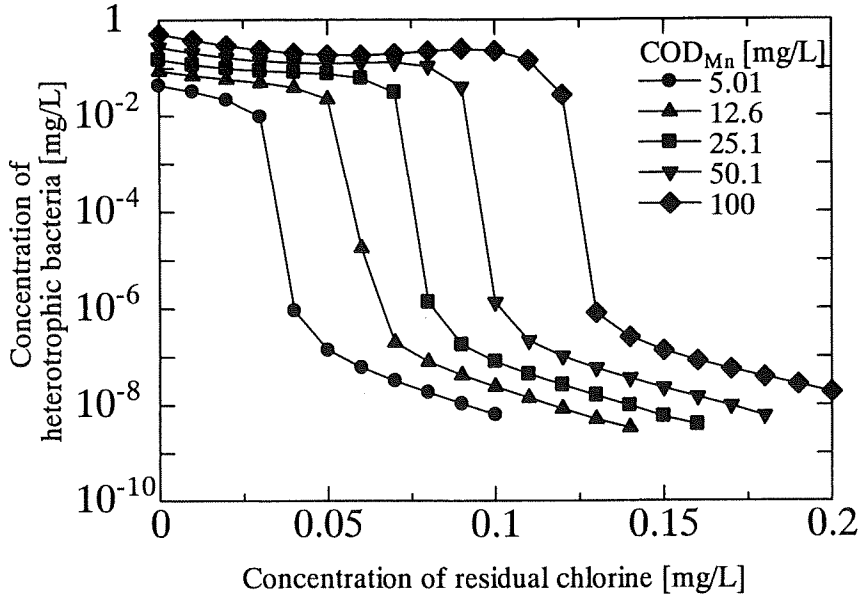

図 8　浄水中の残留塩素濃度と節点 10 における微生物濃度の関係

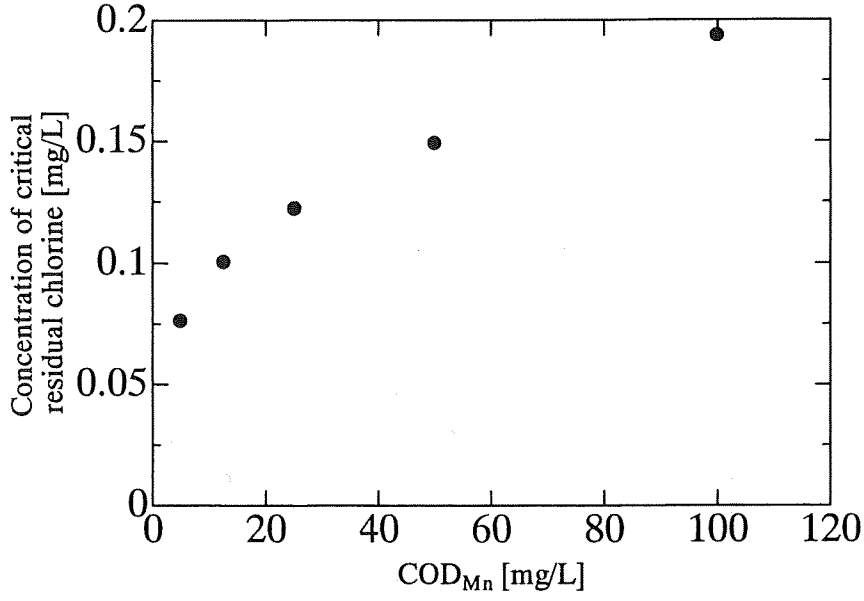

図 9　原水中の有機物濃度と限界塩素濃度の関係

E 結論

より実態に近いシミュレーションモデルを構築するため、給配水系を配管網として計算を行うようにし、また塩素の消費速度に関する有機物との反応モデルを組み入れた。この改良モデルを用いて配管網内での従属栄養細菌の再増殖速度と、原水水質および浄水場における有効塩素濃度との関係について検討を行い、次にあげる結論を得た。

1. 配水池が複数あるような複雑な配水管網に対応した。
2. 各節点における給水量変動に対応した。
3. 残留塩素と有機物との反応を評価できるようになった。
4. 微生物の再増殖について、浄水中の残留塩素濃度と給水栓における微生物濃度の関係を明らかにした。
5. 最も微生物濃度が高い節点に着目することで、原水水質と微生物の再増殖抑制のための必要残留塩素濃度の関係が計算可能であることを示した。

F 健康危険情報

該当なし

G 研究発表

(1) 論文発表

該当なし

(2) 学会発表

該当なし

H 知的財産権の出願・登録状況（予定を含む）

該当なし

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

1. 論文発表

1) Yumiko Ohkouchi, Bich Thuy Ly, and Sadahiko Itoh (2009) Detection of bacterial regrowth in water distribution system using endtoxin as an alternative indicator, Advances in Asian Environmental Engineering, Vol.8, No.1, pp.13-19.
2) Ikuro Kasuga, Hirotaka Nakagaki, Futoshi Kurisu, and Hiroaki Furumai (2010) Abundance and diversity of ammonia-oxidizing archaea and bacteria on biological activated carbon in a pilot-scale drinking water treatment plant with different treatment processes, Water Science and Technology, In press.

2. 学会発表

1) 島崎 大, 国包 章一 (2009) 水道水の残留塩素保持に係る規定および研究の動向, 環境衛生工学研究, 23(3), 16-19.
2) Bich Thuy Ly, Yumiko Ohkouchi, Sadahiko Itoh (2009) Investigation of related factors to biological stability in drinking water distribution system and the possibility of AOC removal by ion exchange, The 8th International Symposium on Water Supply Technology Proceedings, pp.270-278.
3) 伊藤禎彦 (2009) 高度浄水処理水を越える水道水質ニーズとリスク管理のゆくえ, 環境衛生工学研究, Vol.23, No.3, pp.3-9.
4) Yumiko Ohkouchi, Shinya Echigo, Nagahisa Hirayama and Sadahiko Itoh (2009) Our approaches for reducing chlorinous odor to establish satisfactory water supply systems in Japan, Proceedings of 6th Netherlands-Japan Workshop on Water Technology, pp.30-33.
5) Yusuke Aoki, Shinya Echigo, Yumiko Ohkouchi and Sadahiko Itoh (2009) Fate of amino acids in drinking water treatment process, Proceedings of 6th Netherlands-Japan Workshop on Water Technology, pp.98-99.
6) Yasuhiro Asada, Yumiko Ohkouchi and Sadahiko Itoh (2009) Rapid quantification of heterotrophic bacteria in drinking water based on the amount of DNA labeled with Bromodeoxyuridine, Proceedings of 6th Netherlands-Japan Workshop on Water Technology, pp.108-109.
7) Yoshihiro Kawano, Bich Thuy Ly, Suguru Ishikawa, Yumiko Ohkouchi and Sadahiko Itoh (2009) AOC removal during drinking water treatment processes in Japan, Proceedings of 6th Netherlands-Japan Workshop on Water Technology, pp.113-114.
8) Yumiko Ohkouchi, Bich Thuy Ly, Suguru Ishikawa, Yoshihiro Kawano, Sadahiko Itoh (2009) Present AOC levels in drinking water and its required level for biologically stable water with lower chlorine residual, Proceedings of the 17th Seminar of JSPS-MOE Core University Program on Urban Environment, pp. 1-9.
9) 伊藤禎彦, Patrick Smeets, Gertjan Medema (2009) 微生物の定量的感染リスク評価手法日中戦略的国際科学技術協力推進事業 第3回シンポジウム 水の反復利用によるリスク低減のためのモニタリング評価と対策技術に関する研究, pp.74-76.
10) 伊藤禎彦, Patrick Smeets, Gertjan Medema, 宋金姬 (2010) 定量的感染リスク評価における浄水処理プロセスの流入・流出水濃度のデータペアリング方法, 第44回日本水環境学会年会講演集, 75.
11) 伊藤禎彦, Patrick Smeets, Gertjan Medema, (2010) 定量的感染リスク評価の感度解析における非加熱飲料水消費量データの影響, 第44回日本水環境学会年会講演集, 76.

