Practical selection of microorganisms indicating the stability of pathogenic removal in water treatment plants

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Abstract There have been several recent drinking water incidents in connection with detection of water borne pathogens in Korea. This has led to the need for research to find microorganisms that will indicate the stability of pathogenic removal in water treatment plants (WTPs). We investigated seven microorganisms as potential indicators: four for bacteria [*E. coli*, fecal coliform (FC), total coliform (TC) and heterotrophic plate counts (HPC)], two for protozoan [aerobic spore formers (ASF) and *Clostridium perfringens* (CP) spore], and one for viruses (F-specific RNA coliphage). Source water, treated water from each process, and finished water were analyzed periodically from three WTPs, chosen in view of the source water quality and plant size. This study concludes that HPC and ASF appeared to be good indicators of the stability of pathogenic removal. Although this study supports ASF as the best indicating microorganism, the potential use of HPC as an indicating microorganism was demonstrated in this study. From this study, the regrowth problem of HPC in water plants was shown to be negligible depending on the characteristics of the chlorination practice at the WTPs, and temperate climate conditions. The relative importances of each treatment process, for the removal of suggested indicating microorganisms, were found to be as follows: combined process of coagulation and sedimentation with prechlorination > filtration ≈ disinfection, with emphasis on the necessity for the optimization of whole water treatment processes for effective microbial removal.

Keywords Aerobic spore formers; disinfection; heterotrophic plate counts; indicating microorganisms

Introduction

Water treatment requirements are becoming increasingly stringent due to the emergence of pathogens that challenge water treatments, such as *Cryptosporidium*, *Giardia*, and viruses, and their potential presence in potable water (Pontius, 1999). In Korea, incidents of the pathogen detection in drinking water during a national survey, indicated that a comprehensive management plan for drinking water quality was needed, which should include the introduction of treatment regulations similar to the US-SWTR (Surface Water Treatment Rule) (US-EPA, 1991). This was supported by reports that many WTPs in Korea would not meet the CT requirement of the US-SWTR should the worst case scenario occur (Yoon *et al*., 1998, 2001). The rapidly changing environment in drinking water production requires water utilities to strengthen their microbiological monitoring of raw water, and to optimize treatment processes for effective microbial removal.

Treatment by turbidity criteria and CT requirement does not guarantee safety from pathogenic contamination of drinking water. This is because water borne outbreaks have been reported, even when finished water is in full compliance with regulatory requirements, due to the highly variable microbial load and water quality. Detecting pathogens, such as protozoa and viruses, requires a high level of skill and cost, rendering frequent monitoring cost prohibitive. The absence of quick, economical and reliable analytical methods for pathogens has resulted in the necessity for research to find a method for

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effective pathogenic removal from water treatment processes. Much research has been undertaken to find surrogate microorganisms, such as ASF, CP and F-specific phages, in order to evaluate the microbial treatment targeted for specific pathogens (Nieminski *et al.*, 2000; Crockett and Procopio, 1998; Rice *et al.*, 1996; Hinjen *et al.*, 1997; Havelaar, 1986). ASF was recently suggested as a microbial surrogate for evaluating drinking water treatment performance (Nieminski *et al.*, 2000; Rice *et al.*, 1996). There are also reports regarding bacterial treatment efficiency such as coliforms and *E. coli* by HPC monitoring in WTPs (Reasoner, 1990), although these studies did not properly address the fact that ASF is a part of HPC. Analysis of ASF becomes inconvenient due to the heat treatment required when large sample volumes are measured, compared to that of HPC.

This study was performed to find practically useful microorganisms for indicating the stability of pathogenic removal in WTPs, and consisted of three parts. Firstly, the evaluation of several indicating microorganisms for their microbial removal from three water treatment plants. Secondly, the role of each unit treatment in terms of microbial removal was assessed on full-scale WTPs. Thirdly, any relationships between indicating microorganisms were partly discussed.

Materials and methods

Study sites

Three WTPs (IS, WP, and OR) were chosen, according to the characteristics of the water sources [lake or river: high or low biological oxygen demand (BOD)] and the size of plants (small, medium, or large). These test sites represented several watersheds across the southern Korean peninsula (Figure 1). Table 1 shows the general characteristics of source water quality and treatment facilities at each plant. The IS, WP, and OR plants use the Han River, Daechung Lake, and Keum River for source water, respectively. Daechung Lake is located upstream from the Keum River. Water quality of the WP plant is the best in terms of turbidity (2.5–5.8 NTU) and BOD (0.6–1.1 mg/L). The treatment processes of all three plants consisted of coagulation, sedimentation, filtration and free chlorine disinfection. Prechlorination practices were performed at the coagulation step in all three plants for the purpose of meeting the short-term chlorine demand of the source water.

