BIOMEDICAL AND ENVIRONMENTAL SCIENCES **20**, 373-380 (2007)

Synergetic Inactivation of Microorganisms in Drinking Water by Short-term Free Chlorination and Subsequent Monochloramination[1]

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Objective To introduce synergetic inactivation of microorganisms in drinking water by short-term free chlorination for less than 15 minutes followed by monochloramination. **Methods** Indicator microorganisms such as *Escherichia coli, Staphylococcus aureus, Candida albicans,* and spores of *Bacillus subtilis* were used to assess the efficiency of sequential chlorination and free chlorination. **Results** The sequential chlorination was more efficient in inactivating these microorganisms than free chlorination, indicating that synergy was provided by free chlorine and monochloramine. Ammonia addition time, temperature and pH had influences on this synergy. **Conclusion** The possible mechanism of this synergy might involve three aspects: free chlorine causing sublethal injury to microorganisms and monochloramine further inactivating them; different ability of free chlorine and monochloramine to penetrate and inactivate microorganism congeries; and higher concentration of residual chlorine in sequential chlorination than in free chlorination.

Key words: Disinfection; Sequential chlorination; Synergetic effect; Inactivation; Microorganism

INTRODUCTION

Chlorination has played a vital role in protecting the public from epidemics since its first application to drinking water disinfection in the 1900s. To date, it remains a predominant disinfection process in drinking water plants in China, and in the United States as well[1]. Much research has been focusing on chlorination for inactivation of specific pathogens, such as *Legionella*, *Bacillus anthracis*, *Encephalitozoon* spp., *Mycobacterium* spp., *etc*.[2-5].

Since the discovery of disinfection by-products (DBPs) in drinking water chlorination in the 1970s[6], their toxicity has necessitated water professionals to monitor them to ensure proper water quality. Even though the main purpose of the processes of drinking water disinfection is to inactivate pathogens that may bring acute infection to humans, they all should aim to lower the formation of DBPs, which may cause long-term health hazards.

Recently, much research has focused on alternative disinfection techniques, such as chlorine dioxide, ozone and ultraviolet to reduce chlorinated DBPs. However, these techniques have some limitations. Firstly, chlorine dioxide can be transformed into chlorite, and ozone can oxidize bromide into bromate, which are also carcinogenic. Secondly, ozone or UV disinfection cannot maintain residual disinfectants in distribution systems. Finally, these alternative processes are more expensive than chlorination. Thus, they have not been used in drinking water plants on a large scale in China and other developing countries. Therefore, it is of great importance to optimize the chlorine disinfection process so that DBPs control and hygienic indices can be simultaneously guaranteed in a cost effective way.

A sequential disinfection process of short-term free chlorination followed by chloramination was developed in this laboratory and was given trials in practice. After free chlorine disinfection within 15 minutes, ammonia was added to transform the chlorine disinfection into chloramine disinfection. This sequential disinfection process takes advantage of free chlorine's quick inactivation of microorganisms and chloramine's low DBP yield and long-term residual effect. Thus, the dual control of microorganisms and DBPs could be achieved effectively and economically[7]. The mechanism of the sequential chlorination disinfection process is

[1]This work was part of a project (No. 50708050) supported by the National Natural Science Foundation of China.
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0895-3988/2007
CN 11-2816/Q

shown in Fig. 1. We initially believed that the sequential chlorination would provide an efficacy of moderate inactivation, i.e. an efficacy between free chlorination and monochloramination alone, according to their CT values. To our surprise, its efficacy was even better than that of free chlorination, indicating that there was some synergy of free chlorine and monochloramine (Fig. 1a).

