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Effects of Chlorine Application on Bactericidal Efficiency at Municipal Wastewater Treatment Plant

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This research was performed to evaluate the inactivation rate of indicator microorganisms such as total coliform, fecal coliform, fecal streptococcus and the effect of water parameters when chlorine is applied as a disinfectant in the discharged water at the J municipal wastewater treatment. Based on these results, it presented the empirical equation for estimating the number of these indicator microorganisms. The value of LC_{50} (median lethal concentration) for 24 h was 0.8 mg/L by the acute toxicity test of *Oryzias latipse*. The formation of disinfection by-product such as trihalomethanes (THMs) and haloacetic acids (HAAs) which are known as carcinogens was evaluated on chlorine dose in discharged sewage water to examine the sanitary and ecological facts during chlorination.

Key Words: Disinfection, Total coliform, Fecal coliform, Fecal streptococci, Trihalomethanes, Haloacetic acids, LC_{50}.

INTRODUCTION

Surface water is being polluted by domestic sewage, industrial wastewater and other pollutants, which are also used as a raw water source for producing tap water. Fecal-originated pollutants, pathogenic of microorganism and organic matters, were the cause of water-borne disease and deteriorated the quality of recreational water and costal fisheries, after being discharged from the urban sewage plants without a suitable disinfection treatment[1,2]. This water then entered the water distribution systems[3-5].

The purpose of disinfection is to inactivate pathogens and parasites that can spread water-borne diseases[6-9]. The pathogens in finished sewage water at the plant die off naturally. However, some pathogens survive latently over several months. So, the proper sanitary management of sewage water is critical to stem the spread of epidemic diseases.

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Chlorination is one of the methods most often used to disinfect for the treatment of urban wastewater because it is economical, easy to apply and has relatively fair oxidation ability[10]. However, chlorine residuals and their by-products are toxic to fish and plankton in the discharge areas because they remain continuously in water[11,12]. The representative disinfection by-products are trihalomethanes, halogenated ketones, chloripicrin and chlorinated phenols[13-15]. Despite the great damage caused by pathogens occurring increasingly every year, domestic monitoring of the quality of finished sewage water has not been considered in detail. The Ministry of Environment in Korea announced recently that the revised standard of discharged finished water would be regulated for the level of total coliform[16]. So, individual wastewater treatment facilities must establish correspondence plans for the level of regulation.

The harmful effect on living organisms or the toxicity to which chlorine rapidly react with inorganic and organic matters, was seen in chlorinated water[17]. This toxicity results in death, lack of reproduction and metabolism, functional mutation and change of genes such as the generation of tumors. It is especially named inactivation that brings functional incapability, though it does not greatly affect the life of organisms.

This research was performed to evaluate the chlorine disinfection effect for indicator organisms such as total coliform, fecal coliform and fecal streptococcus in finished sewage water from J urban wastewater treatment plant. The relationships between indicator microorganisms and physical and chemical parameters were evaluated to assess the importance of the growth of microorganisms of influenced factors. Also, the variation of fisheries' toxicity levels with *Oryzias latipse* and the formation of disinfectant by-products such as trihalomethanes (THMs) and haloacetic acids (HAAs) were measured to evaluate the additional ecological effect after chlorination.

EXPERIMENTAL

Samples for these experiments were taken by grab sampling methods from discharged sewage water from the J municipal wastewater treatment plant located in Seoul and were kept in a laboratory refrigerator under 4 ºC. The chemical oxygen demand (COD) and dissolved organic carbon (DOC) items were performed in 6 h. Samples of the nitrogen were pretreated with 1 N of HCl and NaOH to control the pH level of between 5 and 8.

All experimental glassware was pretreated with a mixing solution of 10 % $KMnO_4$ and H_2SO_4 for 24 h and then rinsed with tap and distilled water, successively. They were then dried in a 100 ºC controlled oven. They were located at an electric furnace with a temperature of 400 ºC. All glassware for the microbiological experiment was sterilized over 15 min at 120 ºC in an autoclave.

The disinfection experiment was performed in pre-autoclaved bottles as mentioned above. 100 mL of samples were filled in the glass bottles and 3, 5, 10, 15 and 20 mg/L of NaOCl solution was added, successively. One per cent of sodium thiosulfate solution was put for quenching the disinfectant reaction after the set reaction time of 1, 3, 5, 10 and 15 min. The disinfection effect was expressed on time as the survival ratio (the number of microorganisms at reaction time/initial number of microorganisms) of indicator microorganisms such as the total coliform, fecal coliform and fecal streptococcus. Disinfection experiment were evaluated on the seasonal variations which were taken in April, June, August, October, December and February.