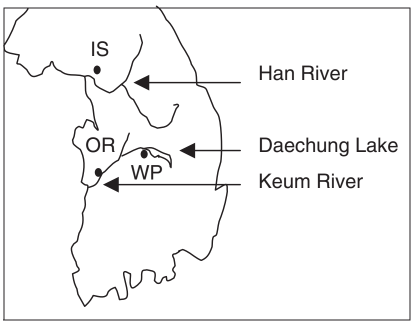

Figure 1 Location of water treatment plants where this study was conducted

Table 1 General characteristics of each water treatment plant (provided by WTP operator)

Plants	IS	WP	OR
Source water	Han River	Daechung Lake	Keum River
Capacity (m^3/day)	250,000	400,000	22,000
Production (m^3/day)	160,000	280,000	17,000
BOD (mg/L)	2.5[2)] (1.3–3.0)	0.9 (0.6–1.1)	2.5 (1.9–3.1)
Turbidity (NTU)	9.6 (2.0–30)	5.3 (2.5–5.8)	19.0 (6.8–51)

[1] Average value

Sample analysis

A total of seven candidate microorganisms were examined as potential indicators: four for bacteria [*E. coli*, fecal coliform (FC), total coliform (TC) and heterotrophic plate counts (HPC)], two for protozoan [aerobic spore formers (ASF) and *Clostridium perfringens* (CP) spore], and one for viruses (F-specific RNA coliphage). The analyses for HPC, *E. coli*, FC, and TC followed those delineated by *Standard Methods for the Examination of Water and Wastewater* (1995). CP and F-specific RNA coliphage were analyzed by the ICR method of the US-EPA (1996), and ASF was measured by the Rice *et al*. method (1996). Concentrations of microorganisms in each sample were measured from replicate tests of each serial 10 fold dilution. As shown in Table 2, the sample volume of raw water used was up to 1 mL for detecting HPC and ASF, and up to 100 mL for detecting CP, F-specific RNA coliphage, TC, FC and *E. coli*. Sample volumes of treated water were increased up to 1–7 L, which is the maximum volume applicable without additional concentration pre-treatment. Further measurements were not attempted for TC, FC and *E. coli* as their detection were not expected in treated water, as deduced from preliminary tests. Sampling for measuring each candidate microorganism was made every month, or every other month, for 20 months, between April 1999 and November 2000, in source and treated water of WTPs.

Data analysis

To evaluate microbial removal in each unit process of WTPs, the log removals of microorganisms were calculated using:

$$\text{log removal} = \log\left(\frac{C_1}{C_2}\right)$$

where C_1 = the microorganisms concentration of influent entering the specific process, and C_2 = the microorganisms concentration of effluent from the specific process. For data analysis, the non-detected values were replaced by the detection limits calculated from the smallest concentration in sum of the tested volumes of replicate samples.

Results and discussion

Three experimental schemes were chosen to find out the indicating microorganisms. First, attempts to find indicating microorganisms were made based on their occurrence characteristics in raw and finished waters. Indicating microorganisms had to partially meet the following considerations: (1) abundant occurrence in raw water; (2) presence in finished water, to give some quantitative information for microbial removal; and (3) correlation between pathogen and non-pathogenic indicating microorganism.

Figure 2 indicates the occurrences of microorganisms in raw water, which are expressed as the geometric mean and standard deviation. Three interesting observations can be made from the results in Figure 2. First, the HPC and ASF in raw water were the most abundant.

Table 2 The detection methods and tested sample volumes[1]

Sample	Microorganism				
	HPC	ASF	CP	F+phage	TC, FC, *E.coli*
Raw water	1 mL PPM[2]	1 mL PPM	100 mL MFM[3]	100 mL PAM[4]	100 mL MFM
Treated water	1 L MFM	1 L MFM	7 L MFM	5 L PAM	–

[1] Maximum sample volume tested
[2] PPM = pour plate method
[3] MFM = membrane filter method
[4] PAM = plaque assay method

