

Influence of disinfection on bacterial regrowth in pilot distribution system

Young-Cheol Chang*, Andrew Andy Randall**, OnYou Choi***, DuBok Choi***,
Hoon Cho****†, and Shintaro Kikuchi*

*Division of Applied Sciences, College of Environmental Technology, Graduate School of Engineering,
Muroran Institute of Technology, 27-1 Mizumoto, Muroran 050-8585, Hokkaido, Japan

**Department of Civil and Environmental Engineering, University of Central Florida,
P.O. Box 162450, Orlando, Florida 32816-2450, USA

***Department of Environmental health, Cho-dang University,
419, Songnam-ri, Muan-up, Muan-gun, Jeonnam 534-800, Korea

****Department of Polymer Science and Engineering, Chosun University, Gwangju 501-759, Korea
(Received 18 May 2010 • accepted 13 July 2010)

Abstract—A correlation between heterotrophic plate count (HPC) and chloramine residual in pilot distribution systems (PDSs) was investigated. The data was derived from an AWWARF (the Awwa Research Foundation) and Tampa Bay Water tailored collaboration project to determine the effect of blending different waters on distribution system water quality. Seven different finished waters were produced from surface, ground, or simulated brackish water sources on site and fed to 18 independent PDSs, either as a single finished water or as a blend of several finished waters. Significantly higher numbers for PDS HPC were observed below 0.06 mg/L of combined chlorine residual. Changes in assimilable organic carbon (AOC) levels between influent and effluent of the PDSs increased as disinfectant dosage decreased in distribution systems. Significant differences between input and output AOC (Δ AOC) were observed when the chloramine residual was less than 1.0 mg/L, and particularly when less than 0.5 mg/L. High HPC counts often occurred when chloramine residual was less than 0.5 mg/L, regardless of AOC levels or AOC stability. However AOC instability could occur at high influent AOC levels even in the presence of residual greater than 0.5 mg/L, with corresponding high HPC counts.

Key words: Assimilable Organic Carbon, Biostability, Chloramine, Pilot Distribution System

INTRODUCTION

One aspect of maintaining water quality in drinking water distribution systems is controlling biofilm that forms on distribution systems' pipe walls. Bacterial numbers tend to increase during distribution and are influenced by a number of factors including the microbiological quality of the finished water entering the system, temperature, residence time, presence or absence of a disinfectant residual, construction materials, and availability of nutrients for growth [1-5]. If the disinfectant residual is sufficient, bacterial regrowth is significantly reduced in bulk water. Nevertheless, it is widely appreciated that bacteria can attach and grow on the surface of pipe materials [6]. Suspended cells are considered to be introduced into the liquid phase from the biofilm through the detachment process during the distribution of drinking water and control of suspended cell concentrations can be assisted by minimizing the number of biofilm cells. Conventional water treatment in North America has traditionally added a secondary disinfectant (chlorine and/or chloramine) to achieve biological stability. The strong trend toward replacing free chlorine with chloramine to meet more stringent disinfection byproduct regulations in drinking water may also have an impact on bacterial regrowth in distribution systems. Chloramine produces

a more stable residual than free chlorine and provides lasting protection against bacterial regrowth. In addition, chloramine is believed to penetrate more deeply than chlorine within biofilm [7]. However, chloramine potentially adds a nutrient, ammonia, to the distribution system [8]. Organic substrates in drinking water can be estimated by the assimilable organic carbon (AOC) assay. AOC is the part of dissolved organic carbon (DOC) that can be easily assimilated by bacteria and converted to cell mass. The biostability of drinking water may be assessed by monitoring AOC since heterotrophic bacterial growth is supported by biodegradable organic matter in drinking water and most distribution systems are carbon limited. Numerous laboratory studies [9-16] as well as pilot plant or field studies [17-20] have demonstrated that residual chlorine can limit biofilm formation in distribution systems. However to ensure effective distribution of biologically safe water, more data need to be collected to assess practical dose and residual levels of disinfectant. This is especially true for chloramine in North America as water treatment plant chloramination has increased dramatically in recent years.

This paper demonstrates a significant correlation between heterotrophic plate count (HPC) and combined chlorine residual, as well as a correlation between changes in AOC levels and combined chlorine residual. High HPC counts often occurred at low residual levels regardless of AOC levels or AOC changes. However, high HPC counts in waters having >0.5 mg/L chloramine residual correlated directly with AOC instability and level.