The *Oryzias latipes* for the fisheries' toxicity test were selected with an average weight of 0.2 g and length of 2.4 cm. Toxicity tests were carried out with 72 h without an exchange of samples. The 10 of *Oryzias latipes* was contacted with a chlorine solution of 0-1 mg/L in a 20 L container. The result of fish fatality was observed after time lapses of 1, 3, 6, 12, 24 and 72 h. The discontinuation condition of brachial respiration was considered as a fish lethal and the value of LD_{50} was analyzed by probit method.

The disinfection by-products were analyzed for THMs and HAAs. Samples were prepared in 3 L flasks and chlorine was injected at a concentration of 1-30 mg/L and then quenched with sodium sulfite after 15 min of reaction. The pH value was controlled between 2 and 3 with sulfuric acid. Samples were taken through a glass column packed with XAD-2 resin to absorb organic matters and 25 mL of diethyl ether was passed through the column to extract organics. These were analyzed with gas chromatography (HP 5890 Series II) by standard method 6232 B[18].

Analytical method: Chlorine with a dilution of 12 % NaOCl was applied as a disinfectant and was measured using amperometric titration methods. The pH level was measured with an Orion 900A pH meter. Total nitrogen was analyzed in the acidic condition by the optical density of ultraviolet absorbance after it was decomposed in alkaline $K_2S_2O_8$ at 120 ºC by the Korean standard method for water pollution control.

The spread plate method was used for anlyzing the number of heterotrophic microorganisms present. The samples was inoculated on plate count agars and then incubated for 48 ± 3 h at 35 ± 1 ºC. After the colonies enumerated on plates and they were expressed by colony forming unit (CFU)/mL.

The multiple tube fermentation method was applied for anlyzing the total coliform level. Samples were inoculated in a lauryl trytose broth and were incubated for 24 ± 2 h at 35 ± 1 ºC for the test of presumptive phase. The positive reaction was checked by acidic production or gas occurrence. The tubes that tested the positive reaction were checked using the confirmed phase test. Samples were transferred again in brilliant green lactose bile

(BGLB) broth with a sterilized loop and it were incubated for 48 ± 3 h at 35 ± 1 ºC. They were then tested for the completed phase of the positive samples representing acidic reaction. The completed test was preceeded by the MacConkey agar, incubation time was 48 ± 3 h and incubation temperature was 35 ± 1 ºC. The typical pinks to dark red colonies with a greenish metallic sheen were treated with gram strain. Also, two well-isolated coliform colonies were picked and growths transferred from each isolate to the lauryl tryptose broth and incubated for 24 ± 2 h at 35 ± 1 ºC. The result was calculated in terms of the most probable number (MPN)/100 mL.

The culture of fecal coliform in tubes showing positive reaction for the presumptive test of total coliform was moved to EC medium with a sterilized loop and incubated at 44.5 ± 0.2 ºC for 24 ± 2 h. The result was expressed as MPN/100 mL after the enumeration for positive tubes showing gas production.

The presumptive phase test for fecal streptococcus was performed in Azid dextroth broth and confirmed by the occurrence of turbidity with an incubation temperature of 35 ± 1 ºC and 24 ± 2 h as the positive samples for the presumptive test and the confirmed test were performed for positive sampes. It was inoculated with a sterilized loop on Pfizer selective entrococcus (PSE), which checked for the positive sign of brownish-black colonies with brown halos after being incubated at 35 ± 1 ºC for 24 ± 2 h. The result of fecal streptococcus was expressed as MPN/100 mL.

RESULTS AND DISCUSSION

Behaviour of indicator microorganisms

The seasonal variation of the indicator microorganisms with total coliform, fecal coliform and fecal streptococcus in treated sewage is presented in Fig. 1. This experiment was performed to observe seasonal distribution and find the limiting factors for multiplication of indicator microorganisms in treated municipal wastewater.