The HPC concentrations in the IS, WP, and OR plants were found to be 36,000, 44,000, and 170,000 cfu/100 mL, respectively. The ASF concentrations found were 3,600, 2,200, and 7,100 cfu/100 mL in each plant, respectively, and were the second most abundant. The HPC concentrations were higher, by more than an order of magnitude, than those of ASF, in raw water. Large concentrations of HPC and ASF provide the possibility of indicating microorganisms for treatment performance evaluation with regard to quantitative microbial removal larger than three or four log removals. Second, despite the different water quality of the three plants, the magnitude of microbial concentration were found in the following decreasing order: HPC > ASF > TC > FC ≥ CP ≥ *E. coli* > F-specific RNA coliphage. One exception was that the CP concentration at the OR plant was slightly larger than the FC concentration. Third, the standard deviations of the ASF and CP concentrations are relatively small compared to the other five microorganisms, indicating the less seasonal variation of the aerobic and anaerobic spores. Next to the ASF and CP, the seasonal variation of HPC was relatively small.

Another important requirement for being an indicating microorganism is presence in finished water, at a detectable concentration. This requirement is essential since it provides quantitative information of the microbial removal performance. Otherwise, the microorgansim removal tends to be underestimated. Figure 3 shows the detection characteristics of the candidate microorganisms in finished waters. The HPC and ASF were the most

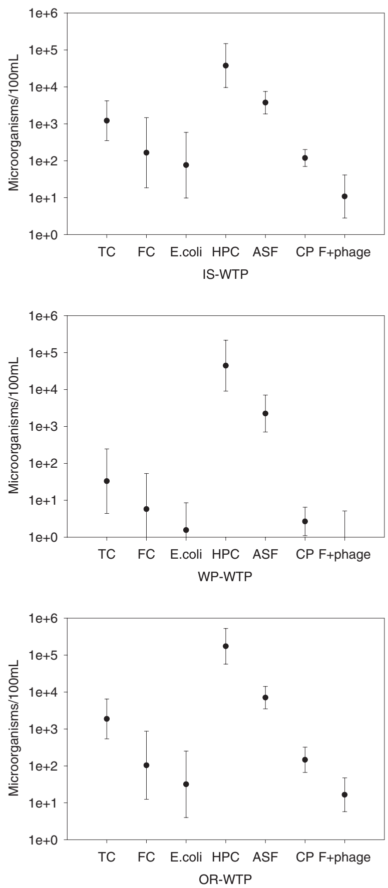

Figure 2 Occurrence of microorganisms in raw water of each WTP: geometric mean and standard deviation

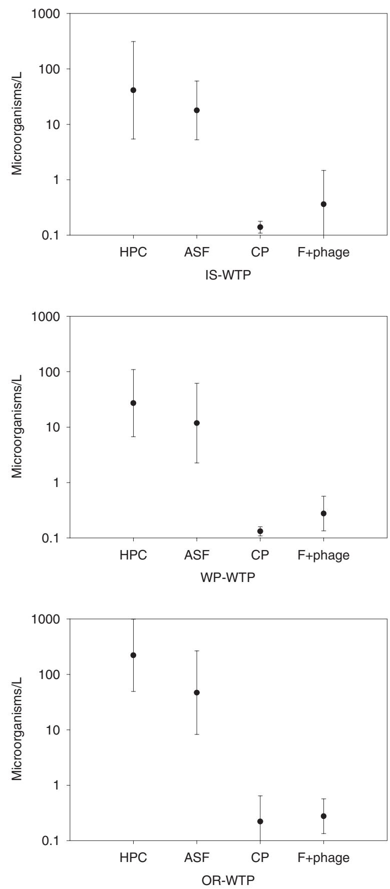

Figure 3 Occurrence of microorganisms in finished water of each WTP: geometric mean and standard deviation

abundant in source waters, and were detected in finished waters at a level of concentration larger than 1 cfu/L. It is worth noting that HPC is a quality parameter for drinking water (100 cfu/mL) and the sample volume of finished water used was increased one thousand times. The HPC concentrations at the IS, WP, and OR plants were found to be 38, 27 and 221 cfu/L, respectively, and those of ASF were found to be 15, 12 and 47 cfu/L. HPC and ASF were detected in all samples from the three plants, with the exception of one non-detected sample of ASF at the WP plant. Conversely, the CP and F-specific coliphage concentrations detected were low in raw water, at a level of 1 cfu/L, and rarely detected in finished waters. Because CP and F-specific coliphage have a non-detectable nature, their real concentrations in finished water are lower than expressed in Figure 3. The results shown in Figures 2 and 3 suggest ASF and HPC have the potential to be indicating microorganisms.