12) 河野圭浩，Ly Bich Thuy，大河内由美子，伊藤禎彦（2010）浄水処理過程における生物分解性有機炭素の除去特性，第44回日本水環境学会年会講演集，178.
13) 大瀧雅寛，溝添倫子，林紗綾佳（2009）紫外線および二酸化塩素処理における大腸菌の細胞損傷レベルの測定，第60回全国水道研究発表会講演集，184-185.
14) 溝添倫子，佐野満実子，Myriam Ben Said，大瀧雅寛（2010）消毒処理による大腸菌および緑膿菌の損傷メカニズムの定量的解析，第44回日本水環境学会年会講演集，302.
15) Ikuro Kasuga, Hirotaka Nakagaki, Futoshi Kurisu, and Hiroaki Furumai (2009) Abundance and diversity of ammonia-oxidizing archaea and bacteria on biological activated carbon in a pilot-scale drinking water treatment plant with different placements of sand filtration, The 3rd IWA-ASPIRE conference Abstracts, p.6.
16) Suwat Soonglerdsongpha, Ikuro Kasuga, Futoshi Kurisu, and Hiroaki Furumai (2009) Comparison of assimilable organic carbon removal by biological activated carbon in different advanced drinking water treatment plants, The 3rd IWA-ASPIRE conference Abstracts, pp.67-68.
17) Ikuro Kasuga (2009) Characterization of ammonia-oxidizing archaea associated with biological activated carbon used for advanced drinking water treatment, International Workshop on water and Wastewater Treatment at National Cheng Kung University, p.16.
18) 春日郁朗，中垣宏隆，栗栖太，古米弘明，関哲雄（2009）生物活性炭立ち上げ時の微生物定着に及ぼす前塩素処理の影響，第60回全国水道研究発表会講演集，pp.164-165.
19) 前田裕太，春日郁朗，栗栖太，古米弘明（2009）培養法と分子生物学的手法を用いた給水末端における細菌群の多様性評価，第60回全国水道研究発表会講演集，pp.462-463.
20) 春日郁朗，中垣宏隆，栗栖太，古米弘明（2009）生物活性炭における硝化微生物の付着過程と硝化能との関係，第9回日中水道技術交流会論文集，pp.15-18.
21) 春日郁朗（2010）水道水における同化性有機炭素の制御と課題，第12回東京大学水環境制御研究センターシンポジウム，講演要旨集.
22) Suwat Soonglerdsongpha, Ikuro Kasuga, Futoshi Kurisu Hiroyuki Katayama, Hiroaki Furumai (2010) Application of stable isotope probing to evaluate AOC assimilating bacteria attached on BAC in drinking water treatment plant, 第 44 回日本水環境学会年会講演集，169.

研究成果の刊行物・別刷

[Original Paper]

Advances in Asian Environ. Eng.
Vol. 8, No. 1, p. 13-20 (2009)

Detection of Bacterial Regrowth in Water Distribution System Using Endotoxin as an Alternative Indicator

Yumiko OHKOUCHI, Bich Thuy LY and Sadahiko ITOH

Graduate School of Engineering, Kyoto University, Kyoto 615-8540, Japan,

ABSTRACT

Endotoxin concentrations and their fractions, which can be measured in a short time, were focused as new indicators for regrown bacteria in distribution systems instead of traditional heterotrophic plate count (HPC) method. It was found that almost all part of endotoxin existed as free endotoxin in tap water. Once chlorine residual was neutralized, HPC in several samples were increased after 7 days incubation. The concentrations of cell-bound and total endotoxin were increased drastically, and the ratios of free endotoxin were decreased relatively. The biofilm accumulation was monitored under continuous flow condition using annular reactors at different concentrations of chlorine residual. There were trends toward increasing HPC numbers in the effluent of AR with biofilm accumulation. The concentrations of cell-bound and total endotoxin were also increased with HPC numbers in effluent, and could be indicators for regrown bacteria only in the situation where significant bacterial regrowth was occurred (HPC > 5000 CFU/mL).

Keywords: Bacterial regrowth, Heterotrophic plate count (HPC), Endotoxin, Assimilable organic carbon (AOC), Water distribution system

INTRODUCTION

Disinfection of finished water is considered as an important treatment to supply microbiologically safe drinking water. In Japan, free chlorine of 0.1 mg/L is required at each end of distribution systems, and it works very effectively to control infectious risks by bacterial agents. However, many people have complaints about odor in drinking water, particularly chlorinous odor (Itoh *et al.*, 2007). The chlorine disinfection also causes formation of disinfection by-products (DBPs), such as trihalomethane and haloacetic acid. It has been widely recognized that the chlorine level should be reduced as low as possible to mitigate odor and DBPs problems. But, in a situation decreased chlorine residual, an advanced monitoring for bacterial regrowth and organic control are highly required at the same time in order to minimize microbiological risks, because bacteria in finished water could grow easily using small amount of biodegradable organics such as assimilable organic carbon (AOC) (van der Kooij, 1981).

At present, in water quality standard for drinking water in Japan, the standard values of standard plate count (SPC; 100 CFU/mL) and *Escherichia coli* (not detected), and the provisional targeted value of heterotrophic plate count (HPC; 2,000 CFU/mL) are established as indicators for bacteria. It is well-known that HPC is an excellent indicator for integrity of water treatment process and hygienic status of water distribution system, HPC data in finished water or during water treatment process therefore have been accumulating currently. However, it usually takes 1 week for HPC measurement, so it seems unrealistic to establish water quality monitoring system based on HPC data in distribution systems, which have relatively short detention time in Japan. Thus, new indicators for regrown bacteria, which can be tested rapidly and in practical use, are highly concerned to manage microbiological drinking water quality.

Endotoxin is an outer membrane component of gram-negative bacteria and cyanobacteria, and one of bacterial toxins acting on human. Endotoxin can be measured using simple procedure within 1 hour. There are several reports about endotoxin concentrations in water resources and drinking water and their removal during drinking water treatment (Anderson *et al.*, 2002; Sykora *et al.*, 1980). Endotoxin could be released from bacterial cells with cell multiplication or cell damage caused by chlorination, and it was cited as free endotoxin. Total endotoxin represents sum of two types of endotoxin, cell-bound endotoxin and free endotoxin. The information on free endotoxin was limited, while the concentrations of total endotoxin were described in many reports. In our previous research, it was found that large part of endotoxin in finished water existed as free endotoxin in dissolved organic fraction (Ohkouchi *et al.*, 2007), because almost all parts of bacteria were inactivated by chlorine disinfection. Conversely, it is expected that increase of cell-bound endotoxin could be a good indicator for regrown bacteria.

In this paper, endotoxin was examined as an alternative indicator for bacterial regrowth. First, tap water samples were collected in two different water distribution areas, and the concentrations of endotoxin and the ratios of free endotoxin to total endotoxin, defined here as the ratio of free endotoxin, were determined. Then those changes along with bacterial regrowth were examined in batch mode experiment, by incubation of tap

water samples after neutralizing chlorine residual. Finally, the biofilm accumulation under continuous flow condition was examined using two annular reactors at different concentrations of chlorine residual, to confirm applicability of endotoxin as an indicator for bacterial regrowth in water distribution system.

MATERIALS AND METHODS

Sampling Methods

Two typical water treatment plants (A and B) and their distribution systems were selected. The water treatment plant A had treatment processes including flocculation, sedimentation, rapid sand filtration, and chlorination. Forty and six water samples were taken in the distribution system from plant A (DS-A) at two different seasons, May - June 2007 and January 2008, respectively. In plant B, the surface water was treated by flocculation, sedimentation, rapid sand filtration, ozonation, biological activated carbon adsorption, and chlorination. Six water samples were taken in distribution system from plant B (DS-B) in January 2008. The water samples after 5 min flashing were collected in the glass bottles treated by heat sterilization at 250 ℃ for 2 hours for chemical or microbiological analyses except for AOC measurement. For AOC measurement, the carbon-free glass bottles were prepared by thermal treatment at 550 ℃ for 4 hours. The samples for chemical or bacterial analyses were processed within 4 h after sampling. For endotoxin assay, water samples were preserved at −80 ℃ after fractionation by centrifugation.

Bacterial Regrowth in Batch Mode Experiments

To neutralize chlorine residual, the autoclaved solution of sodium thiosulfate was added to 25 tap water samples taken in DS-A as a final concentration of 0.03 %. Then, each sample were incubated at 20 ℃ for a week under light-protected condition. The samples were taken at day 0, 1, and 7, respectively, and provided for HPC and endotoxin measurement.

Biofilm Accumulation in Continuous Flow Reactor Systems

Two annular reactors (ARs) Model 1320 LS (BioSurface Technologies Corporation, Bozeman) were used in this investigation. The AR is consisted of an outer glass cylinder and a rotating inner drum. Twenty removable polyvinyl chloride (PVC) coupons are mounted on the drum surface. Each PVC coupon has a wetted surface area of 17.9 cm^2. By rotating inner drums at 84 rpm, it could simulate a shear stress, which was equivalent to a velocity of 0.4 m/s in a PVC pipe with 125 mm diameter. The outside of outer each glass cylinder was covered with aluminum foil. Tap water at Katsura campus of Kyoto University was adjusted their chlorine residuals at 0 and 0.1 mg Cl_2/L with addition of sodium thiosulfate solution, respectively, and was pumped to each AR at flow rate of 8.3 mL/min. These AR systems were operated at 20 ℃. The detention time in each reactor was 2 hours. The flow diagram of these systems was illustrated in **Fig. 1**. The biofilm were accumulated on the surface of 20 PVC coupons, so one or two removed coupons were provided for measurements of HPC and total bacterial cells regularly. The biofilm samples were detached from each coupon by scratching using rubber policeman after loosen by sonication for 2.5 minutes with appropriate volume of autoclaved phosphate buffer.