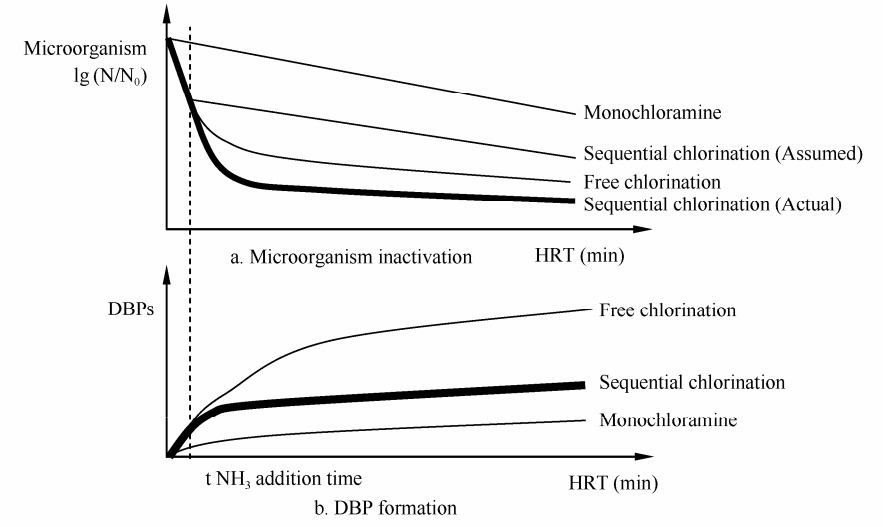

FIG. 1. Mechanism of sequential chlorination disinfection process.

There have been other studies of chlorination optimization which are similar to our work. A pilot study was reported using short-term free chlorine for 10 min as an alternative disinfectant to lower THM formation[8]. Free chlorine was added in the outlet of the coagulation basin and ammonia was added in the inlet of the softening basin. Since THM reactions were enhanced at elevated pH values, the chlorine was converted to a chloramine residual prior to the elevation of water pH in the softening basin. In another pilot test of prechlorination followed by the addition of ammonia, chlorine was added in a rapid mix basin and ammonia was added in 17 minutes later in the pipe after flocculation[9].

There are some differences between these investigations and our study. First, the previous investigations were case studies and did not furnish conclusive practical parameters to guide the subsequent application in other locations. In our study, the parameters and influencing factors were investigated and the efficiency of the process in inactivating microorganisms and controlling the formation of DBPs was tested in order to obtain its comprehensive assessment. Secondly, it was discovered that this sequential chlorination process has better inactivation efficiency than free chlorination with equal chlorine dosage and exposure time, showing that free chlorine and chloramines act synergistically. The synergy of co-existing free chlorine and monochloramine was reported previously[10]. However, the co-existence of free chlorine and monochloramine only existed at a Cl_2:N ratio of more than 5:1, so chlorine was partially wasted and the process using co-existing free chlorine and monochloramine was not practical, as compared with the sequential chlorination process. Moreover, previous investigations focused on prechlorination processes (chlorination before filtration) and our study embraced a post-chlorination process (chlorination after filtration). Although prechlorination used to be popular in conventional drinking water treatment, it cannot be applied before biological treatment, such as biological activated carbon filtration, which will be used in future drinking water treatment plants.

This study included experiments on microorganism inactivation by sequential chlorination. The DBP yield of sequential chlorination was only about 50% of that of free chlorination as reported in another paper[11].

MATERIAL AND METHODS

Microorganism Strains and Growth Conditions

Escherichia coli (CGMCC 1.3373), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231) and spores of *Bacillus subtilis* (ATCC 9372) were used as indicators in this study. All these standard strains, obtained from China General Microbiological Culture Collection (CGMCC), were inoculated in broths separately and incubated for 18 h to gain cultures in stationary phase. Before treatment,

microorganisms were centrifuged at 4 000×g for 10 min at 20℃. The broth was removed, and the cell pellets were rinsed and re-suspended in phosphate buffer solution (PBS = 0.03 mol/L, pH=7). This washing procedure was repeated three times to wash off the residual nutrients to prevent chlorine demand. The concentration of the final pure culture was determined by measuring the absorbance at 620 nm. Before each experiment, absorbance measurements were confirmed by plate counting.

Disinfectants

The free chlorine disinfectant was sodium hypochlorite (analytical reagent). The stock sodium hypochlorite was diluted to about 500 mg/L before testing. The monochloramine was produced by the reaction of hypochlorite with ammonia sulfate at a Cl_2/N ratio of 5:1 as previously described[12]. By this method, chlorine reacts with ammonia to form monochloramine in a virtually complete manner. In the sequential chlorination experiments, hypochlorite solution was added to the water first, and ammonia sulfate was added at a set time later to form chloramines. Under conditions of pH=7.0 and Cl_2/N=4:1, free chlorine could be transformed entirely into monochloramine since there was slightly more ammonia than the stoichiometric concentration.