The second part of this study examined the extent of microbial removal by the selected microorganisms in each unit treatment process at the WTPs. This investigation was designed to gain an understanding of the removal behaviours of the indicating microorganisms, and to find the relative importance of each unit treatment process in terms of microbial removal. Figure 4 shows the total log removal of the microorganisms at the three water plants, and indicates that more than three log removals of HPC and ASF, expressed as a geometric mean, can be achieved at all three plants. Other microorganisms could not be assessed for removals larger than three log removals because they were found to be lower than 100 cfu/100 mL in raw waters, and were undetected in finished waters (sample volume of 1 L). Although much research has suggested CP to be a good indicator for treatment resistant pathogens, such as *Cryptosporidium and Giardia* (Crockett and Procopio, 1988; Hinjen *et al.*, 1997), it appeared to give high removals in comparison with HPC and ASF at some WTPs.

Figures 5 and 6 show the contribution of the combined processes of coagulation, sedimentation and filtration, in the removal of microorganisms. As mentioned earlier, these three plants had similar treatment processes. The coagulation and sedimentation in water plants successfully achieved more than a 1.5 log removal of HPC and ASF, in most cases (Figure 5). However, in half the samples, less than one log removal was achieved at the filtration step (Figure 6). It was surprising to observe larger microbial removal from the combined processes of coagulation and sedimentation as compared to filtration only, because filtration is considered more important for microbial removal than the aforementioned (Rice *et al.*, 1996). This result emphasizes the necessity for optimizing the whole water treatment process for effective microbial removal. This further supports the validity of the multiple barrier concept. One possible explanation for the larger microbial removal by coagulation and sedimentation is prechlorination, because all three plants performed prechlorination at the coagulation step. This explanation partly agrees with the large microbial removal at the OR plant, where higher chlorine dosage are used (1.3–17.8 mg/L

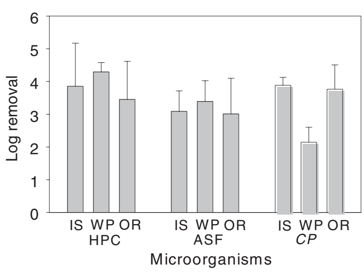

Figure 4 Total log removal of microorganisms through water treatment process

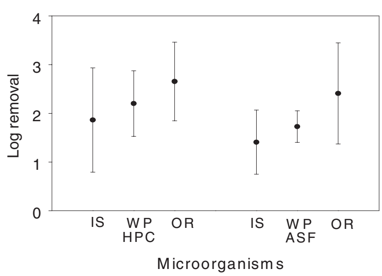

Figure 5 Log removal of microorganisms in coagulation and sedimentation

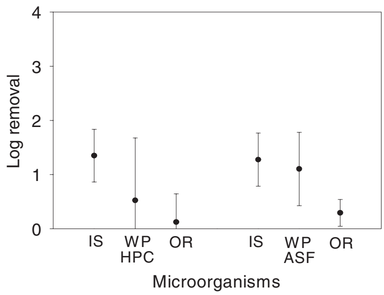

Figure 6 Log removal of microorganisms in filtration step

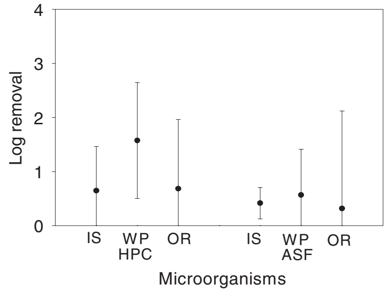

Figure 7 Log removal of microorganisms in disinfection step

measured as short-term chlorine demand) to remove ammonia present in the source water using breakpoint chlorination. However, this explanation needs to be investigated by an independent examination of the prechlorination from coagulation and sedimentation.

Figure 7 shows the log removal of microorganisms in the disinfection process. More than one log removal of microorganisms was rarely observed in the disinfection step. Such low log removal reflects the strong resistance of HPC and ASF to chlorine disinfection, even though slightly better inactivations of HPC than ASF were observed. The inactivation of ASF in this study agrees well with the results of Rice *et al*. (1996), that also reported high resistance of ASF compared to that of *Giardia lamblia* cyst.

Figure 8 shows the seasonal characteristics of microbial removals by the disinfection process. HPC removal by the disinfection process is quite low, approximately 0.3 log (geometric mean), but the difficulty with the inactivation of ASF got worse, and it was not removed at all in many cases. This observation is satisfactorily explained by the inactivation of these microorganisms, by chlorine, is more difficult at low temperature, as reported in many laboratory scale disinfection studies (US-EPA, 1991). However, little research demonstrating the low inactivation in winter was available for full scale WTPs (Reasoner, 1990). Low inactivation of microorganisms is important since it mainly determines the design of disinfection facilities at WTP. The potential regrowth of HPC, the criticizing feature as indicating microorganisms, was not observed significantly when HPC was tested in the unit processes. Only one piece of data, which occurred in summer at the IS plant, was supposed for microbial regrowth out of 38 samples.