Analytical Methods

Culture-based and direct enumeration of bacteria

HPC bacteria were enumerated by pour plate procedure with R2A agar incubated at 20 ℃ for 7 days. Total bacterial cells were enumerated by staining with 4',6-diamidino-2-phenylindole (DAPI). The bacterial cells in 1 mL water samples or culture broth were collected on 0.2 μm black polycarbonate membrane filter (Nihon Millipore K.K., Tokyo) and then the DAPI solution (1 μg/mL) were added onto the filter. After 10 min staining, the DAPI solution was removed by vacuum filtration. The filters were air-dried and mounted on slide glasses with cover slips. The fluorescence images were observed using epifluorescence microscope,

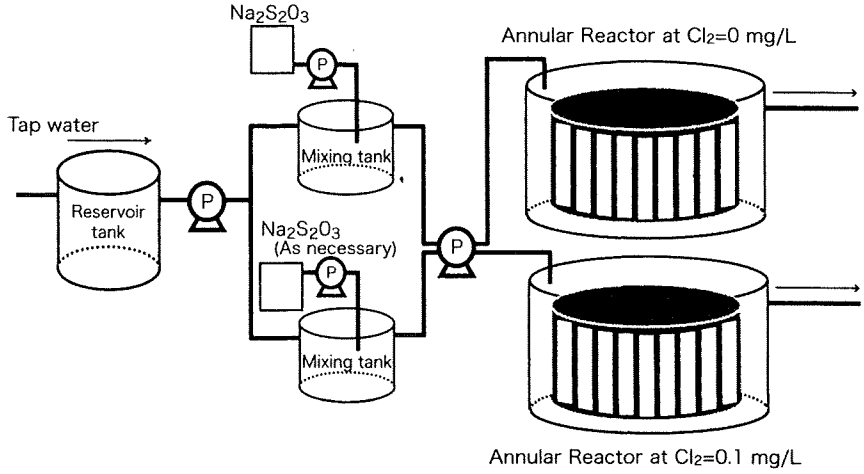

Fig. 1 Flow diagram of annular reactors for biofilm accumulation test.

MICROPHOTO-FX (Nikon, Tokyo) under UV excitation. Each image was captured by digital camera PDMC IIi (Nikon, Tokyo) and the image processing was carried out with software, ImagePro version 4.1 for Windows.

Endotoxin Endotoxin in water samples was fractionated by centrifugation at 14,000 rpm for 10 min, and the supernatant fraction was used for free endotoxin determination. Each endotoxin was determined by endpoint-colorimetric microplate method using Endospecy ES-50M Set and Toxicolor DIA-MP Set (Seikagaku Kogyo, Tokyo). The formation of diazo-compounds was monitored using microplate reader (Model 550, Bio-Rad, Tokyo) at 545 nm with reference wavelength at 650 nm. Endotoxin from *E. coli* strain O113:H10 was used for calibration. The samples were diluted serially using endotoxin-free water. The pipet chips and microplates guaranteed of endotoxin-free were used for this analysis.

Organic carbon Total organic carbon was analyzed using TOC 5000 analyzer (Shimadzu, Kyoto). Assimilable organic carbon (AOC) was determined by slightly modified procedure reported by van der Kooij *et al.* (1982). One mL mineral solution was added to 100 mL sample pasteurized at 70 ℃ for 30 min. Then, *Pseudomonas fluorescens* strain P17 (ATCC 49642) and *Aquaspirillum* sp. strain NOX (ATCC 49643) were inoculated. The inoculated samples were incubated at 20 ℃. With interval of a few days, the bacterial cells were enumerated by counting colonies formed on R2A agar plate separately. The AOC-P17 and AOC-NOX concentrations were calculated from the maximum colony counts divided by each yield factor, which were determined preliminary using acetate as a sole carbon source, respectively. The yield factors in this study were determined as 4.53×10^6 CFU/μg-C for P17, 1.54×10^7 CFU/μg-C for NOX, respectively.

Chlorines Free and combined chlorines were determined by DPD-Ferrous titration method according to the Standard Methods (1998).

Statistical Analysis

All statistical analyses were performed with GraphPad Prism ver. 4.0 for Macintosh (GraphPad software Inc., San Diego). To compare the differences between two groups of samples, a nonparametric *t*-test was performed. Significant differences were determined with a level of $p < 0.01$ in all analyses.

RESULTS AND DISCUSSION

Water Quality Parameters Associated with Bacterial Regrowth

The averages of water quality parameters in two different distribution systems were compared in **Table 1**. In both distribution systems, total chlorine residuals not less than 0.3 mg/L in average were detected, and HPCs were inactivated sufficiently. The average TOC content in DS-A was higher than that in DS-B. It seemed that TOC was removed effectively by ozonation and biological activated carbon adsorption processes in DS-B. The average AOC content in DS-B, however, was slightly lower than that in DS-A, but the difference was not significant. This result suggested that advanced water treatment system consisted of ozonation and GAC or BAC had only a definite improvement in AOC removal. It has been well-known that ozonation increased AOC concentration by degrading high molecular weight organics to low molecular weight and polar compounds (Hammes *et al.*, 2006). Then, some parts of AOC was removed during BAC process (Hu *et al.*, 1999), but the overall efficiency of AOC removal was not improved significantly.

Total and free endotoxins in DS-B were higher than those in DS-A as opposing to organic carbons. The reason has not been identified yet, but in our previous research, the same level of endotoxin, 10 EU/mL approximately, was found in finished water at other water treatment plant located in the same river basin of Plant B, which has a similar treatment processes including ozonation and BAC (Ohkouchi *et al.*, 2007). Two possible explanations are following; 1) Endotoxin concentration in raw water of Plant B is higher than that of Plant A, 2) BAC process causes to increase endotoxin in finished water. The average ratio of free endotoxin to total endotoxin was slightly lower in DS-B, but the difference was not significant.

On the other hand, in comparison of AOC concentrations in summer and winter in DS-A, the average AOC in winter was three-fold greater than that in summer. In winter, biological activities in water and biofilm were decreased because of low water temperature, so larger amount of AOC was remained through the water distribution. van der Kooij (1992) has proposed that AOC level for prevention of bacterial regrowth was less than 10 μg-C/L, but all our AOC data exceeded that biologically stable level substantially. Besides, there were no differences in the average endotoxin levels and the average ratios of free endotoxin in both seasons.

These results suggested that the ratios of free endotoxin to total endotoxin were usually very high in the water distribution systems which were sufficiently

Table 1 Average of parameters associated with bacterial regwowth in tap water.

Parameter	DS-A		DS-B
	May-June 2007	January 2008	January 2008
Sample numbers	n=40	n=6	n=6
TOC (mg-C/L)	1.8±0.25	–	1.3±0.13*
AOC (μg acetate-C/L)	59.8±15.6	173.9±43.5*	136.0±36.2*
AOC-P17	39.5±13.8	135.6±41.7*	104.7±37.1*
AOC-NOX	20.3±9.6	38.3±11.0*	31.2±3.9
Chlorine residual (mg Cl_2/L)	0.52±0.13	0.30±0.06*	0.31±0.11*
Free chlorine	0.40±0.12	0.25±0.06	0.21±0.10*
Combined chlorine	0.12±0.02	0.088±0.03	0.096±0.02
HPC (CFU/mL)	0.3±0.5	0.0±0.0	0.8±1.9
Total endotoxin (EU/mL)	1.48±0.69	1.16±0.13	6.72±1.85*#
Free endotoxin (EU/mL)	1.46±0.56	1.08±0.23	5.52±0.95*#
Ratio of free endotoxin	1.01±0.15	0.93±0.18	0.85±0.19

Values are the means with standard deviation.

* $p < 0.01$, compared with the samples taken in early summer in distribution system supplied by DS-A.

# $p < 0.01$, compared with the samples taken in winter in distribution system supplied by DS-A.

chlorinated, while concentrations of total endotoxin were relatively constant in each distribution system. But total endotoxin could vary depending on source water or treatment processes.

Bacterial Regrowth in Batch Mode Experiments

Among examined 25 samples, the bacterial regrowth phenomena after 7 days incubation were observed in only 6 samples. In these samples, HPC numbers ranged from 2.3×10^3 to 4.7×10^5 CFU/mL. Endotoxin levels and the ratios of free endotoxin between regrown samples and not-regrown samples were compared in **Fig. 2**. On day 1 after neutralizing chlorine residual, there were no significant differences in all parameters (data not shown). On day 7, it was found that total endotoxin concentrations were increased up to 10 - 40 EU/mL in water samples observed regrowth, while free endotoxin showed little increase. Next, the cell-bound endotoxin concentrations were determined by subtracting free endotoxin from total endotoxin. They were significantly higher in regrown samples ($p < 0.01$), and they represented large proportion of total endotoxin increases. Then, the ratios of free endotoxin were decreased significantly ($p < 0.01$) in regrown samples. It has been believed that free endotoxin was released from bacterial cells with multiplication or environmental stresses. Although almost all part of endotoxin existed as free endotoxin in coexistence of some stressors, such as disinfectant residual in tap water, the cell-bound endotoxin seemed to be increased along with bacterial regrowth once the stressor was removed. These results indicated that cell-bound endotoxin or the ratios of free endotoxin could be considered as useful indicators for regrown bacteria.

