systems (Sung *et al.* 2000; Rossman *et al.* 2001; Gallard & Gunten 2002; Nikolaou *et al.* 2004; Rodriguez *et al.* 2004; Sohn *et al.* 2004). On the other hand, DBPs formed by chlorine dioxide including inorganic by-products such as chlorite and chlorate ions have also been examined (Chang *et al.* 2000*a*,*b*; Dabrowska *et al.* 2003; Veschetti *et al.* 2005). In addition, a few studies show the change or persistence of DBPs formed by chlorine dioxide in distribution systems (Korn *et al.* 2002; Hoehn *et al.* 2003). It is widely believed that increasing the levels of typical DBPs mentioned above imply the increase in the toxicity of drinking water, although this is not clearly described in most studies.

However, we have to pay attention to numerous other DBPs in addition to typical ones formed during disinfection. From this point of view, *in vitro* short-term genotoxicity tests are useful, because they can evaluate the combined action of DBPs present in drinking water as complex mixtures. Actually, many investigations have already been carried out on the mutagenicity in chlorinated drinking water (Rapson *et al.* 1980; Meier *et al.* 1983; Wilcox & Williamson 1986; Donald *et al.* 1989; Kopfler *et al.* 1990; Tanaka *et al.* 1991). As a result, some characteristics on the mutagenicity of chlorinated water have been clarified. One of the representative characteristics is that the mutagenicity easily changes over time after disinfection depending upon pH and temperature of water (Rapson *et al.* 1980; Meier *et al.* 1983; Kinae *et al.* 1992; Itoh *et al.* 2001). These findings suggest that the direction of change in the mutagenicity is inconsistent with that of typical DBPs such as THMs and HAAs in drinking water, which indicates genotoxicity tests are of value for the toxicity detection of water.

There have been some studies on the mutaganicity formation by chlorine dioxide and the comparison between waters treated with chlorine dioxide and chlorine (Donald *et al.* 1989; Anderson *et al.* 1990; Itoh *et al.* 2001; Guzzella *et al.* 2004; Onarca *et al.* 2004), however, no studies have been conducted on the change in the mutagenicity formed by chlorine dioxide over time after the water treatment. We have to consider that there are some differences in the mutagenicity level and the change rate of the mutagenicity over time after disinfection between chlorination and chlorine dioxidation.

This study compares the toxicity of water treated with chlorine and chlorine dioxide. In addition, we examined changes in the mutagenicity of disinfected water in order to estimate the total toxicity of drinking water in distribution systems. Based on the obtained results, we evaluate the advantages of chlorine dioxide. Finally, we also discuss the limitations of chlorine dioxide treatment, which would differ from the generally accepted evaluation.

MATERIALS AND METHODS

Chlorination of humic acid

Commercial humic acid (Wako Pure Chemical Industries, Ltd.) dissolved in water was used as a model substrate in this study. The total organic carbon (TOC) of the humic acid solution was 1,030 mg/L. A sodium hypochlorite stock solution (Wako Pure Chemical Industries, Ltd.) was used for chlorination. Available chlorine in the stock solution was analyzed by the iodometric method (Clesceri *et al.* 1998) just prior to use. In order to measure the mutagenicity of chlorinated water and its change without concentrating a disinfected water, the TOC (1,030 mg/L) of the humic acid solution and the concentration of added chlorine were high. A problem of these reaction conditions and the applicability of the obtained results will be discussed in Estimation of the change in mutagenicity. Chlorination was performed by addition of the desired amount of sodium hypochlorite solution diluted with chlorine demand-free water. Chlorine dosage typically used in practice would be approximately 1.0 of Cl_2/TOC. Therefore, 1,000 mg/L of chlorine was added as approximately 1.0 of Cl_2/TOC. In addition, 2,000, 3,000, 3,500, and 4,000 mg/L of chlorine were also added as higher Cl_2/TOC cases. The pH was adjusted to 7.0 by a phosphate buffer with a final concentration of 200 mM, followed by HCl or NaOH. The reaction proceeded in the dark at 20°C. Dechlorination was not carried out so as not to change the activity that induces chromosomal aberrations in the chlorinated water (Donald *et al.* 1989). It was confirmed that chlorine had no influence on the chromosomal aberration test up to a concentration of 50 mg-Cl_2/L in the culture media, which means residual chlorine up to a concentration of 500 mg-Cl_2/L in a sample solution had no influence on the test since substances in a solution were

diluted to one tenth in the media as described in Chromosomal aberration test.