The variation of total coliform was shown to be in a between 8.6×10^3 and 3.9×10^5 MPN/100 mL. The highest value of total coliform recorded in August fell 45 times compared with that of December, which had the lowest value in this experiment. Water temperature at August was shown to be highest during this experiment. The value of total coliform was also high between June and September. The multiplication of microorganism has to be monitored carefully in the treatment facility especially between June and September. However, the value of total coliform was exceptionally high (1.6×10^5 MPN/100mL) in November when temperature was low (14.6 ºC) during the winter season.

The values of fecal coliform were found to relate closely with the water temperature. A high value of fecal coliform was detected while the temperature was high. The range of fecal coliform was between 2.8×10^3 and 1.8

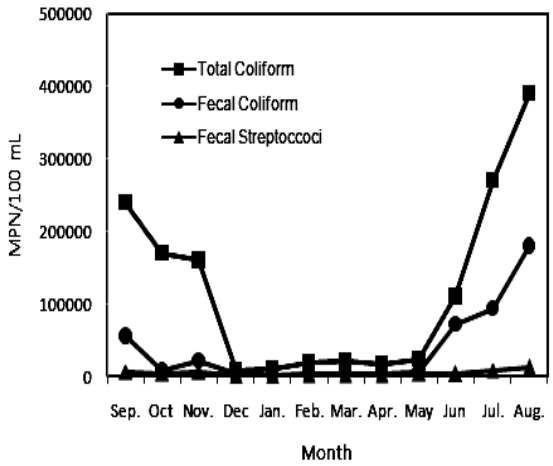

Fig. 1. Seasonal variation for total coliform, fecal coliform and fecal streptococcus in discharged water from J municipal wastewater plant

$\times 10^5$ MPN/100 mL. The amount of total coliform was always higher than that of fecal coliform. The ratio of fecal coliform to be total coliform was shown to be between 4.8 and 65 % for these finished sewage samples. During September, June and July, the values of fecal coliform were higher than that of the mean value for all samples, 3.8×10^4 MPN/100 mL. The lowest fecal coliform value appeared in December and the highest was seen in August.

The number of fecal streptococcus ranged between 1.1×10^3 and 1.2×10^4 MPN/100 mL. The values were low between December and April when the temperature was low. However, the number of fecal streptococcus was found to be high during summer months. The highest values were seen in August and the lowest in January. The number of fecal streptococcus is relatively low compared to total coliform. The ratio of fecal streptococcus to total coliform was between 3.1 and 19.6 %. However, the variation for fecal streptococcus in finished municipal wastewater was similar to that of total coliform.

The multiple regression analysis was used to evaluate the order of importance of independent water variables related to the proliferation of numbers of total coliform, fecal coliform and fecal streptococcus in discharged sewage water to surface water. DOC was the most important factor for the existence and growth of all indicator microorganisms which were selected in this analysis. The item of COD_{Cr} was also an important factor by which to measure the existence of total coliform and fecal streptococcus in this experiment. However, COD_{Mn} was not important to the multiplication of total coliform, fecal coliform, fecal streptococcus in finished sewage water,

so it was not included when formulating a prediction equation. Organic matters and nitrogen sources were the most influential factors for the growth of indicator microorganisms in these sewage samples.

The regression equation was formulated with the critical water parameter as follows; (eqn. 1-3). The water valuables, COD_{Cr}, DOC, total nitrogen and nitrate were used to predict the existing number of indicator microorganisms in this finished sewage water. The multiple correlation coefficients for total coliform (FS), fecal coliform (FC) and fecal streptoccoci (TC) were 0.96, 0.94 and 0.96, respectively.

$$TC(MPN/100mL) = 8.17(COD_{Cr}) - 87.2(DOC) + 32.05(T-N) - 0.93(NO_3 - N) \quad (1)$$

$$FC(MPN)/100mL) = 2.38(COD_{Cr}) - 32.66(DOC) + 14.06(T-N) - 4.039(NO_3 - N) \quad (2)$$

$$FS(MPN/100mL) = 0.22(COD_{Cr}) - 1.69(DOC) + 0.71(T-N) - 0.1(NO_3 - N) \quad (3)$$

Disinfection with chlorine

The dose concentration of chlorine with 3 and 5 mg/L, respectively in the finished sewage water was evaluated on contact time in Fig. 2. The log of survival ratio for total coliform has a linear relationship with time for this finished sewage water while chlorine was applied. The constant of k in Chick equation[6] was 0.098 and 0.092 for 3 and 5 mg/L.