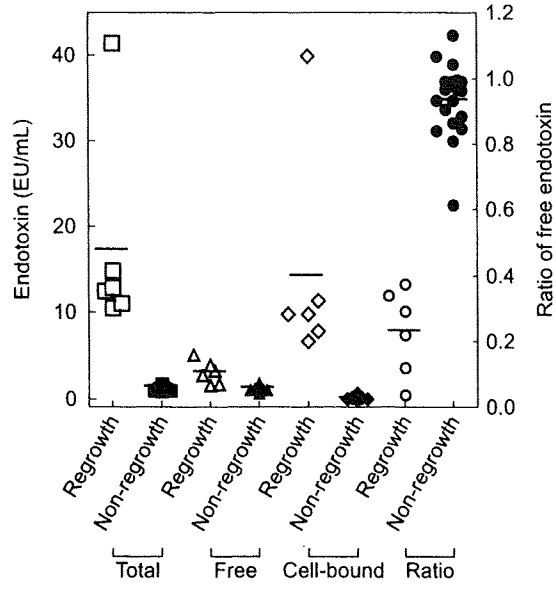

Fig. 2 Comparison of endotoxin between regrown and non-regrown samples.

Biofilm Accumulation in Continuous Flow Reactor Systems

Effects of chlorine residual on biofilm accumulation on PVC coupons The biofilm accumulation on PVC coupons were examined under continuous flow conditions. The time-dependent changes of HPC and total bacterial cells in biofilm were shown in **Fig. 3**. The HPC in biofilm on PVC coupons in AR without chlorine residual reached a stationary phase after 100 days operation, and the maximum biofilm density was approximately 5×10^5 CFU/cm^2. In AR with chlorine residual of 0.1 mg/L, the actual concentrations of chlorine residual were 0.07 $\pm$0.05 mg/L. The HPC in biofilm fluctuated after 85 days operation, but the maximum density of biofilm was approximately 2-log lower than that in AR without chlorine residual. At initial phase of biofilm accumulation, the total bacterial cells in AR without chlorine residual remained at higher level than corresponding HPC, but the percentages of HPC were increased drastically. In AR with chlorine residual, the total bacterial cells remained 10 times higher than HPC in average for the entire period.

The biofilm accumulation rates were calculated based on each HPC data until around day 110. The biofilm accumulation rate of 0.078 day^{-1} in AR without chlorine residual was twofold greater than that in AR at chlorine residual of 0.1 mg/L (0.040 day^{-1}). Pederson (1990) has reported that the doubling time of total number of microorganisms in biofilm was 11 days using biofilm reactors fed tap water with chlorine residual of

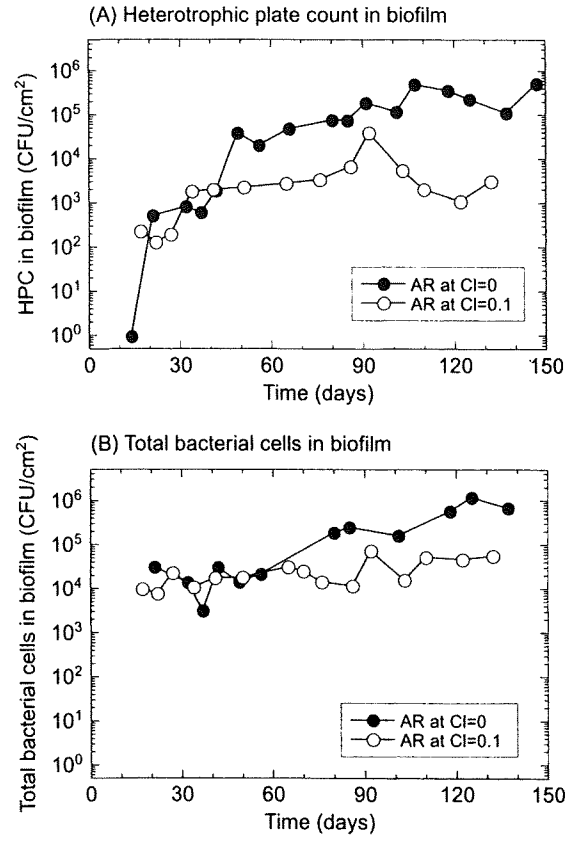

Fig. 3 Changes of HPC and total bacterial ells in biofilm.

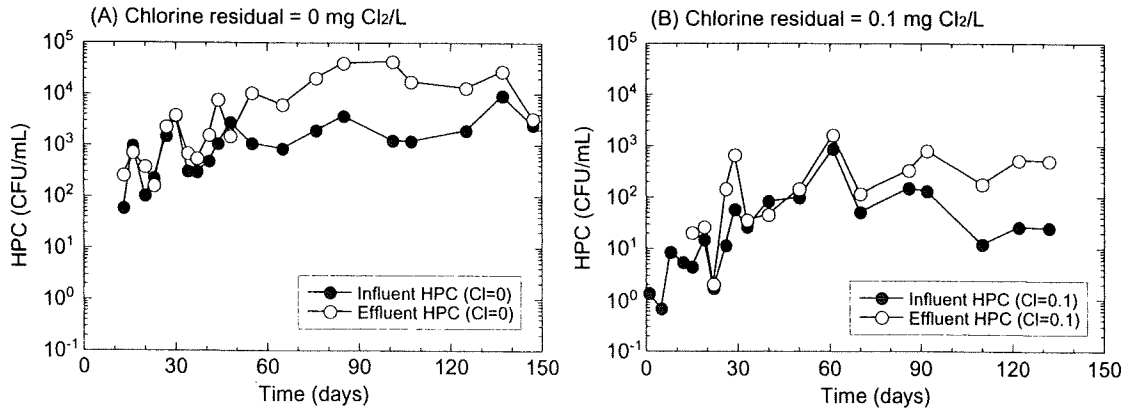

Fig. 4 Changes of HPC in influent and effluent water of each AR.

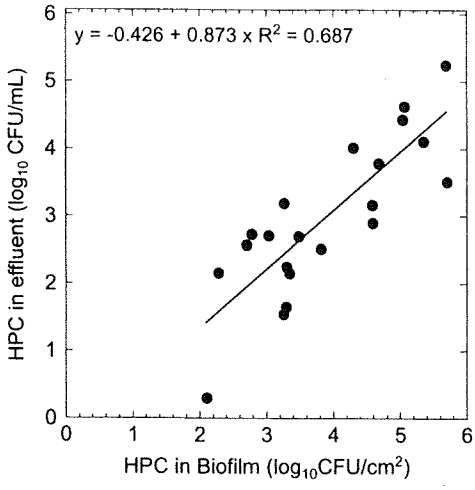

Fig. 5 Relationship between HPC in biofilm and HPC in effluent of AR.

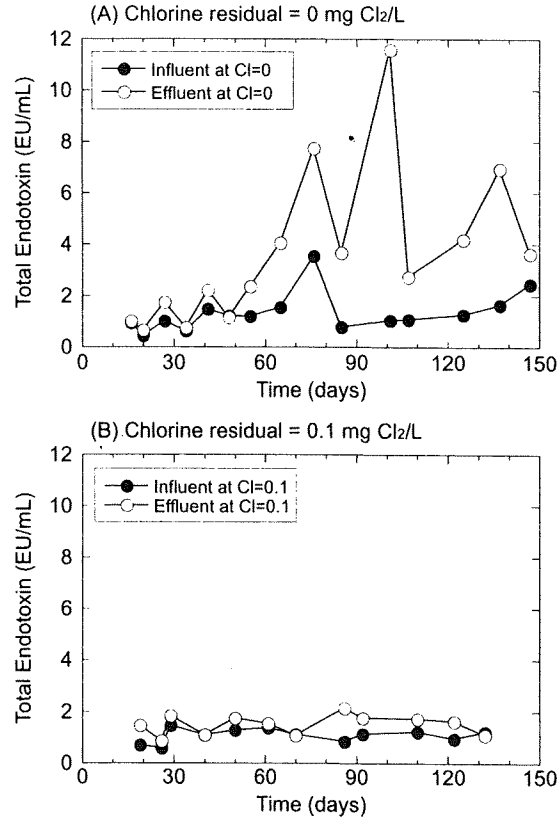

Fig. 6 Changes of endotoxin in influent and effluent of AR.