Procedures

The inactivation of indicator microorganisms was carried out in a sterile laboratory. The polluted water for microbe inactivation tests was made by dissolving an indicator microorganism culture into sterile 0.03 mol/L phosphate buffer solution at pH 7 to obtain a concentration of about 1×10^8 CFU/mL. All experiments were performed in chlorine-demand-free glassware. Colorimetric tubes with lids and a volume of 10 mL were used as disinfection reactors. Tubes containing 10 mL polluted water were placed in a water bath at 20℃ or other certain reaction temperature. Disinfectant was added to the polluted water at definite concentrations. After definite contact times (Figs. 2-4 and Tables 1-2), disinfection was quenched by excessive sodium thiosulfate solution (0.05 mol/L). The initial and residual concentrations of microbes were assayed to determine the efficacy of disinfectants. Each point of data was obtained by three dilution ratios and a blank control. Parallel tests were repeated several days later to confirm the accuracy of the tests.

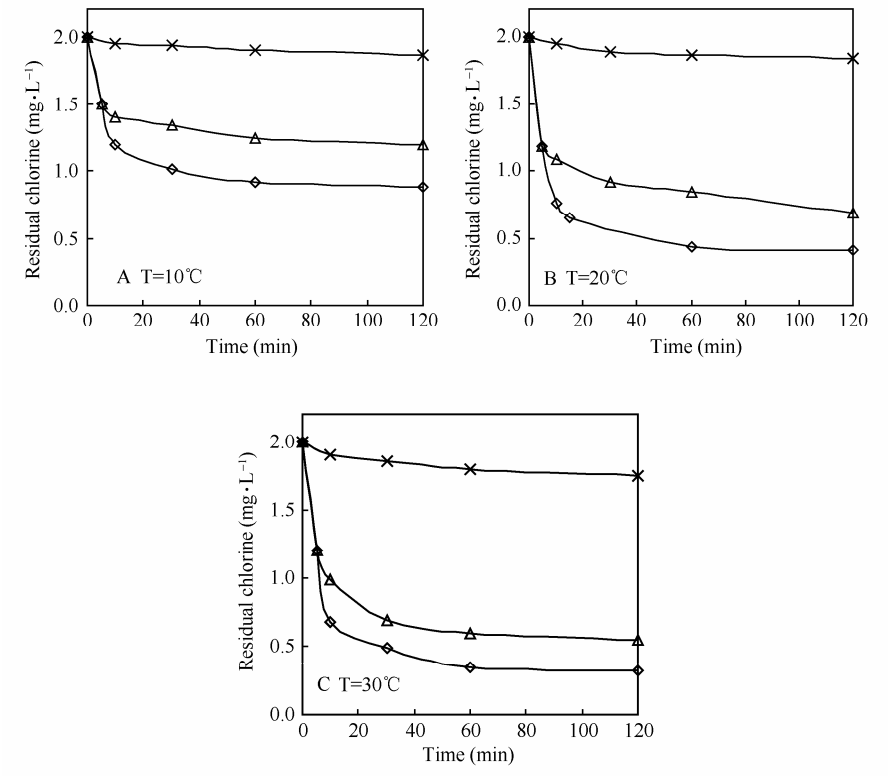

FIG. 2. Comparison of chlorine decay in chlorination processes at pH=7. × monochloramine, addition concentration=2.0 mg/L; □ free chlorine, addition concentration=2.0 mg/L; △ free chlorine for 5 min and monochloramine afterwards, addition concentration=2.0 mg/L.