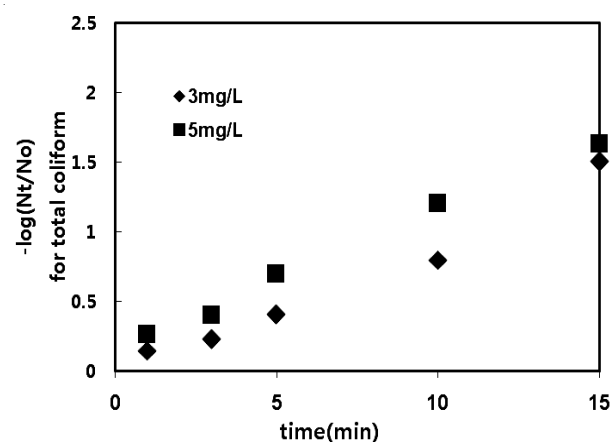

Fig. 2. log survival ratio for total coliform with 3 and 5 mg/L chlorine disinfection in finished water at J municipal wastewater plant

Seasonal variation in the number of total coliform with 3 mg/L of chlorine is shown in Fig. 3. The total coliform number before chlorination was 3.9×10^5 MPN/100 mL in August, which was over the regulated limit for discharge water in Korea. Therefore, it is thought that water should be disinfected in this treatment plant to catch up to the microbial standard level in August.

The initial disinfection effect observed in 1 min appeared depending on temperature. The log survival ratio at 1 min was -0.196, -0.102, -0.114, -0.189, -0.089 and -0.10 for April, June, August, October, December and February, respectively. The total coliform survival ratio varied according to seasonal change. At 20 min, the log survival ratio was -2.30, -2.11, -1.89, -2.63, -2.70 and -2.28 for April, June, August, October, December and February, respectively. The effect of temperature on disinfection was not distinctive at 20 min. The lowest disinfection effect appeared in August, when the temperature was highest for a 20 min contact with chlorine. Therefore, the initial disinfection effect with chlorine was related mainly with temperature, it was gradually affected as the contact times passed by the other physicochemical factors in the water such as DOC and total nitrogen.

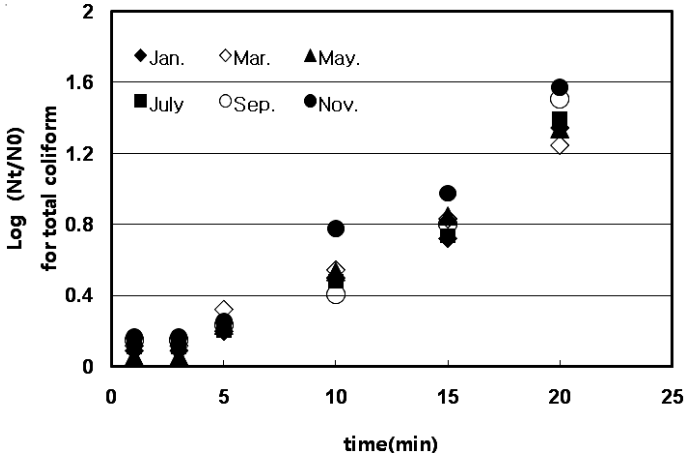

Fig. 3. Seasonal variation of total coliform inactivation with 3 mg/L chlorine for the finished treatment water at J municipal wastewater plant

The inactivation rate on the chlorine concentration for total coliform in finished sewage water is presented in Fig. 4. Samples in May were collected for this experiment. The inactivation rate was 98.6 % with 3 mg/L of chlorine at 20 min. However, the disinfection effect could reach over 99.99 % with 5 mg/L of chlorine dosage after 20 min.

The log inactivation was -0.11, -0.45, -1.00 and -1.85 for 3, 5, 10, 15 mg/L of chlorine dose at 1 min, respectively. 99.99 % of the inactivation rate could get to 20, 5 and 3 min for 5, 10 and 15 mg/L of chlorine dose, respectively. When chlorine doses increased, the times to reach the target inactivation levels have shortened. It was found that the range of chlorine levels between 5 and 15 mg/L of chlorine would be applicable to reach over 99.99 % of the inactivation rate for discharged water in these treated