0.1 mg/L. The biofilm accumulation rate in his research was calculated as 0.063 day^{-1}, and our results were nearly equal to his result. These results indicated that low concentration of chlorine residual such as 0.1 mg/L could not prevent biofilm formation inside distribution pipes, but it could slow progression of biofilm in terms of accumulation rate, total amount of bacterial cells, and culturable bacterial cells in biofilm.

Changes of HPC in effluent from AR The time-dependent changes of HPC in effluent from each reactor were shown in **Fig. 4**. During all phases of biofilm accumulation, there were trends toward increasing HPC numbers in the effluent from both ARs. The correlation between HPC in biofilm and HPC in effluent of ARs was shown in **Fig. 5**. The measurement dates of HPC in biofilm were not always corresponding to those of HPC in effluent, therefore only the data, whose time intervals were within 1day, were used for this analysis. After logarithmic transformation of all HPC data, the HPC in effluent from ARs was increased proportionally with HPC on biofilm, and the slope of linear regression line and the correlation coefficient (R^2) were 0.873 and 0.687, respectively. It is not practical to take biofilm samples from surface of pipes and provide HPC test frequently. This correlation suggested that HPC in bulk water phase, which could be taken easily and frequently, could be a surrogate indicator for progression of biofilm inside pipes.

Endotoxin The changes of total endotoxin in effluent were shown in **Fig. 6**. In effluent from AR without chlorine, total endotoxin was increased and fluctuated significantly after 50 days, while no significant increase of total endotoxin was observed in case of AR without chlorine. The relationship between HPC in water phase and cell-bound endotoxin was shown in **Fig. 7 (A)**. In fact, bacterial regrowth was occurred immediately after adjusting chlorine residual, therefore, all HPC data in both influent

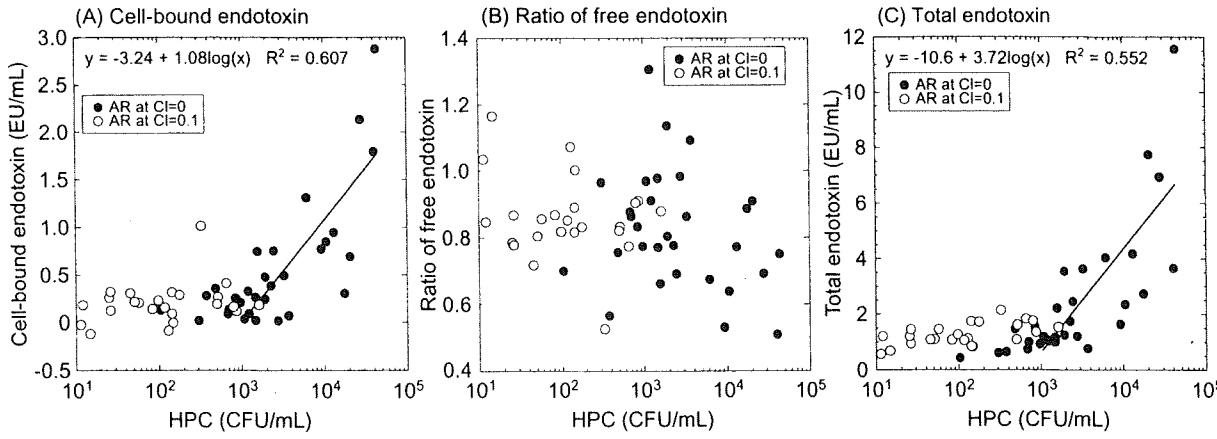

Fig. 7 Relationship between endotoxin and HPC in effluent.

and effluent were used for the following analysis. When HPC numbers in water phase were 1000 CFU/mL or higher, the cell-bound endotoxin tended to increase with HPC numbers. Especially in the range of over 5000 CFU/mL, the concentrations of cell-bound endotoxin were greater than 0.5 with one exception. However, these increased amounts were smaller than those observed in regrown samples in batch mode experiment described above. These results suggested that growth of bacteria in water phase contributed little to the increases of HPC in effluent because of short detention time, although Manuel *et al.* (2007) have reported that the specific growth rate in bulk water phase was much greater than that in biofilm. It was estimated paradoxically that HPC numbers in effluent were increased by HPC came from biofilm. As a result, it seemed that the amount of cell-bound endotoxin in effluent was affected by the difference in membrane integrities of bacterial cells in biofilm under continuous flow condition and in stagnant water as in batch mode experiment.

On the other hand, a poor correlation between HPC in water phase and ratios of free endotoxin (**Fig. 7 (B)**) was obtained. One possible explanation is that not only cell-bound endotoxin, but also free endotoxin were increased simultaneously under continuous flow condition, because there were not only chlorine residual but also more other stressors for bacteria. Total endotoxin followed a similar pattern with cell-bound endotoxin as shown in **Fig. 7 (C)**. The concentrations of total endotoxin were greater than 2.0 with one exception at HPC levels of over 5000 CFU/mL. And the regression slope of total endotoxin was greater than that of cell-bound endotoxin. Based on above results, it is recognized that total endotoxin could be a better and more sensitive indicator for regrown bacteria rather than indicators related to cell-bound endotoxin including ratios of free endotoxin. However, it should be also noted that these indicators of endotoxin could work effectively only in the situation that significant bacterial regrowth, such as over 5000 CFU/mL, was occurred.

CONCLUSIONS

As an indicator for regrown bacteria in distribution system, the applicability of endotoxin, which can be measured quickly, was examined in this study. In tap water samples taken in two different distribution systems, there was a difference in total endotoxin concentrations. It was suggested that endotoxin levels in water source or BAC treatment process could affect the concentrations of total endotoxin in finished water. It was also confirmed that large part of endotoxin in tap water existed as free endotoxin. Once chlorine residual was neutralized, bacteria started to grow in several water samples. In these samples, the concentrations of cell-bound endotoxin were increased drastically. At the same time, the concentrations of total endotoxin were also increased, and the ratios of free endotoxin were decreased relatively. The biofilm accumulation was also monitored using two annular reactors under continuous flow conditions. It was found that HPC in effluent of ARs changed reflecting the progression of biofilm accumulation. There was a positive correlation between the concentrations of cell-bound endotoxin or total endotoxin and HPC numbers in water phase, therefore cell-bound or total endotoxin could be indicators for regrown bacteria only when significant bacterial regrowth, such as over 5000 CFU/mL, was occurred. However, a poor correlation between the ratios of free endotoxin and HPC numbers in water phase was observed.

ACKNOWLEDGEMENT

We thank Mr. Tsukasa Kusano for sampling of tap water and their analysis. We also thank Mr. Suguru Ishikawa and Mr. Kentaro Kobayashi for helping another 12 sampling. This work was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Health, Labour and Welfare (H17-Health-025).

REFERENCES

Anderson, W.B., Slawson, R.M. and Mayfield, C.I., "A review of drinking-water-associated endotoxin, including potential routes of human exposure", *Can. J. Microbiol.*, **48**(7), 567-587 (2002).

Hammes, F., Salhi, E., Köster, O., Kaiser, H-P., Egli, T. and von Gunten, U., "Mechanistic and kinetic evaluation of organic disinfection by-product and assimilable organic carbon (AOC) formation during the ozonation of drinking water", *Water Res.*, **40** (12), 2275-2286 (2006).

Hu, J.Y., Wang, Z.S., Ng, W.J. and Ong, S.L., "The effect of water treatment processes on the biological stability of potable water", *Water Res.*, **33**(11), 2587-2592 (1999).

Itoh, S., Shiro, S., Hirayama, N., Echigo, S. and Ohkouchi, Y., "Factors related to citizens' satisfaction with tap water and analysis of improvement needs in water supply system" (in Japanese), *Environ. Sanit. Eng. Res.*, **21**(1), 9-19 (2007).

Manuel, C.M., Nunes, O.C. and Melo, L.F., "Dynamics of drinking water biofilm in flow/non-flow conditions", *Water Res.*, **41**(3), 551-562 (2007).

Ohkouchi, Y., Ishikawa, S., Takahashi, K. and Itoh, S., "Factors associated with endotoxin fluctuation in aquatic environment and characterization of endotoxin removal in water treatment process " (in Japanese), *Environ. Eng. Res.*, **44**, 247-254 (2007).

Pedersen, K., "Biofilm development on stainless-steel and PVC surfaces in drinking water", *Water Res.*, **24**(2), 239-243 (1990).

Standard Methods for the Examination of Water and Wastewater, 20th edn., American Public Health Association/ American Water Works Association/ Water Environment Federation, Washington DC, USA (1998).

Sykora, J.L., Keleti, G., Roche, R. and Volk, D.R., "Endotoxins, algae and *Limulus* amoebocyte lysate test in drinking water", *Water Res.*, **14**, 829-839 (1980).

van der Kooij, D., "Multiplication of bacteria in drinking water", *Antonie van Leeuwenhoek,* **47**(3), 281-283 (1981).

van der Kooij, D., "Assimilable organic carbon as indicator of bacterial regrowth", *Journal AWWA,* **84** (Feb.), 57-65 (1992).