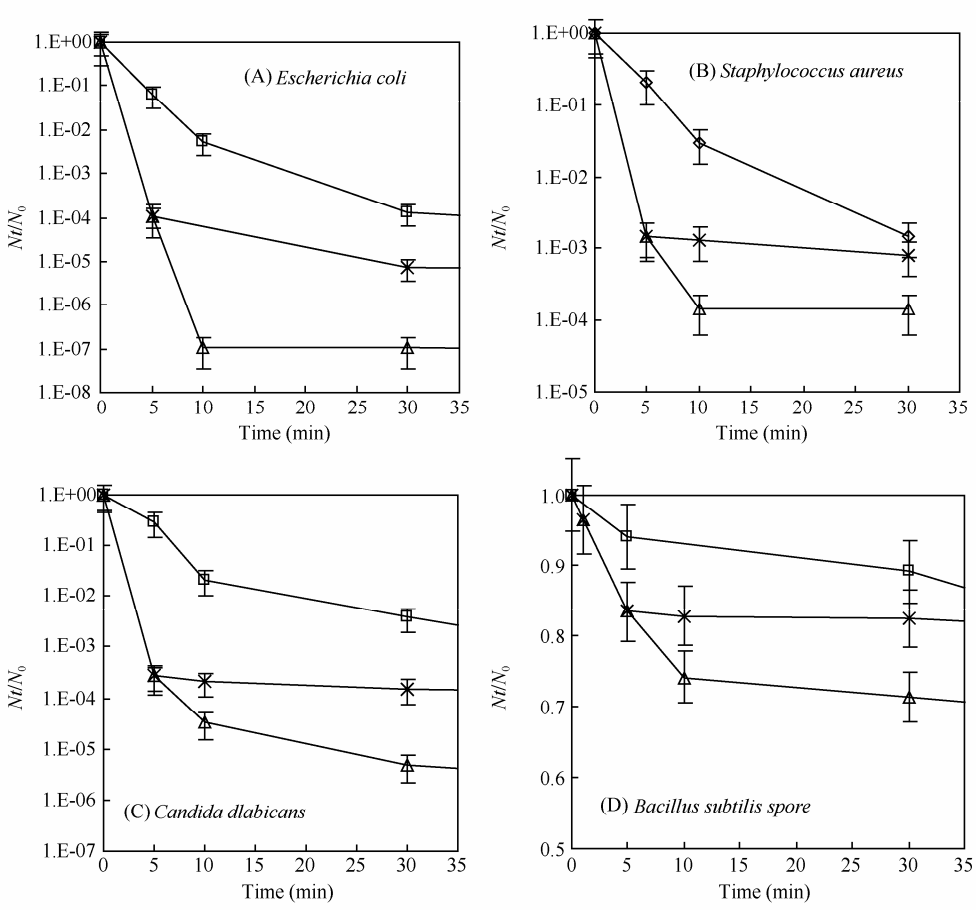

FIG. 3. Comparison of different disinfection processes in inactivating index microorganisms at pH=7 and T=20℃. □ monochloramine, addition concn =2.0 mg/L; × free chlorine, addition concn =2.0 mg/L; △ free chlorine for 5 min and monochloramine afterwards, addition concn =2.0 mg/L.

