of HPC was not influenced by the phosphate-based inhibitor on copper pipes. PVC material for installations such as water tanks, connecters and valves was ubiquitously used in the water supply system. The effect of this material on the various facilities in the water supply system should be evaluated in future work. Bacterial growth was limited by phosphorous on copper pipes. Therefore, the raw water quality and removal efficiency on the unit process for nutrients such as carbon, phosphorus, nitrogen, *etc*., should be evaluated to effectively manage the microbial quality of tap water. In particular, bacterial regrowth according to the nutrient source could be expected in relation to a nutrient increase, while in the case of water reuse in public spas and swimming pools, inappropriate treatment of water, or a dose of corrosion inhibitor to control corrosion in the building might have such an effect. Thermal disinfection was not effective in controlling biofilm formation on water pipe systems. Considering THM formation in relation to the dose of free chlorine in water, substitute methods such as UV irradiation and flushing should be considered, as well as nutrient source management in tap water to control microbial regrowth.

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