Chlorine dioxidation of humic acid

An aqueous solution of chlorine dioxide was produced by mixing sodium chlorite (Wako Pure Chemical Industries, Ltd.) solution with HCl (1 + 3) (White 1999). Chlorine dioxidation of the humic acid was carried out by adding either a 2% or 4% chlorine dioxide solution prepared just prior to use. In order to measure the mutagenicity of water treated with chlorine dioxide and its change without concentrating a disinfected water, the TOC (1,030 mg/L) of the humic acid solution and the concentration of added chlorine dioxide were high. 800 mg/L of chlorine was added as approximately 0.8 of ClO_2/TOC. In addition, 1,600, 2,000, and 4,000 mg/L of chlorine were also added as higher ClO_2/TOC cases. The pH was adjusted to 7.0 by a phosphate buffer with a final concentration of 200 mM, followed by HCl or NaOH. The reaction proceeded in the dark at 20°C. Residual chlorine dioxide was not removed so as not to change the activity that induces chromosomal aberrations in treated waters. Chromosomal aberration test for some samples cannot be carried out, because cytotoxicity resulted from chlorite and chlorate ions formed during the treatment is strong. The pH of the treated water was adjusted to 2.5 using HCl, and the solution was allowed to stand in the dark at 20°C for 5 days to allow a decrease in the concentration of chlorite ion under acidic conditions. We were able to carry out chromosomal aberration tests using samples containing less than 110 mg/L of chlorite ion.

Chromosomal aberration test

Chromosomal aberration tests using Chinese hamster lung cells (CHL/IU, Dainihon Pharmaceutical Co., Ltd.) were carried out to evaluate mutagenicity (Sofuni 1999). Cells were cultured in Eagle's MEM (Nissui Pharmaceutical Co., Ltd) supplemented with 10% fetal bovine serum (Gibco Oriental Co., Ltd). CHL cultures were grown in 18 ml media in glass silicon-capped bottles. Two ml of chlorinated water was added to 1-day-old cultures. As a result, substances in the treated water were diluted 1:10 in the media. Bacteria in the treated water were eliminated by 0.22 μm filteration. Only activity that induced chromosomal aberrations without activation was measured in this study. Chromosome preparations were made 24 hours after addition of the treated water (Sofuni 1999).

To evaluate the results of the chromosomal aberration test objectively, the shapes of chromosomes were examined with an image analyzer (Nikon LUZEX 2D), as described previously (Itoh *et al.* 1992). Chromosomal aberrations are divided into two categories: broken and exchanged. Exchanged aberrations were detected by the developed method. 50 metaphases in a specimen were analyzed. As a CHL cell has 25 chromosomes, 1,250 of chromosomes were analyzed by each specimen. Image analysis of negative control gave a mean of 4.5 chromosomes/50 metaphases and a standard deviation of 2.6. The activity that induces chromosomal aberrations is expressed as a mean value of test results of triplicate specimens. When the activity that induces chromosomal aberrations of certain chemicals has to be judged, and when a test result has to be compared with data obtained by other laboratories, the standard method (Sofuni 1999) should be used and the method developed by ourselves could not be used. The developed method is effective in order to compare the relative intensity of the activity that induces chromosomal aberrations only in this study.

Analytical procedures

Chloroform in the water treated with chlorine or chlorine dioxide was extracted with hexane, and the concentration was determined by gas chromatography with an electron capture detector (Shimadzu GC-14B) using a 2 m × 2.6 mm i.d. column packed with silicone GE SE-30 on Chromosorb W AW-DMCS 80/100 mesh. The standard operating conditions were as follows. Injector temperature, 150°C; detector temperature, 200°C. The column oven temperature was initially held at 70°C for 3 min, ramped to 145°C at 15°C /min, and held at 145°C for 2 min. Chlorite and chlorate ions were measured by ion chromatography. The standard analytical and operating conditions were as follows. Detector, TOSOH CM25 μSFS; column, TSKgel IC-PW; eluent, 2 mM benzoic acid (pH5.5); eluent flow, 1.2 ml/min; injection volume, 100 μl; column oven temperature, 35°C. Total organic halogen (TOX) was measured by a TOX-10Σ

analyzer (Mitsubishi Chemical Corporation). TOC was measured using a TOC-5000A analyzer (Shimadzu).