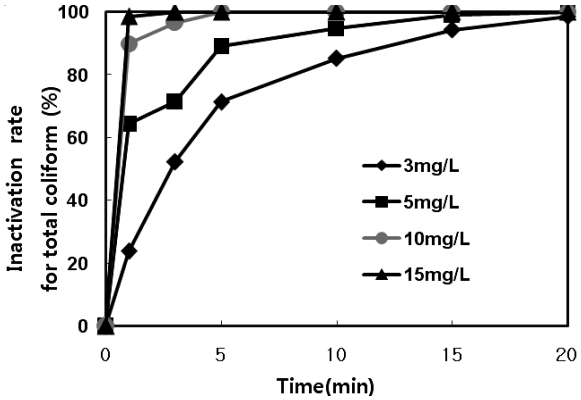

Fig. 4. Inactivation rate for total coliform on chlorine concentration

samples with suitable contact time. However, additional consideration for ecological and sanitary factors is needed to determine a reasonable disinfectant dosage and attains disinfection management strategies in this facility.

The disinfection effect of fecal coliform evaluated on contact time with 3 and 5 mg/L dose of chlorine is seen in Fig. 5. The log survival ratio at 20 min was -2.97 and -4.45 for 3 and 5 mg/L, respectively. Inactivation effect for fecal coliform was higher than that of total coliform with the same dose of chlorine. The log survival ratio for fecal coliform for 3 and 5 mg/L doses of chlorine was between 1.6 and 2.1 times higher than that of total coliform at 20 min.

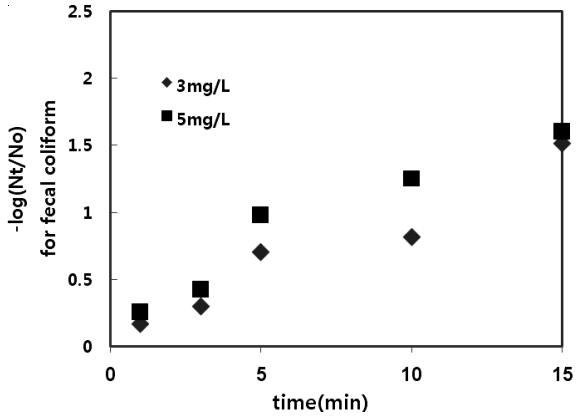

Fig. 5. log survival ratio for fecal coliform with 3 and 5 mg/L chlorine disinfection in finished water at J municipal wastewater plant

The variation of fecal coliform inactivation on chlorine concentration in the finished water is presented in Fig. 6. The inactivation rate with a chlorine dose of 3, 5, 10 and 15 mg/L was found to be 19.3, 43.6, 87.1 and

98.2 % at 1 min, respectively. A fecal coliform inactivation efficiency with a dose 15 mg/L could reach over 99.9 % at 5 min.

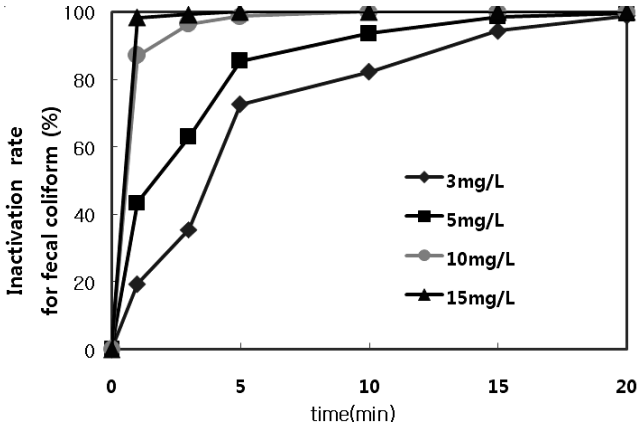

Fig. 6. Inactivation rate for fecal coliform on chlorine concentration

The log survival ratio for fecal coliform inactivation at 1 min was -0.11, -0.29, -0.77 and -2.21 for 3, 5, 10 and 15 mg/L of chlorine dosage, respectively. The level of the inactivation effect of chlorine on fecal coliform and total coliform was similar. It appeared that the selection in both microorganisms as an indicator for monitoring the disinfection effect in the municipal sewage treatment facility might have no significant difference.

Seasonal variation of inactivation efficiency for fecal coliform with 3 mg/L of chlorine is presented in Fig. 7. The fecal coliform inactivation rate increased with time, but was not significantly high before 5 min. Until 5 min, the fecal coliform inactivation could achieve 7-11 % of the final attained inactivation level at 20 min. The log survival ratio for fecal coliform inactivation at 20 min was -1.45, -1.53, -1.25, -1.50, -1.52 and -1.30 for January, March, May, July, September and November, respectively. The tendency of inactivation for fecal coliform was not evident by season. The inactivation efficiency for fecal coliform could achieve over 99 % with 3 mg/L chlorine through all months. It should be noted that the initial level of fecal coliform before chlorination in July was very high, 34 times than that of January.