[Received: June 23, 2008]
[Accepted: December 1, 2008]

Abundance and diversity of ammonia-oxidizing archaea and bacteria on biological activated carbon in a pilot-scale drinking water treatment plant with different treatment processes

Ikuro Kasuga [1]*, Hirotaka Nakagaki [1], Futoshi Kurisu [2] and Hiroaki Furumai [2]

[1] Department of Urban Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo, 113-8656, Japan
(E-mail:* kasuga@env.t.u-tokyo.ac.jp, nakagaki@env.t.u-tokyo.ac.jp)
[2] Research Center for Water Environment Technology, The University of Tokyo,
7-3-1, Hongo, Bunkyo, Tokyo, 113-8656, Japan
(E-mail:kurisu@env.t.u-tokyo.ac.jp, furumai@env.t.u-tokyo.ac.jp)

Abstract

The effects of different placements of rapid sand filtration on nitrification performance of BAC treatment in a pilot-scale plant were evaluated. In this plant, rapid sand filtration was placed after ozonation-BAC treatment in Process (A), while it preceded ozonation-BAC treatment in Process (B). Analysis of amoA genes of ammonia-oxidizing archaea (AOA) and bacteria (AOB) combined with nitrification potential test was conducted. BAC from Process (A) demonstrated slightly higher nitrification potential at every sampling occasion. This might be due to higher abundances of AOB on BAC from Process (A) than those on BAC from Process (B). However, AOA rather than AOB could be predominant ammonia-oxidizers in BAC treatment regardless of the position of rapid sand filtration. The highest nitrification potential was observed for BAC from both processes in February when the highest abundances of AOA-amoA and AOB-amoA genes were detected. Since rapid sand filtration was placed after BAC treatment in Process (A), residual aluminum concentration in BAC influent was higher in Process (A). However, adverse effects of aluminum on nitrification activity were not observed. These results suggest that factors other than aluminum concentration in different treatment processes could possibly have some influence on abundances of ammonia-oxidizing microorganisms on BAC.

Keywords

ammonia-oxidizing archaea, ammonia-oxidizing bacteria, biological activated carbon

INTRODUCTION

Ozonation followed by biological activated carbon (BAC) is one of the advanced drinking water treatment technologies. Some organic matters are degraded by ozone oxidation and then adsorbed by granular activated carbon. In addition to the physicochemical removal, microorganisms associated with granular activated carbon are known to be involved in the treatment. One of the biological contributions is removal of ammonium-nitrogen through nitrification (Andersson *et al.*, 2001; Simpson, 2008; Wert *et al.*, 2008). When ammonium-nitrogen in raw water reacts with chlorine, trichloramine which causes smell of chlorine could be produced as a by-product. Since consumers are sensitive to smell or odor of drinking water, waterworks are required to suppress production of trichloramine. Biological nitrification in BAC treatment can efficiently treat ammonium-nitrogen which resulted in the reduction of the precursor of trichloramine. Though trichloramine is not included in the Japanese water quality standard for drinking water, Tokyo metropolitan government has established the original drinking water standard in which trichloramine concentration should be controlled at 0 mg/L. To achieve this standard, ozonation-BAC treatment will be introduced to almost all major drinking water treatment plants in Tokyo by 2013.

Though some studies investigated bacterial community associated with BAC by using

culture-dependent (Suffet and Pipes, 1986) and culture-independent techniques (Kasuga *et al.*, 2007; Kasuga *et al.*, 2009), the mechanisms of biological treatment including diversity, abundance and activity of attached microorganisms remain unknown. Little information is available especially for nitrifying microorganisms on BAC. So far, ammonia-oxidizing bacteria (AOB) have been regarded as only players for oxidation of ammonium-nitrogen. However, the recent discovery of ammonia-oxidizing archaea (AOA) capable of oxidizing ammonium-nitrogen has dramatically changed our understanding (Könneke *et al.*, 2005; Treusch *et al.*, 2005; Nicol and Schleper, 2006). AOA have been found in various environments including ocean, soil, and water treatment processes (Francis *et al.*, 2005; Leininger *et al.*, 2006; Park *et al.*, 2006; Wuchter *et al.*, 2006; de Vet *et al.*, 2008). Contribution of AOA to nitrogen cycle needs to be considered, though ecology and physiology of AOA have not been elucidated yet. Thus, microbiological diagnosis of AOA as well as AOB is required to evaluate nitrification performance of BAC treatment.

In this study, nitrification performance of BAC treatment in a pilot-scale plant was investigated by analyzing abundance and diversity of AOA and AOB associated with BAC. This pilot-scale plant compared two treatment processes in which rapid sand filtration was placed before or after ozonation-BAC treatment. In Japan, both treatment processes have been put in practical use in advanced drinking water treatment plants. However, effects of different placements of rapid sand filtration on treatment performance especially for biological nitrification have not been investigated. Here, we discussed vertical and seasonal variations of nitrification performance of BAC treatments in both processes. In addition, since rapid sand filtration preceding BAC treatment can efficiently remove residual polyaluminum chloride used for coagulation, effects of aluminum on nitrification activity were evaluated.

METHODS

Pilot-scale plant for advanced drinking water treatment

A pilot-scale plant capable of treating 200 m^3/day with two different processes is shown in **Figure 1**. After river water was treated by coagulation and sedimentation, two treatment processes (A) and (B) were compared. In Process (A), rapid sand filtration was placed after ozonation-BAC treatment. On the other hand, rapid sand filtration was followed by ozonation-BAC treatment in Process (B). Operational conditions of ozonation-BAC treatment were summarized in **Table 1**.

Sampling

BAC samples were collected from the surface layer of BAC beds in Process (A) (BAC-A) and Process (B) (BAC-B) on 21 September and 26 November of 2007 and 21 February of 2008. In addition to the surface layer samples, middle layer (1 m from the surface) and bottom layer (2 m from the surface) samples from both processes were also obtained on 21 September of 2007.

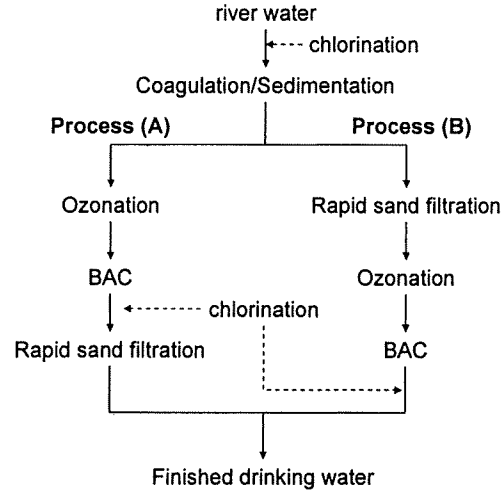

Figure 1 Treatment processes in a pilot-scale plant

Nitrification potential test

Nitrification potential of BAC samples was evaluated by batch experiment. In an Erlenmeyer flask, 100 g-wet of BAC sample was mixed with 200 mL of inorganic media containing 5 mg NH_4-N/L as the sole nitrogen source. It was incubated at 20°C with constant agitation. After 0, 8,

Table 1 Operational conditions of ozonation and BAC treatments

	Operational conditions	unit	Process (A)	Process (B)
Ozonation	Nominal capacity	m^3/day	66	45
	Dissolved ozone concentration	mg/L	0.1	0.1
	Contact time	min	5	10
	Retention time	min	5	5
BAC	Nominal capacity	m^3/day	55	34
	Bed volume	m^3	0.46	0.28
	Bed depth	m	2	2
	Flow regime	-	down flow	down flow
	Empty bed contact time	min	12	12
	Linier velocity	m/day	240	240
	Effective diameter of AC	mm	1.2	0.7
	Backwashing interval	hr	72	72
Rapid Sand Filtration	Grain size: anthracite	mm	1.3	1.3
	Grain size: sand	mm	0.6	0.6
	Line velocity	m/day	120	120
	Backwashing interval	hr	168	72

16, 24 and 48 hours, ammonium-nitrogen was determined spectrophotometrically using the indophenol-blue method. Nitrite-nitrogen and nitrate-nitrogen were measured with an ion chromatograph (761 Compact IC, Metrohm).