†To whom correspondence should be addressed.
E-mail: hcho@chosun.ac.kr

MATERIALS AND METHODS

1. Heterotrophic Plate Count (HPC)

HPC measurements were performed by the spread plate method using R2A agar incubated at 22 °C for 7 days, according to *Standard Method* 9215B [21]. Results were expressed in colony-forming units per milliliter (cfu/mL). The procedure was performed entirely inside a laminar flow hood (model 62674, Enviroco Corporation, Albuquerque, NM, USA) equipped with a HEPA filter.

2. Assimilable Organic Carbon (AOC)

AOC was measured using the rapid method of LeChevallier et al. [22], except that plate counts were used to enumerate bacteria rather than ATP fluorescence, in conjunction with *Standard Methods* 9217 [21] and the method of Van der Kooij [23]. The procedure used a temperature of 25 °C for sample incubation as detailed by Escobar and Randall [24]. Quality control for the AOC bioassay was performed using blank controls, 100 µg/L sodium acetate standards, and duplicate samples. The 100 µg/L sodium acetate standards inoculated with P17 produced an average AOC of 93.80 ± 20.00 µg/L as acetate-C, while for NOX, they produced an average AOC of 77.20 ± 12.53 µg/L as acetate-C. Experimental yield values from acetate standards for P17 ($4.08 \pm 0.81 \times 10^6$ cfu/µg of acetate-C) and NOX ($9.26 \pm 1.50 \times 10^6$ cfu/µg of acetate-C) compared reasonably well with the literature values as specified in *Standard Methods* (4.1×10^6 and 1.2×10^7 cfu/µg of acetate-C for P17 and NOX, respectively).

Literature yield values were used for the AOC calculations to conform to the *Standard Method* [21]. A significant correlation between the AOC concentration and the density of heterotrophic bacteria in distribution water supplies has previously been reported [4,23].

3. Experimental Setup

Waters produced from seven different treatment systems (aeration (G1), softening (G2), Blended softening (G3), Blended NF (G4), CSF-O₃-BAC (S1), IMS (CSF-NF or S2) and high pressure RO were blended and distributed to 18 different pilot distribution systems



Fig. 1. Pilot distribution system for bacterial regrowth.

Table 1. Operation protocol

PDS	Sources and (%)	PDS	Sources and (%)
01-Hybrid	G1(100)	10-Hybrid	G2(50), S1(50)
02-Hybrid	G2(100)	11-Hybrid	G2(62), S1(24), RO(14)
03-Hybrid	S1(100)	12-Hybrid	G3(100)
04-Hybrid	G4(100)	13-Hybrid	S2(100)
05-Hybrid	RO(100)	14-Hybrid (High Freq.)	G1(62), S1(27), RO(11)
06-Hybrid	G1(55), S1(45)	15-Unlined Cast Iron	G1(23), S1(45), RO(32)
07-Hybrid	G1(68), RO(32)	16-Lined Cast Iron	G1(23), S1(45), RO(32)
08-Hybrid	G1(23), S1(45), RO(32)	17-PVC	G1(23), S1(45), RO(32)
09-Hybrid	G1(60), S2(30), RO(10)	18-Galvanized	G1(23), S1(45), RO(32)

Table 2. Pilot unit description

Symbol	Source water	Unit processes
G1	Ground water source	Treatment by aeration, disinfection by free chlorine with a residual of 4.0 mg/L after a 5 minute contact time, 4.0 mg/L chloramine residual
G2	Ground water	Treatment by lime softening to total hardness of 120 mg/L CaCO ₃ , Disinfection by free chlorine with a residual of 4.0 mg/L after a 5 minute contact time, 4.0 mg/L chloramine residual
G3	Blend of finished G1, S1 and RO water source	Treatment by lime softening to total hardness of 120 mg/L CaCO ₃ or alkalinity of not less than 50 mg/L, 4.0 mg/L chloramine residual
G4	Blend of finished G1, S1 and RO water source	Treatment by membrane nanofiltration aeration, 4.0 mg/L chloramine residual
S1	Surface water	Treatment by ferric sulfate coagulation flocculation settling filtration disinfection by azonation biologically activated carbon filtration, 4.0 mg/L chloramine residual
S2	S1 after sedimentation	Followed by nanofiltration, aeration and disinfection by free chlorine with a residual of 4.0 mg/L after a 5 minute contact time, 4.0 mg/L chloramine residual
RO	Ground water	Treatment by membrane reverse osmosis aeration disinfection by free chlorine with a residual of 4.0 mg/L after a 5 minute contact time, 4.0 mg/L chloramine residual