RESULTS AND DISCUSSION

Chlorinated water

Figure 1 shows the changes in the activity that induces chromosomal aberrations in chlorinated water. Figure 2 shows the residual chlorine concentration in the chlorinated water. Chromosomal aberration tests could not be carried out for some samples that contained greater than 3,500 mg/L of added chlorine because residual chlorine concentrations were greater than 500 mg/L even two or three days following chlorination, as shown in Figure 2. An activity that induced chromosomal aberrations was produced by chlorination; however, this activity was unstable and gradually decreased over time after the treatment. It must be noted that the activity decreased even under conditions where residual chlorine could be detected in the solution.

Figures 3 and 4 show the levels of TOX and chloroform, respectively, in the chlorinated water. It is known that the levels of typical by-products, such as THMs and HAAs increase after chlorination in distribution systems. This direction of change is not consistent with the direction of change of the activity that induces chromosomal aberrations

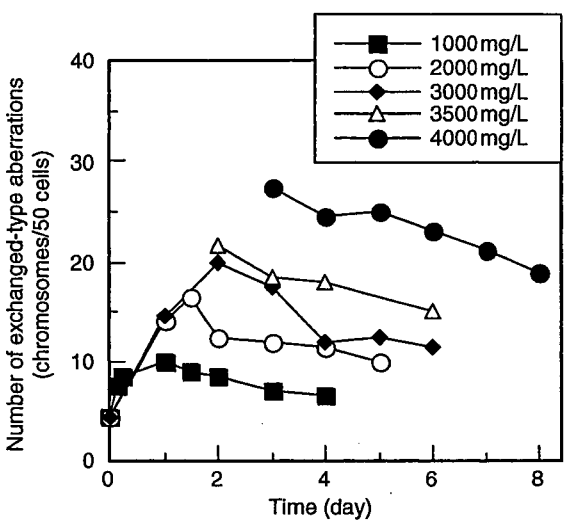

Figure 1 | Changes in the activity that induced chromosomal aberrations in chlorinated humic acid.

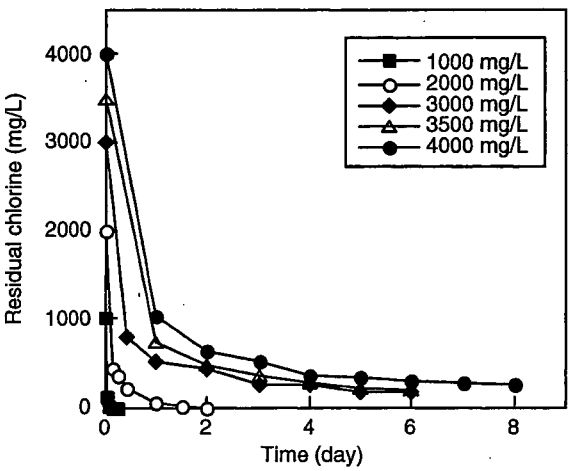

Figure 2 | Residual chlorine in chlorinated water. Initial concentrations of chlorine were 1,000 mg/L, 2,000 mg/L, 3,000 mg/L, 3,500 mg/L, and 4,000 mg/L.

shown in Figure 1. In addition to TOX and chloroform, we measured the concentrations of carbonyl group and low-molecular weight aldehydes (formaldehyde, acetaldehyde, propionaldehyde, and butylaldehyde) (data are not shown). The direction of change of these by-products was also inconsistent with that of the activity that induced chromosomal aberrations. Thus, we were not able to identify a by-product with the same direction of change as the direction of change of the activity that induced chromosomal aberrations. On the other hand, Itoh *et al.* (2006) have discussed the possibility of MX (3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) as an index for comparing the carcinogenicity of tap water near and far from a water purification plant.

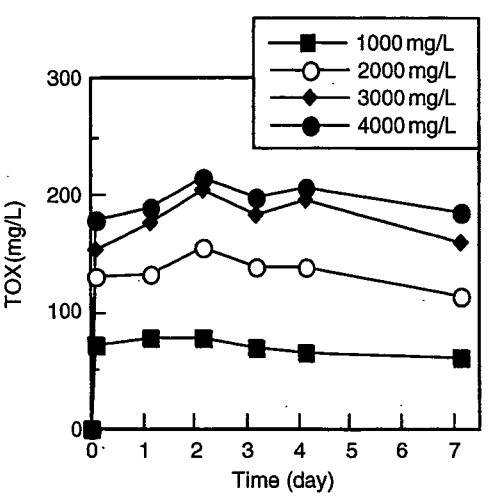

Figure 3 | TOX produced by chlorination.