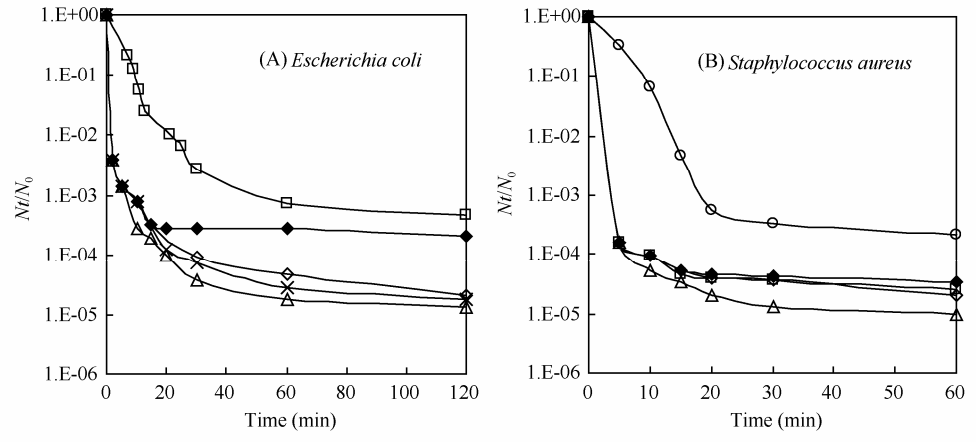

FIG. 4. Influence of ammonia addition time on sequential chlorination at pH=7 and T=20℃. □ monochloramine, addition concn = 2.0 mg/L; ◆ free chlorine, addition concn = 2.0 mg/L; △ free chlorine for 5 min and monochloramine afterwards, addition concn = 2.0 mg/L. × free chlorine for 10 min and monochloramine afterwards, addition concn = 2.0 mg/L. ◇ free chlorine for 15 min and monochloramine afterwards, addition concn = 2.0 mg/L. Note: the relative error was silimar to that in Fig. 3. The error bar is not shown in this figure to improve readability.

Analytical Methods

Free chlorine, monochloramine, dichloramine, and trichloramine residuals were determined by the 4500-Cl.F, DPD ferrous titrimetric method[13]. The initial and residual coliform in the inactivation test was assayed using membrane filtration with Endo's culture media. The other three kinds of microorganisms were assayed by plate count with nutrient agar culture media.

DATA AND RESULTS

Chlorine Decay in Chlorination Processes

Disinfectant decay is very important for disinfection, so residual chlorine in free chlorination, sequential chlorination and monochloramination were monitored with different water temperatures in the inactivation tests, as shown in Fig. 2. The ammonia addition time in sequential chlorination was 5 minutes.

As is well known, free chlorine decays faster in water because it reacts quickly with reductive matter. Only 16%-44% of residuals remained in free chlorination after 120 min, the usual hydraulic retention time of clear wells in large water plants in China. The higher the temperature was, the less residual chlorine remained. Monochloramine decays much slower than free chlorine. In the monochloramination process, 88%-93% of residual chlorine remained after 120 min. With sequential chlorination, chlorine decay was decelerated after ammonia addition and about 0.5 mg/L more residual chlorine was maintained than when free chlorination was used.

Microorganism Inactivation Test

The efficiency of microorganism inactivation by the three chlorination processes, i.e., sequential chlorination, free chlorination and monochloramination, was compared in the laboratory (Fig. 3).

The inactivation curves of each indicator microorganism had almost the same shape. Monochloramine had the poorest inactivation efficiency. Free chlorination had a fast disinfection effect in about the first 5 minutes. However, its inactivation curve later leveled off, and the final inactivation efficiency was not greatly improved. The inactivation efficiency of sequential chlorination was expected to be between that of monochloramine and free chlorine according to their usual CT values. However, the sequential chlorination process, in which 5 minutes free chlorination was followed by monochloramination, had even better efficiency than free chlorination, showing that there was synergy between free chlorine and monochloramine during this process.

The quantitative method we used for assessing synergy was that of Gyürék[14]. If sequential chlorination has a higher inactivation rate than the sum of each component disinfectant's inactivation rate, it can be ensured that there was synergy. The synergy (E_{syn}) can be quantified by the difference between the inactivation rate of sequential chlorination and the sum of each disinfectant's inactivation rate, which is shown in Equation 1. Another method for quantifying synergy, Berenbaum's equation[15], is more suitable for combined disinfection with two components together, and thus it is not applicable to this sequential chlorination process.

$$E_{syn}=I_r - (I_{r1}+I_{r2}) \quad \text{Equation 1}$$

I_r –inactivation rate of sequential disinfection (in lg scale)

I_{r1}, I_{r2} –each component disinfectants's inactivation rate (in lg scale)

A 10-minute disinfection time was set as the point of calculation. According to the data in Fig. 3, 5 minutes of free chlorination plus 5 minutes of monochloramination resulted in an inactivation rate of 6.96 lg on *E. coli*; 5 minutes of free chlorination and 5 minutes of monochloramination had inactivation rates of 3.96 lg and 1.14 lg respectively. Thus, the synergy of sequential chlorination on *E. coli* was 6.96-(3.96+1.14) =1.86 lg.

Sequential chlorination disinfection had different synergy values for different index microorganisms. Its synergy on *Staphylococcus aureus, Candida albicans* and *Bacillus subtilis* spores were 0.33 lg, 0.368 lg, and 0.0248 lg, respectively.