Effects of aluminum on nitrification

The BAC sample collected from the surface layer of the BAC bed in Process (B) on 21 September of 2007 was mixed with the media containing 5 mg NH_4-N/L used for nitrification potential test. Additionally, polyaluminum chloride was added at the final concentrations of 0.02, 0.2 and 2.0 mg Al/L, respectively. The control without addition of aluminum (0 mg Al/L) was also prepared. After incubating them at 20°C with constant agitation for one month, all media were replaced with newly prepared ones without aluminum. Thereafter, nitrification potential was evaluated as described above. The first-order reaction model expressed in the equation (1) was applied to estimate the rate constants by the least-square regression.

$$C = C_0 e^{-kt} \quad (1)$$

where C=concentration of ammonium-nitrogen (mg N/L), C_0=Initial concentration of ammonium-nitrogen (mg N/L), k=the first-order rate constant (1/hr), and t=time (hr)

DNA extraction and PCR amplification

DNA was extracted from BAC samples by using a PowerSoil™ DNA kit (Mo Bio Laboratories). Arch-amoAF and Arch-amoAR primers were used for amplification of AOA-*amoA* genes (Francis *et al.*, 2005). On the other hand, amoA-1F and amoA-2R primers specific to *amoA* genes of betaproteobacterial AOB were used for amplification of AOB-*amoA* genes (Rotthauwe *et al.*, 1997). The 5′ ends of the forward primers (Arch-amoAF and amoA-1F) were labeled with 6-carboxyfluorescein (6-FAM). After thermal reactions, PCR products were checked by agarose gel electrophoresis.

Terminal-restriction fragment length polymorphism

Since we could not obtain enough amounts of AOB-*amoA* genes by PCR amplification, terminal-restriction fragment length polymorphism (T-RFLP) analysis was applied to only

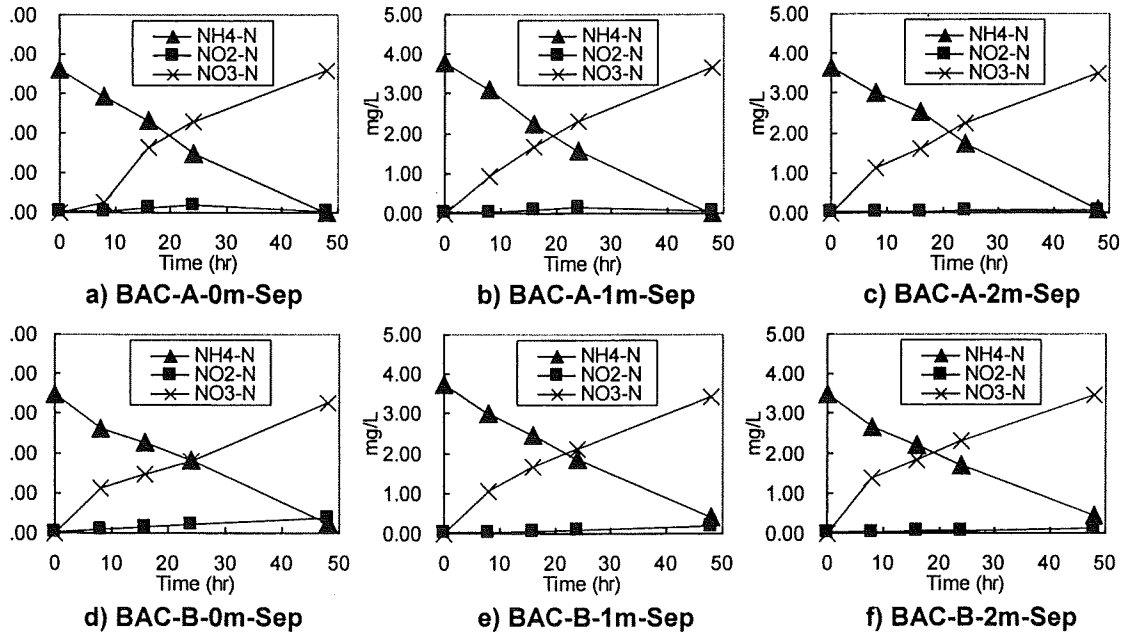

Figure 2 Nitrification potential of BAC-A and BAC-B on 21 September 2007 collected from surface (0m), middle (1m) and bottom (2m) layers of the BAC beds

AOA-*amoA* genes. After the PCR products of AOA-*amoA* genes were digested with *Hha*I (TaKaRa BIO), the 6-FAM-labeled fragments were separated with an ABI Prism® 310 Genetic Analyzer (Applied Biosystems). Fragment analysis was carried out by using GeneMapper™ v3.0 software (Applied Biosystems).

Real time PCR quantification

Quantification of AOA-*amoA* genes and AOB-*amoA* genes were conducted following the protocol described previously (Leininger *et al.*, 2006). Copy numbers of AOA-*amoA* genes were determined with primers amo196F and amo277R and probe amo247. The 5′ end of amo247 was labeled with 6-FAM while the 3′ end was labeled with carboxytetramethylrhodamine (TAMRA). Reaction mixture was prepared by using iTaq Supermix with ROX (Bio-Rad). On the other hand, AOB-*amoA* genes were quantified with primers amoA-1F and amoA-2R using QuantiTect SYBR Green PCR Master Mix (Qiagen). All real time PCR reactions were carried out with a LightCycler 2.0 (Roche).

RESULTS AND DISCUSSION

Removal of ammonium-nitrogen by pilot-scale plant operation

As water temperature decreased from 25.1°C on 21 September of 2007 down to 6.8°C on 21 February of 2008, ammonium-nitrogen concentration in raw water was increased from less than 0.02 mg N/L on 21 September of 2007 to 0.20 mg N/L on 21 February of 2008, which was in accordance with deterioration of natural nitrification activity in river. On the sampling occasions in September and November of 2007, ammonium-nitrogen was completely treated by BAC filtration in both processes. However, on 21 February of 2008, 0.14 mg N/L and 0.07 mg N/L of ammonium-nitrogen were detected in BAC effluent in Process (A) and Process (B), respectively. Such an incomplete ammonium-nitrogen removal by BAC treatment was frequently observed when water temperature decreased to less than 10°C (data not shown). Ammonium-nitrogen concentrations in BAC influent of both processes were same throughout the investigation period. It indicated that nitrification did not occur in the precedent rapid sand filtration in Process (B). We can assume that the same volume load of ammonium-nitrogen for BAC treatment in both processes.

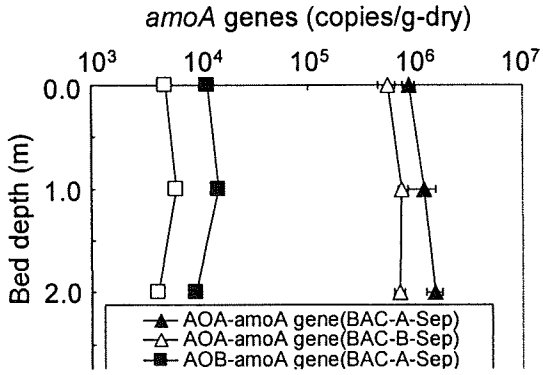

Figure 3 Vertical distributions of AOA-*amoA* and AOB-*amoA* genes in the BAC beds in Process (A) and Process (B) (21 September, 2007)

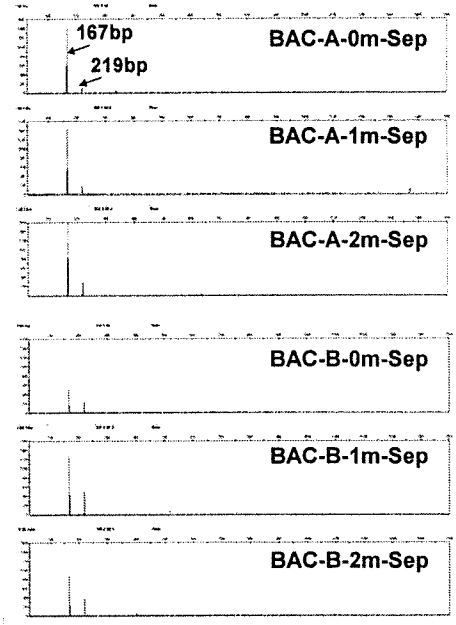

Figure 4 T-RFLP profiles of AOA-*amoA* genes in the BAC beds in Process (A) and Process (B) (21 September, 2007)

Vertical variations of nitrification performance

Figure 2 shows vertical variations of nitrification potential of BAC-A and BAC-B collected on 21 September of 2007. Similar nitrification kinetic profiles were observed for BAC-A and BAC-B at different layers. BAC-A could treat almost all ammonium-nitrogen within 48 hours while 0.27, 0.41, and 0.46 mg N/L of ammonium-nitrogen remained after 48 hours for BAC-B-0m, BAC-B-1m, and BAC-B-2m, respectively. This result demonstrated nitrification potential of BAC-A was slightly better than that of BAC-B.

Figure 3 shows the vertical distribution of AOA-*amoA* and AOB-*amoA* genes in the BAC beds in Process (A) and Process (B) quantified by real time PCR. In both processes, abundances of AOA-*amoA* genes detected at all layers of the BAC beds were in the range of 10^5-10^6 copies/g-dry, which were one to two orders of magnitude higher than those of AOB-*amoA* genes. The result suggests that AOA rather than AOB could be predominant ammonia-oxidizers in BAC treatment regardless of the position of rapid sand filtration in the treatment process train. AOA-*amoA* and AOB-*amoA* genes were distributed in the BAC beds uniformly in both processes probably due to mixing of BAC beds by regular backwashing. At all layers, abundances of AOA-*amoA* and AOB-*amoA* genes of BAC-A were approximately two times higher than those of BAC-B. The higher nitrification potential of BAC-A in September could probably be due to higher abundances of ammonia-oxidizing microorganisms on BAC. Since AOB-*amoA* genes were not enough in number for the analysis, only AOA-*amoA* genes were characterized by the T-RFLP analysis. As shown in **Figure 4**, digestion of the PCR products of AOA-*amoA* genes produced two distinctive T-RF peaks of 167 bp and 219 bp in all BAC samples. This composition did not change at all layers in both processes, suggesting that the diversity of AOA associated with BAC is not affected by the position of rapid sand filtration.