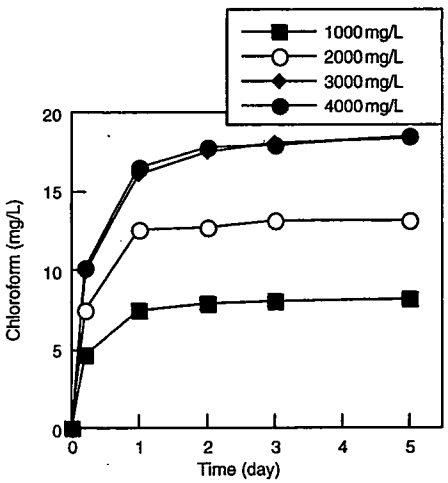

Figure 4 | Chloroform produced by chlorination.

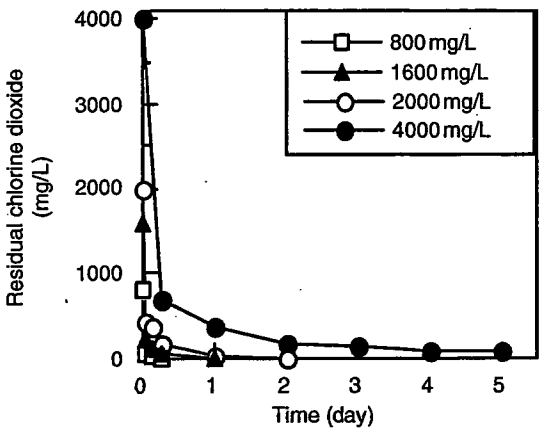

Figure 6 | Residual chlorine dioxide in water treated with chlorine dioxide. Initial concentrations of chlorine dioxide were 800 mg/L, 1,600 mg/L, 2,000 mg/L, and 4,000 mg/L.

Water treated with chlorine dioxide

Figure 5 shows the changes in the activity that induces chromosomal aberrations in water treated with chlorine dioxide. Figure 6 shows residual chlorine dioxide concentrations in the treated water. Comparison of Figures 1 and 5 shows that the activity that induces chromosomal aberrations is approximately 1.3 times greater in chlorinated water than in water treated with chlorine dioxide. An activity that induced chromosomal aberrations was produced by chlorine dioxidation; however, this activity was unstable and gradually decreased over time after treatment. In addition, this activity decreased even under conditions where residual chlorine dioxide could be detected in the solution following a chloride dioxide dose of 4,000 mg/L. These results are qualitatively the same as those obtained in tests of chlorinated water.

Figure 7 shows the concentrations of chlorite and chlorate ions in the water treated with 2,000 mg/L and 4,000 mg/L of chlorine dioxide. The drinking water quality standards (DWQSs) in Japan have been revised in 2003 (Wakayama 2004). The new DWQSs system includes DWQSs (50 items), complementary items to set the targets

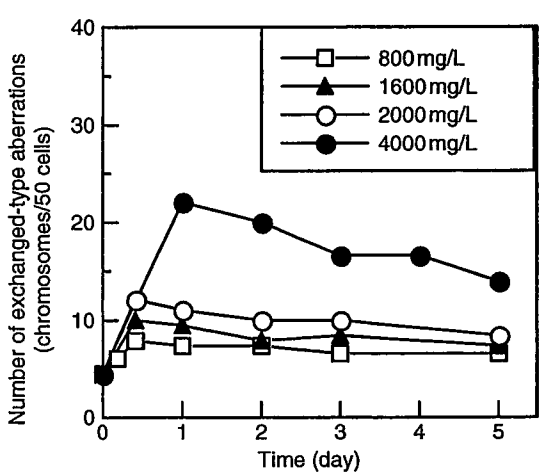

Figure 5 | Changes in the activity that induced chromosomal aberrations in humic acid treated with chlorine dioxide.