Influence on Synergy by Ammonia Addition Time

Ammonia addition time is one key parameter of sequential chlorination. 5, 10, and 15 minutes were set as the ammonia addition times in the experiments since shorter spans of time are not applicable in water treatment plants. As shown in Fig. 4, the efficiency of sequential chlorination for all three ammonia addition times was better than that of free chlorination. Unexpectedly, the inactivation efficiency of the 5 minutes' ammonia addition test was even better than those of the 10 or 15 minutes' tests.

As seen in the curve for free chlorination, short-term quick log inactivation was followed by a long-term tailing off period and the efficiency was not enhanced further. As with ammonia addition, the slopes of these curves increased on the basis of the tailing off period. The final efficacy of

sequential chlorination was 1-2 lg higher than free chlorination.

Influence of pH and Temperature on Synergy

The synergy was influenced by pH and temperature, as shown in the data listed in Tables 1 and 2. Synergy increased with the increase of pH and the decrease of temperature. These phenomena mean that sequential chlorination could improve the inactivation efficiency in the unfavorable conditions of high pH and low temperature under free chlorination.

TABLE 1

pH Influence on Synergy of Sequential Chlorination

Disinfection	Inactivation Efficiency at Different pH*					Conditions
	6	6.5	7	7.5	8	
Cl_2	−4.26	−4.15	−3.96	−3.79	−3.73	$[Cl_2]$=2 mg/L, 5 min
NH_2Cl	−1.52	−1.22	−1.14	−1.00	−0.86	$[NH_2Cl]$=2 mg/L, 5 min
Cl_2+ NH_2Cl	−5.79	−5.37	−5.10	−4.79	−4.59	Sum of the Above
Sequential Chlorination	−5.57	−6.79	−6.96	−7.26	−7.26	$[Cl_2]$=2 mg/L, 5 min + $[NH_2Cl]$=2 mg/L, 5 min
Synergy#	−0.21	1.41	1.86	2.47	2.68	----

Note. *The inactivation efficiency was calculated in lg scale. #The synergy was the improvement of inactivation efficiency compared with the sum of Cl_2+ NH_2Cl.

TABLE 2

Temperature Influence on Synergy of Sequential Chlorination

Disinfection	Inactivation Efficiency at Different T (℃)*				Conditions
	5	10	20	30	
Cl_2	−3.53	−3.64	−3.71	−3.74	$[Cl_2]$=2 mg/L, 5 min
NH_2Cl	−0.46	−0.63	−0.97	−1.60	$[NH_2Cl]$=2 mg/L, 5 min
Cl_2+ NH_2Cl	−3.99	−4.28	−4.67	−5.34	Sum of the Above
Sequential Chlorination	−4.51	−4.71	−4.72	−4.76	$[Cl_2]$=2 mg/L, 5 min +$[NH_2Cl]$=2 mg/L, 5 min
Synergy#	0.52	0.43	0.05	−0.58	----

Note. *The inactivation efficiency was calculated in lg scale. #The synergy was the improvement of inactivation efficiency compared with the sum of Cl_2+ NH_2Cl.

DISCUSSION

Factors of Synergy

The synergy was influenced by the ammonia addition time, temperature and pH. In the span of 5 to 15 minutes, the earlier ammonia was added to transform free chlorine into monochloramine, the higher efficiency could be obtained. Low temperature and high pH also benefited the synergy to some degree.

The influence of ammonia addition time could be explained by the fact that the residual chlorine consumption speed was decelerated by the ammonia addition and transformation into monochloramine, as shown in Fig. 2. The earlier ammonia was added to be transformed into chloramines, and the more residual chlorine was retained in the subsequent process of disinfection.

The influence of pH on synergy could be explained by the influence of pH on monochloramine formation. Higher pH, within the range of pH 6 to 9, benefits the quick formation of monochloramine.

Higher temperatures benefit the inactivation by free chlorine or monochloramine alone according to the Arrhenius law. Therefore, the synergy of sequential chlorination decreased with the rise of temperature.

Hypothetical Mechanism of Synergy in Sequential Chlorination

The widest difference in the inactivation mechanism between our process and previous ones was lying in the fact that synergy was discovered in the sequential process of free chlorination and subsequent chloramination, instead of between two co-existing disinfectants.