(PDSs) (Fig. 1 and Table 1). The preceding acronyms are defined accordingly: G: groundwater; S: surface water; NF: nanofiltration; CSF: coagulation-sedimentation-filtration; BAC: biological activated carbon; IMS: integrated membrane system; RO: reverse osmosis (Table 2). G1, G2, G3, G4 and RO finished waters are produced from the same ground water (Cypress Creek Water Treatment Plant, Polk County, Florida, USA). Salts are added in RO permeate to simulate typical finished water from a desalination process. The S1 and S2 finished waters are produced from the same surface water (Hillsborough River Water Treatment Plant, Hillsborough County, Florida, USA). The PDSs consist of combined PVC, galvanized, lined ductile iron and cast iron pipes taken from actual distribution systems and have a 5-day hydraulic residence time (HRT). Hypochlorite (NaOCl) and ammonium chloride (NH_4Cl) were used to adjust the total chlorine concentration to 4.5 mg/L using a 4 : 1, Cl_2 to NH_3 -N mass ratio.

RESULTS AND DISCUSSION

Experiments were conducted for about nine months. The samples were taken five times, February 12th, March 28th, May 16th, June 20th, and August 1st, respectively, from the influent and effluent sampling port of each PDSs during the study at 21.6 °C (average temperature). HPCs and chlorine residuals were monitored. The relationship between effluent HPCs and chloramine residuals is given

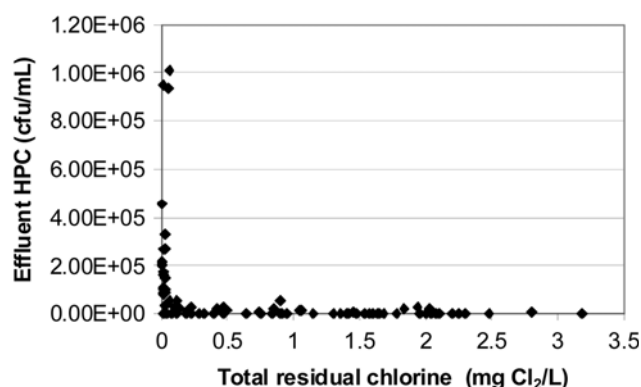


Fig. 2. Correlation between effluent HPC and total residual chlorine.

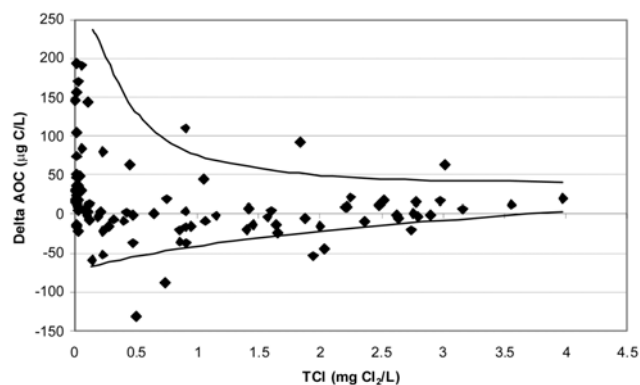


Fig. 3. Correlation between delta AOC and total residual chlorine (TCI).

in Fig. 2. Significantly higher numbers of HPC were observed below 0.06 mg/L of total chlorine residual (Fig. 2). The difference between AOC influent and AOC effluent (ΔAOC) was determined and the relationship between delta AOC and total chlorine residual is shown in Fig. 3. Relatively stable delta AOCs ($\pm 50 \mu\text{g C/L}$) were observed at chloramine residuals of 1.0 to 4.0 mg/L. However, AOC responded dramatically at low chloramine residuals. A gradual increase of delta AOC was observed as the chloramine residual was less than 1.0 mg/L. This included not only consumption of AOC, which increased at residuals below 1.0 mg/L, but also production of AOC starting below 2 mg/L, and accelerating below 1.0 mg/L. Similar increases of AOC levels in warm distribution systems have been observed [24,25] and fermentative yeast were implicated [26].