The disinfection effect with 3 and 5 mg/L chlorine for the fecal streptococcus is presented on contact time in Fig. 8. The log ratio of fecal streptococcus at 10 min was found to be -1.53 and -2.64 for 3 and 5 mg/L, respectively. This is 2.1 and 2.2 times for the total coliform and fecal coliform for the chlorine dose of 5 mg/L. Therefore, the resistance of fecal streptococci against chlorine is weaker than those of total coliform and fecal coliform.

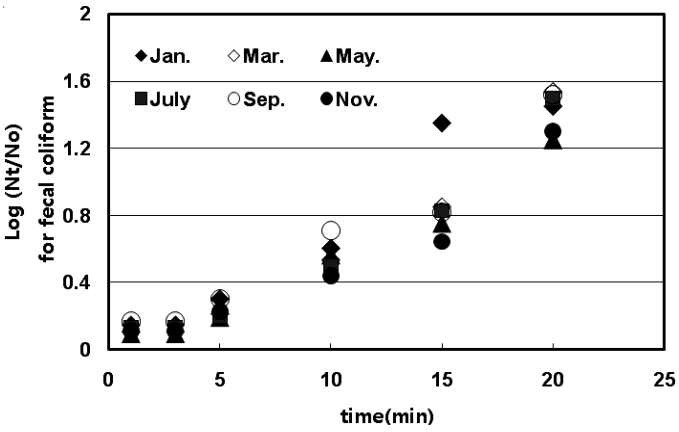

Fig. 7. Seasonal variation of fecal coliform inactivation with 3 mg/L chlorine for the finished treatment water at J municipal wastewater plant

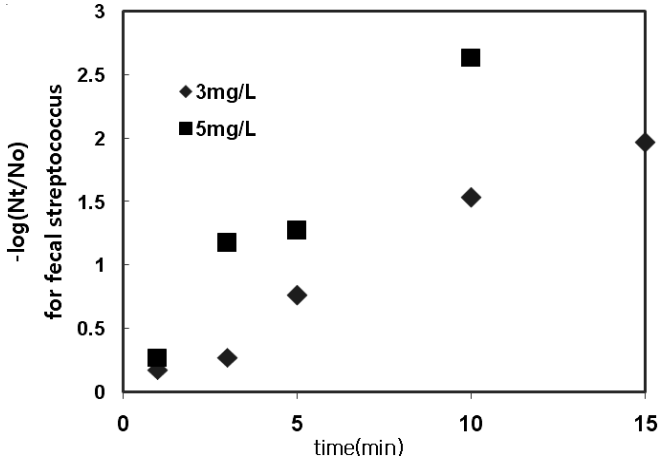

Fig. 8. log survival ratio for fecal streptococcus with 3 and 5 mg/L chlorine disinfection in finished water at J municipal wastewater plant

Seasonal variation for the disinfection of fecal streptococcus is presented in Fig. 9. It did not show the close relationship to temperature for the inactivation of fecal streptococcus. The log survival ratio of fecal streptococcus at 20 min for January, March, May, July, September and November was -2.40, -1.85, -1.77, -2.02, -1.97 and -3.53 with the application of chlorine, respectively. The highest efficiency was shown in November and the lowest was found in May.

The contact time had to be 10, 5 and 3 min for attaining the fecal streptococcus inactivation level of over 99.9 % for the chlorine dosage of 5, 10 and 15 mg/L, respectively. The inactivation rate at 1 min was found

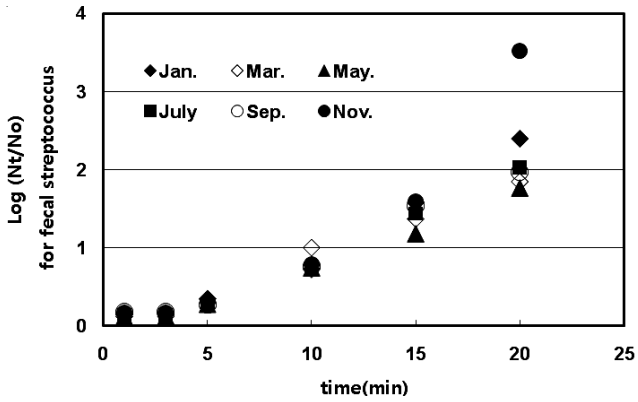

Fig. 9. Seasonal variation of fecal streptococcus inactivation with 3 mg/L chlorine for the finished treatment water at J municipal wastewater plant