In previous studies, Kouame and Haas[10] investigated the synergy of co-existing free chlorine

and monochloramine, and Straub *et al*.[16] reported the synergistic inactivation of *Escherichia coli* and MS-2 coliphage by chloramines and cupric chloride. Rennecker *et al*.[17] also reported synergy in sequential inactivation of *Cryptosporidium parvum* with ozone/free chlorine and ozone/monochloramine. In their hypothesis, the main disinfectant caused sublethal injuries or physiological malfunctions in microorganisms, thereby enhancing the sensitivity to secondary disinfectants.

Based on the classical theory of microbiology and former investigations, the possible explanations of synergy in sequential chlorination are as follows.

Firstly, free chlorine may cause sublethal injuries to residual microorganisms, and monochloramine inactivates these microorganisms further. A principal mode of bacterial destruction by free chlorine is disruption of the integrity of the cell membrane[18]. This action severely impairs cellular respiration, destroys membrane permeability, and causes irreparable damage to essential metabolic functions. This action may be fatal to bacteria or may cause sublethal injuries of bacteria[19-20]. Although it is not as reactive as free chlorine, monochloramine can also cause irreversible denaturation of proteins, oxidation of sulphydryl-containing enzymes[21] and can irreversibly harm metabolism of a cell[22]. Monochloramine is also thought to inactivate bacteria cells by damaging the nucleic acid.

In sequential chlorination, a short-term free chlorine process could inactivate a majority of the microorganisms and bring sublethal injuries such as membrane damage and metabolic malfunction to the residual. With the physiological barrier damaged, monochloramine can easily enter the interior, damage the nucleic acids and finally kill the bacteria. However, Vitro *et al*.[20] recently reported that the exposure of bacterial cells to chlorine in distilled water caused extensive permeabilization of the cytoplasmic membrane, but the concentrations required were much higher than those needed to inactivate cells. Thus, our hypothesis will be verified by using PI uptake, FISH technology and other such techniques in later investigations.

Secondly, free chlorine and monochloramine are different in their ability to penetrate and inactivate microorganism congeries. Cellular aggregation, part of the cell-mediated mechanism of resistance, could provide physical protection to internal cells[21]. Free chlorine may decay quickly by the microorganisms on the surface of aggregation and their wreckage and excreta such as glucide and protein. Thus, microorganism congeries or biofilm present much higher resistance to free chlorine, and a tailing off period exists in the inactivation curve. Chloramines decay much slower than free chlorine and can penetrate deeper into congeries to inactivate internal microorganisms. LeChevallier *et al*.[23] found that chloramines were more effective in controlling biofilm microorganisms because they interacted poorly with capsular polysaccharides.

By comparing different chlorination processes (Figs. 3 and 4), the efficiency of free chlorination was not elevated in the tailing off period due to the possible interference of microorganism congeries. However, the efficiency of sequential chlorination improved with ammonia addition during the tailing off period, showing that the subsequent chloramination had better inactivation efficacy on congeries. This explanation will be confirmed by experiments using membrane filtration of 1.0 μm to remove congeries before disinfection.

Finally, more residual chlorine was maintained in sequential chlorination than in free chlorination. According to the data in Fig 2, with sequential chlorination, chlorine decay was slowed down and about 0.5 mg/L more residual chlorine was retained than with free chlorination, which also benefited the improvement of inactivation efficacy during the tailing off period. The earlier ammonia was added to be transformed into chloramines, the more residual chlorine was retained in the subsequent process of disinfection and the higher inactivation efficiency was obtained (Fig. 4).

ACKNOWLEDGEMENT

The authors wish to thank the Tianjin Drinking Water Company for its participation in this project.