Seasonal variations of nitrification performance

In **Figure 5**, seasonal variations of nitrification potential of BAC-A and BAC-B (surface layer)

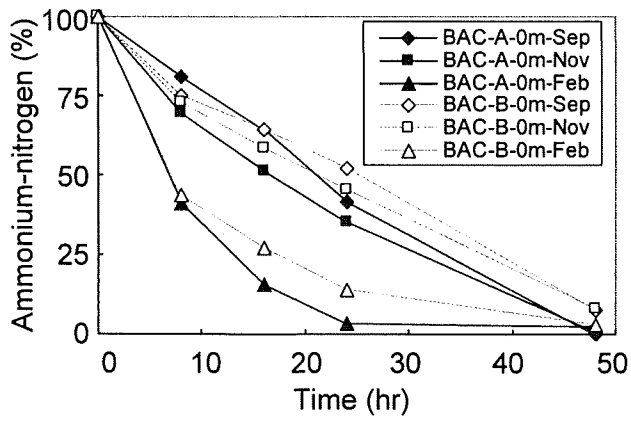

Figure 5 Seasonal changes of nitrification potential of BAC-A and BAC-B

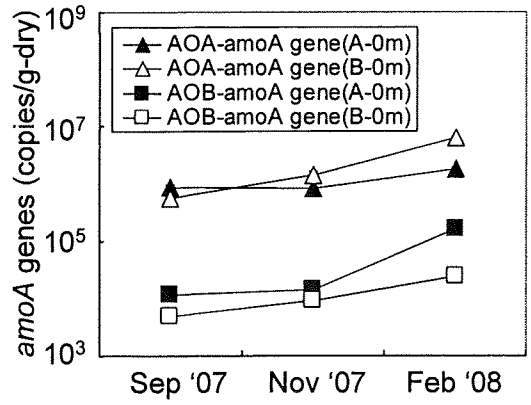

Figure 6 Seasonal changes in abundances of AOA-*amoA* and AOB-*amoA* genes of BAC-A and BAC-B

were compared. The oxidation rates of ammonium-nitrogen for both BAC samples were obviously faster in February than those in September and November. BAC-A had slightly higher potential than BAC-B at every sampling occasion.

Figure 6 shows seasonal changes in abundances of AOA-*amoA* and AOB-*amoA* genes of BAC-A and BAC-B (surface layer). AOA-*amoA* and AOB-*amoA* genes of both BAC samples were gradually increased from September to February. Ammonium-nitrogen concentrations in BAC influent in both processes were increased from less than 0.02 mg N/L in September to 0.20 mg N/L in February. This trend was in accordance with changes in abundances of AOA-*amoA* and AOB-*amoA* genes. The highest nitrification potential of BAC-A and BAC-B in February could be attributed to the highest abundances of ammonia-oxidizing microorganisms on BAC in February. Abundances of AOA-*amoA* genes of BAC-B were lower than those of BAC-A in September, but 1.7 times and 3.6 times higher in November and February, respectively. On the other hand, AOB-*amoA* genes of BAC-A were always approximately 1.5-6.8 times more abundant than those of BAC-B. Some factors of Process (A) could be associated with higher abundances of AOB on BAC-A. The fact that BAC-A demonstrated slightly higher nitrification potential at every sampling occasion might be associated with higher abundances of AOB rather than those of AOA during the investigation period. Diversity of AOA-*amoA* genes observed in BAC-A and BAC-B in November and February was identical to that in September (data not shown), indicating that composition of AOA on BAC was stable in both processes. Though the highest nitrification potential and the highest abundances of AOA-*amoA* and AOB-*amoA* genes were observed in February for both BAC samples, the monitoring data of the pilot-scale plant indicated that ammonium-nitrogen was detected in BAC effluent in both processes in February. The actual activity of AOA and AOB were probably suppressed under the low water temperature conditions despite the highest abundances and potential they had in February.

Effects of aluminum on nitrification activity

Since rapid sand filtration was placed before ozonation in Process (B), suspended solids and residual coagulants such as polyaluminum chloride can be removed before BAC treatment in Process (B). Average concentration of total aluminum after sedimentation was 0.21 mg Al/L. The BAC influent in Process (A) contained almost the same concentration of aluminum. On the other hand, rapid sand filtration preceding ozonation-BAC treatment reduced total aluminum concentration to 0.02 mg Al/L in Process (B). Since aluminum is toxic to bacteria (Wood, 1995),

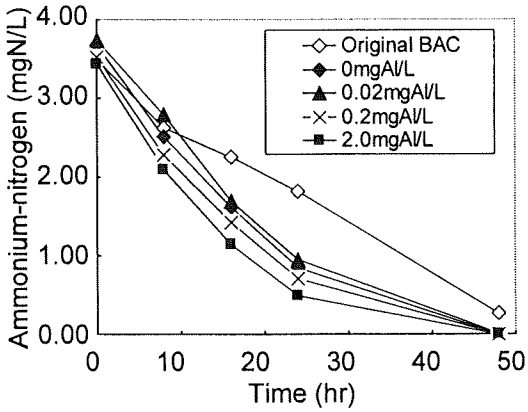

Figure 7 Effects of aluminum on nitrification potential. Original BAC indicates BAC sample before one-month incubation

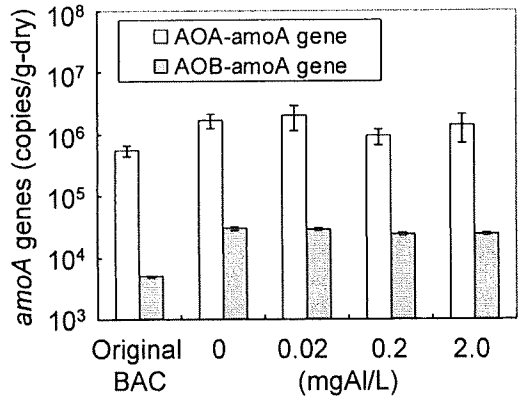

Figure 8 Effects of aluminum on abundances of AOA-*amoA* and AOB-*amoA* genes. Original BAC indicates BAC sample before one-month incubation.

effects of aluminum on nitrification activity were evaluated. After incubating BAC samples collected from Process (B) on 21 September of 2007 (original BAC) with 0 (control), 0.02, 0.2 and 2.0 mg Al/L of polyaluminum chloride for one month, nitrification potential was determined (as shown in **Figure 7**). Compared with nitrification potential of the original BAC sample before one-month incubation, all batches including the control demonstrated faster oxidation rates after one-month incubation. The first-order reaction rate constants were 0.14, 0.13, 0.14 and 0.16 (1/hr) for the batches with 0, 0.02, 0.2, and 2.0 mg Al/L, respectively. Relationship between aluminum concentration levels and nitrification potential were not observed.

Figure 8 shows abundances of AOA-*amoA* and AOB-*amoA* genes after incubation with aluminum. Compared with the original sample before one-month incubation included in **Figure 8**, abundances of AOA-*amoA* and AOB-*amoA* genes in all batches after one-month incubation were increased 1.7-3.7 times and 4.8-6.0 times, respectively. Improvement of nitrification potential could be attributed to growth of AOA and AOB during one-month incubation. This result indicates that aluminum concentration levels tested here do not inhibit AOA and AOB associated with BAC.

CONCLUSIONS

BAC-A collected from Process (A), where rapid sand filtration was placed after ozonation-BAC treatment, demonstrated slightly higher nitrification potential at every sampling occasion. This might be associated with higher abundances of AOB on BAC-A than those on BAC-B, though AOA could be predominant ammonia-oxidizers in BAC treatment regardless of the position of rapid sand filtration. The highest nitrification potential was observed in February for BAC-A and BAC-B when the highest abundances of both AOA-*amoA* and AOB-*amoA* genes were observed. Diversity of AOA-*amoA* genes was not different between BAC-A and BAC-B during the investigation period. Although higher residual aluminum concentration in BAC influent was observed in Process (A), there were no adverse effects of aluminum on nitrification activity. These results suggest that factors other than aluminum concentration in different treatment processes could possibly have some influence on abundances of ammonia-oxidizing microorganisms on BAC.

ACKNOWLEDGEMENT

This work was partially financed by Grant-in-Aid for Young Scientists (B) (no. 20760358) from