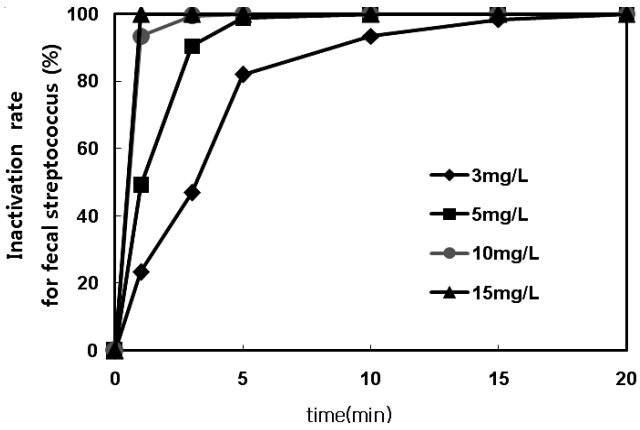

Fig. 10. Inactivation rate for fecal streptococcus on chlorine concentration

to be 23.40, 48.94, 93.40 and 99.96 % for chlorine dosage of 3, 5, 10 and 15 mg/L, respectively. The inactivation of fecal streptococcus could be attained sufficiently within the CT (disinfectant concentration × time) ranges for total and fecal coliform.

Evaluation of toxicity

The formation of THMs on two different temperatures are presented in Fig. 11. Kim *et al.*[19] reported THM formation increased with higher temperatures. Lee *et al.*[20] reported the concentration of THMs precursors is relatively low in winter compared to summer. However, the rate of THM formation does not show distinct consistency with temperature in this case. The level of THM formation in November which is a cold month was higher

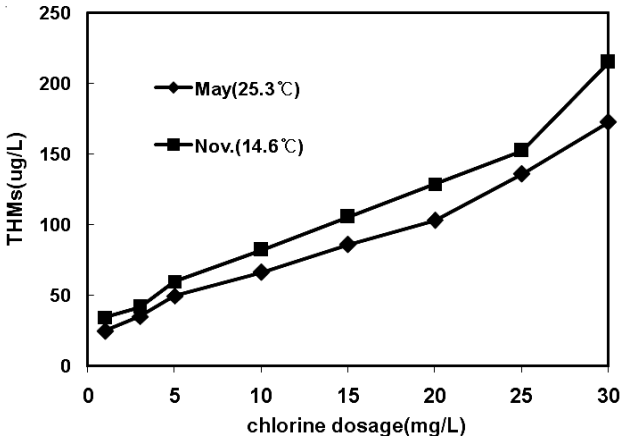

Fig. 11. THMs formation on chlorine concentration

than in May, a warm month. The condition of temperature in May was also higher than in November. It is thought that the formation of THMs is more affected by the condition of nutrition sources in samples than by temperature. The level of DOC in May is similar to that of November. But the level of COD_{Cr} in November was 2.8 times higher than in April. Nitrate concentration was 2.1 and 8.5 mg/L for May and November, respectively and NH_3-N was 1.66 and 1.37 mg/L for May and November, respectively. THM formation increased with chlorine dosage. The formation of THM increased 2.6 times when the level of chlorine dose increased 3 times for both months.

The formation of HAAs on water temperature is evaluated in Fig. 12. The formation rate for HAAs was not notably changed with the increase of temperature due to seasonal change. The level of HAAs formed increased with increasing dosages of chlorine. The formations of HAAs for May and November with a 30 mg/L chlorine dose were 2.1, 2.4 times that of a 10 mg/L of chlorine dose.

Oryzias latipes used to evaluate ecosystem toxicity and the influence of residual chlorine after the application of chlorine is presented in Fig. 13. 100 % of *Oryzias latipes* survived after 1 h with a 0.8 mg/L chlorine dose. 90 % of the survival rate for *Oryzias latipes* was measured after 12 h with a 0.7 mg/L chlorine dose and continued to 72 h. The survival rate of *Oryzias latipes* declined after 6 h with a 0.8 mg/L dose of chlorine. The survival rate for a 0.8 mg/L dose of chlorine was 50 % after 24 h and decreased steadily after the contact time. The survival ratio was 40 % after 72 h with a 0.8 mg/L chlorine dose. Finally, all *Oryzias latipes* were killed by chlorination after 24 and 48 h at 0.9 and 1 mg/L, respectively.