REFERENCES

1. AWWA water quality division disinfection systems committee (2000). Committee report: disinfection at large and medium-size systems. *American Water Works Association Journal* **92**(5), 32-43.
2. Kima B R, Andersona J E, Muellera S A, *et al*. (2002). Literature review—efficacy of various disinfectants against *Legionella* in water systems. *Water Research* **36**, 4433-4444.
3. Rice E W, Adcock N J, Sivaganesan M, *et al*. (2005). Inactivation of Spores of *Bacillus anthracis* Sterne, *Bacillus cereus*, and *Bacillus thuringiensis* subsp. *israelensis* by Chlorination. *Applied and Environmental Microbiology* **71**, 5587-5589.
4. Falkinham III J O (2003). Factors Influencing the Chlorine Susceptibility of *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*. *Applied and Environmental Microbiology* **69**, 5685-5689.
5. Rose L J, Rice E W, Jensen B, *et al*. (2005). Chlorine Inactivation of Bacterial Bioterrorism Agents. *Applied and Environmental Microbiology* **71**, 566-568.
6. Rook J J (1974). Formation of haloforms during chlorination of natural water. *Water Treatment and Examination* **23**, 234-236.
7. Chen C, Zhang X (2004). Reduction of disinfection by-products with short-term free chlorine plus chloramines disinfection

process. 4th World Water Congress and Exhibition. Marrakech, Morocco. Paper ID 24973.
8. Hubbs S A, Amundsen D, Olthius P (1981). Use of chlorine dioxide, chloramines, and short-term free chlorine as alternative disinfectants. *American Water Works Association Journal* **73**(2), 97-101.
9. Means III E G, Tanaka T S, Otsuka D J, *et al*. (1986). Effects of chlorine and ammonia application points on bactericidal efficiency. *American Water Works Association Journal* **78**(2), 62-69.
10. Kouame Y, Haas C N (1991). Inactivation of *E. coli* by combined action of free chlorine and monochloramine. *Water Research* **25**(9), 1027-1032.
11. Chen C (2005). Studies on the Development of Sequential Chlorination Disinfection Process and the Optimization of Water Purification Processes to Control Disinfection By-products. PhD thesis. Department of Environmental Science and Engineering, Tsinghua University, Beijing, China.
12. Diehl A C, Speitel Jr G E, Symons J M, *et al*. (2000). DBP formation during chloramination. *American Water Works Association Journal* **92**(6), 76-90.
13. APHA, AWWA, WEF (1995). Standard methods for the examination of water and wastewater. 19th edition. Am. Pub. Health Assoc., Washington DC, USA.
14. Gyürék L L, Finch G R, Belosevic M (1997). Modeling chlorine inactivation requirements of *Cryptosporidium parvum* oocysts. *Journal of Environmental Engineering* **123**(9), 865-875.
15. Berenbaum C M (1981). Criteria for analyzing interactions between biologically active agents. *Advanced Cancer Research* **35**, 269-335.
16. Straub T M, Gerba C P, Zhou X, *et al*. (1995). Synergistic inactivation of *Escherichia coli* and MS-2 coliphage by chloramines and cupric chloride. *Water Researc*h **29**(3), 811-818.
17. Rennecker J L, Driedger A M, Rubin S A, *et al*. (2000). Synergy in sequential inactivation of *Cryptosporidium parvum* with ozone/free chlorine and ozone/monochloramine. *Water Research* **34**(17), 4121-4130.
18. Wolfe R L, Ward N R, Olson B H (1984). Inorganic chloramines as drinking water disinfectants: a review. *American Water Works Association Journal* **86**(5), 74-88.
19. Preez M du, Kfir R, Coubrough P (1995). Investigation on injury of coliforms after chlorination. *Water Science and Technology* **31**, 115-118.
20. Virto R, Manas P, Alvarez I, *et al*. (2005). Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. *Applied and Environmental Microbiology* **71**, 5022-5028.
21. Stewart M H, Olson B H (1996). Bacterial resistance to potable water disinfectants. In "*Modeling disease transmission and its prevention by disinfection*". Cambridge University Press, Cambridge, UK, pp.140-192.
22. Jacangelo J G, Olivieri V P, Kawata K (1991). Investigation of the mechanism of inactivation of *Escherichia coli* B by monochloramine. *American Water Works Association Journal* **83**(5), 80-87.
23. LeChevallier M W, Lowry C H, Lee R G (1990). Disinfecting biofilm in a model distribution system. *American Water Works Association Journal* **82**(5), 85-99.

(*Received November* 30, 2006 *Accepted August* 13, 2007)