When residual chloramine level was less than 0.5 mg/L, high HPC values were observed in samples with low or high AOC, and with stable or unstable AOC as shown in Fig. 2. However, when the chloramine residual was above 0.5 mg/L high HPCs were typically associated with unstable AOC as shown in Fig. 4. The data points above $|\Delta\text{AOC}| 40 \mu\text{g C/L}$ in Fig. 4 had an average residual of 1.29 mg/L and influent AOC of $122 \mu\text{g C/L}$. Below $40 \mu\text{g C/L}$ the residual was similar at 1.45, but influent AOC was $76 \mu\text{g C/L}$. This implies exceeding an influent AOC value somewhere between 76 and $122 \mu\text{g C/L}$ promotes biostability in systems maintaining a residual. For samples with chloramine residuals above 0.5 mg/L HPCs correlated better with the absolute value of delta AOC rather than delta AOC or AOC levels (Fig. 4). However, it should be noted that the absolute value of delta AOC correlated well with AOC levels as shown in Fig. 5. The values of first-order regression obtained in

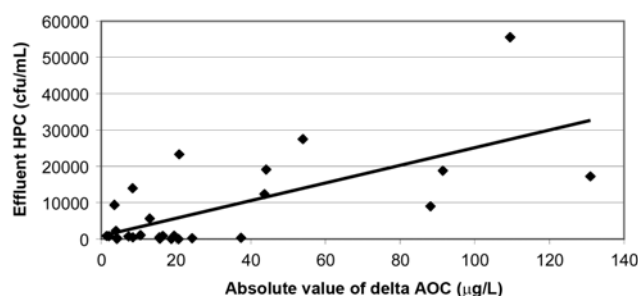


Fig. 4. Correlation between effluent HPC and absolute value of Delta AOC.

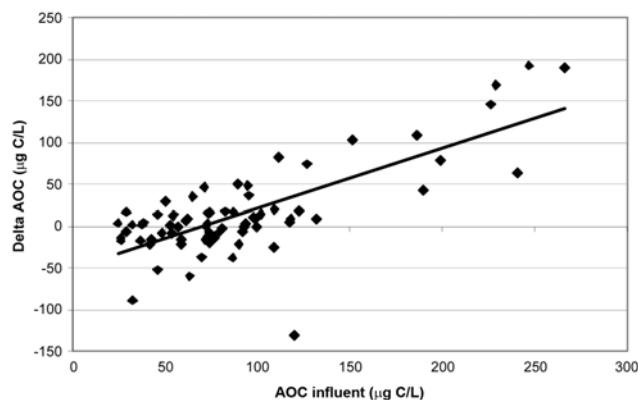


Fig. 5. Correlation between delta AOC and AOC influent.

Figs. 4 and 5 were within 95% confidence intervals. Taking another look at Fig. 3 and 4, it is notable that the points outside both the top and bottom boundaries in Fig. 3 had high HPC levels. These data points, with only one exception, are the same data points above 40 $\mu\text{g C/L}$ shown in Fig. 4. Those above the top boundary had average $\text{AOC}_{\text{inf}}=167 \mu\text{g C/L}$ in Fig. 3. Those below typically had unusually low or high influent AOCs but there was no truly consistent pattern. If AOC increased because of fermentation of non-AOC organic carbon to AOC, the fermentable carbon would not be observed in the AOC_{inf} sample. Thus, it might be expected to not correlate with influent AOC values. The implications are that even when AOC concentration declined to low levels, high HPC counts occurred if the chloramine residual was depleted. However, when residuals were maintained above 0.5 mg/L, reduced AOC levels made it less likely for high HPC counts to occur. Chlorine residual was the most important single parameter for suppressing high HPC counts (Fig. 2), but AOC was also significant and could result in elevated HPC counts even in the presence of residuals ranging from 0.9 to 3.02 mg/L (Fig. 3 and 4). However, high chloramine residuals suppressed AOC transformation and HPC count to some extent (Fig. 3). In this study, a concentration of total residual chlorine lower than 1.0 mg/L, and particularly below 0.5 mg/L, as well as delta AOC levels higher than $\pm 50 \mu\text{g C/L}$, promoted regrowth of resident bacteria in the distribution systems.