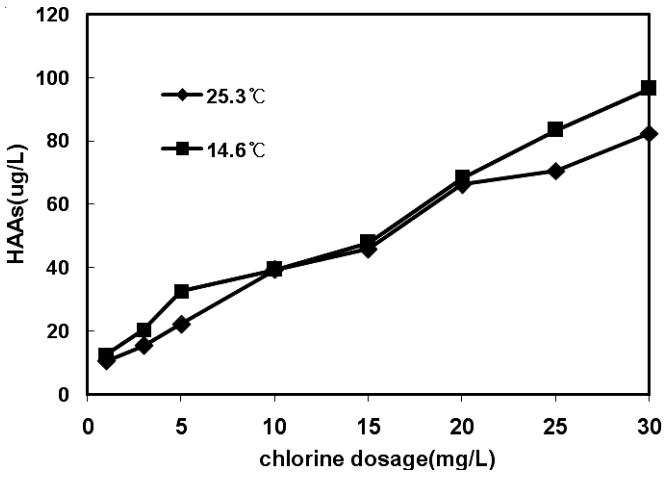

Fig. 12. HAAs formation on chlorine concentration

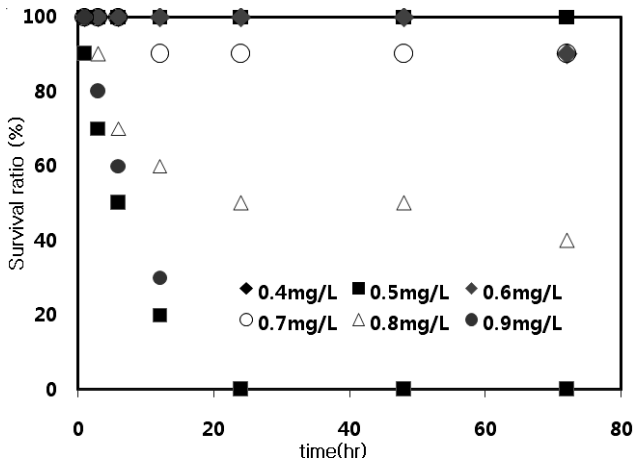

Fig. 13. Survival ratio of *Oryzias latipes* after chlorination with the discharged sewage samples in May

This test shows that chlorination for the sewage effluent can bring the harmful effects in ecosystem. Specifically, chlorination of the discharged sewage can cause detrimental results for the ecosystem and organisms at the chlorine dose over 0.8 mg/L with 1 h of contact time. The values of LC_{50} during 24 and 48 h are 0.784 and 0.778 mg/L, respectively according to the Korean Industrial Standard.

Conclusion

The present results performed to assess the inactivation effect for indicator organisms and evaluated crucial problems associated with chlorination, such as the occurrence of toxicity and disinfection by-products in the ecosystem. The following important conclusions were made in this research.

The empirical model with important factors such as NH_3-N, DOC and COD_{Cr} was made to predict the number of total coliforms, fecal coliforms and fecal streptococcus in finished sewage water. The correlation coefficients were over 0.94 for the individual equations and indicated that the multiplication of microorganisms was closely related to the chemical nutrient factor in finished sewage.

The initial inactivation effect was affected by seasonal changes related to temperature. While sufficient contact time was given, the effect on temperature was not evident. The resistance of fecal streptococcus against chlorine was weaker than total and fecal coliform. So, within the required scope of dose chlorine and the contact time for total coliform and fecal coliform, fecal streptococcus could be securely inactivated.

The mean number of total coliform and fecal coliform in finished water was 1.20×10^5 and 3.81×0^4 MPN/100 mL. The disinfection process is needed to reach the legal standard and secure the sanitary safety for public health in summer month. The problem with the formation of THM was not significantly observed under a dose of 15 mg/L with the consideration of a dilution effect with nature surface water while it is discharged. However, it was found that LC_{50} for 24 h was measured as 0.8 mg/L by toxicity test for *Oryzias latipse*.

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