Figure 9 shows the overall relative contribution of each treatment process in removing HPC and ASF. The relative importance of each treatment process are as follows: the combined process of coagulation and sedimentation with prechlorination [HPC 59% (48–77%), ASF 59% (45–80%)] > filtration [HPC 17% (3–35%), ASF 28% (10–41%)] ≈ disinfection [HPC 24% (17–37%), ASF 14% (11–17%)].

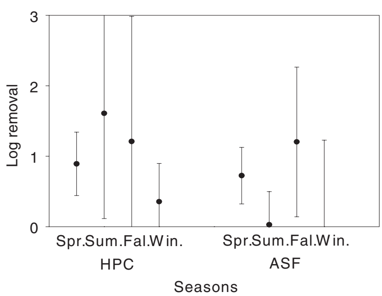

Figure 8 Seasonal variations of log removal in disinfection step

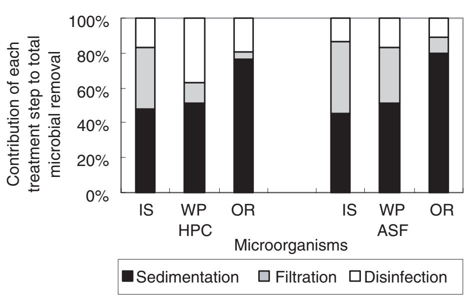

Figure 9 Contribution of each treatment step to total microbial removal in water treatment plants

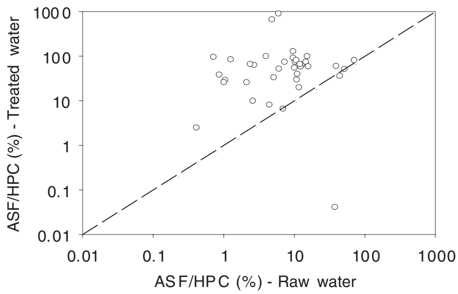

Figure 10 ASF/HPC(%) ratio between raw water and finished water

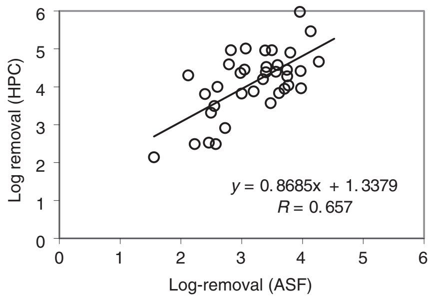

Figure 11 Relation between log removal of HPC and ASF

In the third part, relations of HPC with ASF were examined, as ASF was reported to be a good indicator compared to pathogens in previous studies. Figure 10 shows the ASF/HPC (%) ratio between raw and finished waters. This result indicates that HPC is better than ASF at microbial removal by about one order of magnitude, and is as expected from the microbial nature of HPC and ASF. HPC could be divided into two major subpopulations depending on its resistance to environmental stress. One is the vegetative bacteria being vulnerable in chlorine disinfection. The other is the so-called spore forming bacteria ASF, which is resistant to chlorine disinfection. The percentage of spore forming bacteria in HPC at each WTP ranged from 4–10% in raw water, but increased to 21–40% after treatment.

Figure 11 shows the relationship between HPC and ASF log removal. HPC appeared to be the compromised choice from the results with a good linear relation to ASF ($R = 0.657$).

Conclusion

In the field evaluation, HPC and ASF appeared to be the best indicating microorganisms for achieving the stability of pathogenic removal. Although this study confirms previous reports of ASF being a good indicating microorganism, the potential use of HPC has been demonstrated in this study. However HPC, as a potential indicating microorganism, has been criticized due to the associated regrowth problems in water treatment processes. This regrowth problem can be negligible if WTPs perform prechlorination and are located in temperate climate zones. Before widespread use of HPC in WTPs as a routine microbiological monitoring parameter can be considered, further research will be needed to examine the extensive integrity of HPC as an indicating microorganism. The relative importance of each treatment process for the removal of suggested indicating microorganisms were found to be as follows: the combined process of coagulation and sedimentation with prechlorination > filtration ≈ disinfection, emphasizing the necessity for optimization of whole water treatment processes for effective microorganism removal.

Acknowledgement

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