CONCLUSION

This research led to the following conclusions: A trend between delta AOC and total chlorine residual was observed. Increasingly stable delta AOCs ($\pm 50 \mu\text{g C/L}$) were observed for residual chlorine concentrations from 1.0 to 4.0 mg/L. However, gradual instability of AOC levels was found with less than 1.0 mg/L of total residual chlorine. AOC consumption increased dramatically below 0.06 mg/L of total chlorine residual as did HPC. Delta AOC reflected proliferation of bulk liquid HPCs. When the chloramine residual was not maintained above 0.5 mg/L, even low AOC waters often had high HPC levels. When the chloramine residual was above 0.5 mg/L, low AOC waters were biologically stable, but high AOC waters sometimes showed AOC instability and elevated HPC counts. A combination of both low AOC level and maintenance of a chloramine residual yielded the most biostable water. However, chloramine residual by itself was beneficial to biostability, whereas low AOC levels alone could not maintain biostability.

REFERENCES

1. E. E. Geldreich, H. D. Nash, D. J. Reasoner and R. H. Taylor, *J. Am. Water Works Assoc.*, **64**, 596 (1972).
2. A. Maul, E. L. Shaarawi and J. C. Block, *Sci. Total Environ.*, **44**, 201 (1985).
3. A. Maul, E. L. Shaarawi and J. C. Block, *Sci. Total Environ.*, **44**, 215 (1985).
4. M. W. Lechevallier, T. M. Babcock and R. G. Lee, *Appl. Environ. Microbiol.*, **53**, 2714 (1987).
5. J. P. Candy and M. L. Angels, *Water Res.*, **35**, 2677 (2001).
6. P. J. Ollos, R. M. Slawson and P. M. Huck, *Wat. Sci. Tech.*, **38**, 275 (1998).
7. M. W. Lechevallier, C. D. Lowry, R. G. Lee and D. L. Gibbon, *J. Am. Water Works Assoc.*, **82**, 87 (1990).
8. J. Skadsen, *J. Am. Water Works Assoc.*, **85**, 95 (1993).
9. K. Power and L. A. Nagy, Report No. 4 Urban Water Research Association of Australia, Melbourne, Vic. 16 (1989).
10. D. G. Neden, R. J. Jones, J. R. Smith, G. J. Kirmeyer and G. W. Foust, *J. Am. Water Works Assoc.*, **84**, 80 (1992).
11. D. Debeer, R. Srinivasan and P. S. Stewart, *Appl. Environ. Microbiol.*, **60**, 4339 (1994).
12. D. Van der kooij, H. R. Veenendaal, C. Baars-Lorist, D. W. Van der kluft and Y. C. Drost, *Water Res.*, **23**, 1313 (1995).
13. K. Jones and S. B. Bradshaw, *J. Appl. Bacteriol.*, **80**, 458 (1996).
14. C. D. Norton and M. W. Lechevallier, *J. Am. Water Works Assoc.*, **89**, 66 (1997).
15. M. N. B. Momba, T. E. Cloete, S. N. Venter and R. Kfir, *Water Sci. Technol.*, **38**(8-9), 283 (1998).
16. P. J. Vikesland and R. L. Valentine, *Environ. Sci. Technol.*, **34**, 83 (2000).
17. S. E. Smith, T. Ta, D. M. Holt, A. Delanoue, J. S. Colbourne, A. H. L. Chamberlain and B. J. Lloyd, *J. Inst. Water Environ. Manage.*, **13**, 7 (1999).
18. N. B. Hallam, J. R. West, C. F. Forster and J. Simms, *Water Res.*, **35**, 4063 (2001).
19. P. Niquette, P. Servais and R. Savoir, *Water Res.*, **35**, 675 (2001).
20. W. Zhang and F. A. Digiano, *Water Res.*, **36**, 1469 (2002).
21. American Public Health Association: Standard Methods for the Examination of Water and Wastewater, 19th Ed., Washington, DC (1995).
22. M. W. Lechevallier, C. D. Lowry, R. G. Lee and D. L. Gibbon, *J. Am. Water Works Assoc.*, **85**, 111 (1993).
23. D. Van der kooij, *J. Am. Water Works Assoc.*, **84**, 57 (1992).
24. I. Escobar and A. A. Randall, *J. Am. Water Works Assoc.*, **91**, 76 (1999).
25. I. Escobar and A. A. Randall, *Water Res.*, **34**, 1680 (2000).
26. I. Escobar and A. A. Randall, *Water Res.*, **35**, 4444 (2001).

1. E. E. Geldreich, H. D. Nash, D. J. Reasoner and R. H. Taylor, *J. Am.*