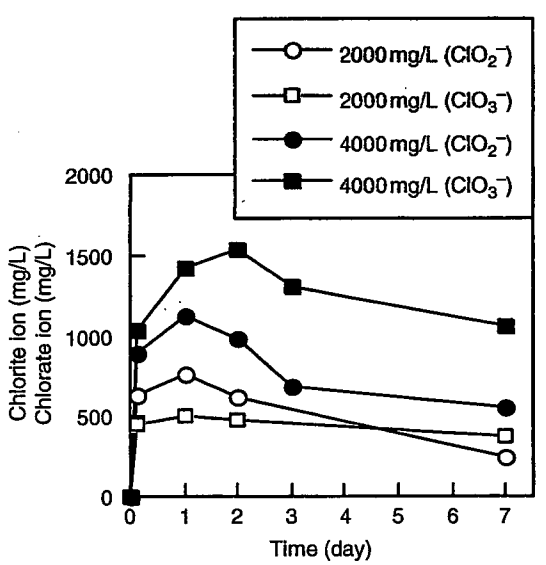

Figure 7 | Chlorite and chlorate produced by chlorine dioxidation. Initial concentrations of chlorine dioxide were 2,000 mg/L and 4,000 mg/L.

for water quality management (27 items), and items for further study (40 items). The target values of chlorine dioxide, chlorite ion, and chlorate ion have been set at 0.6 mg/L in complementary items. Therefore, these inorganic by-products must be monitored after chlorine dioxidation. It must be noted that the concentrations change over time after the treatment, as shown in Figure 7.

Figures 8 and 9 show the levels of TOX and chloroform, respectively, in the treated water. Chloroform and TOX produced by chlorine dioxidation were approximately 1% and 5–7%, respectively, of those produced by chlorination.

A major advantage of chlorine dioxide over chlorine is that it produces significantly lower levels of halogenated organic compounds. However, Figure 5 shows that the level of activity that induces chromosomal aberrations in water treated with chlorine dioxide is greater than would be expected based on the quantity of by-products. Therefore, it is important to note that the use of chlorine dioxide instead of chlorine as an alternative disinfectant does not dramatically reduce the mutagenicity of the treated water.

In addition to TOX and chloroform, the concentrations of carbonyl group and low-molecular weight aldehydes (formaldehyde, acetaldehyde, propionaldehyde, and butylaldehyde) were measured (data are not shown). The directions of changes of by-products measured were not consistent with the direction of the change in the activity that induces chromosomal aberrations.

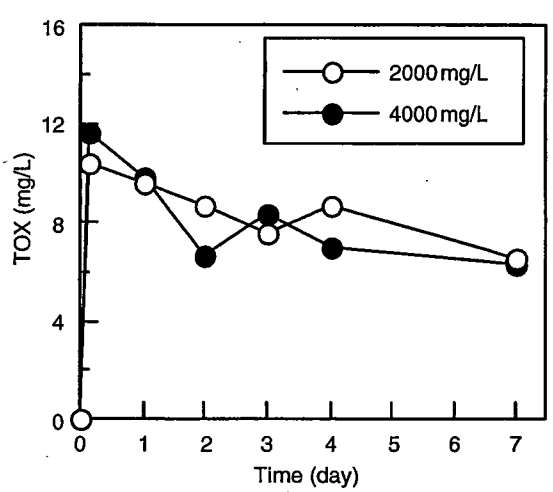

Figure 8 | TOX produced by chlorine dioxidation.

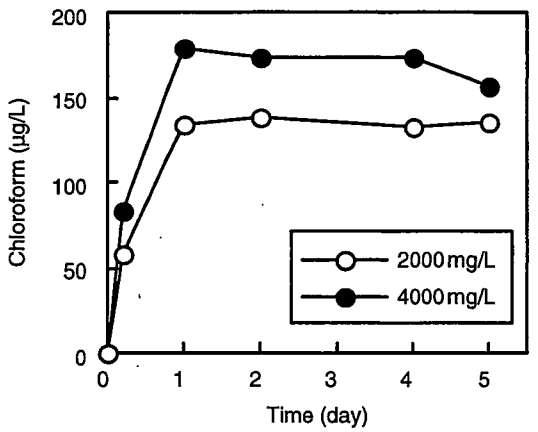

Figure 9 | Chloroform produced by chlorine dioxidation.

Estimation of the change in mutagenicity

Changes in the activity that induced chromosomal aberrations were estimated to compare the safety of drinking water treated with chlorine and chlorine dioxide in distribution systems. Pseudo-first-order rate constants K_{obs} (day^{-1}) were obtained using the integrated first order rate equation:

$$\ln(P_t/P_0) = -K_{obs} \cdot t \quad (1)$$

where P_0 and P_t are the activity that induced chromosomal aberrations in treated water at time 0 and t, respectively. K_{obs} was taken as the slope of the initial decrease.

Figure 10 shows decreasing rate constants for the activity that induced chromosomal aberrations obtained

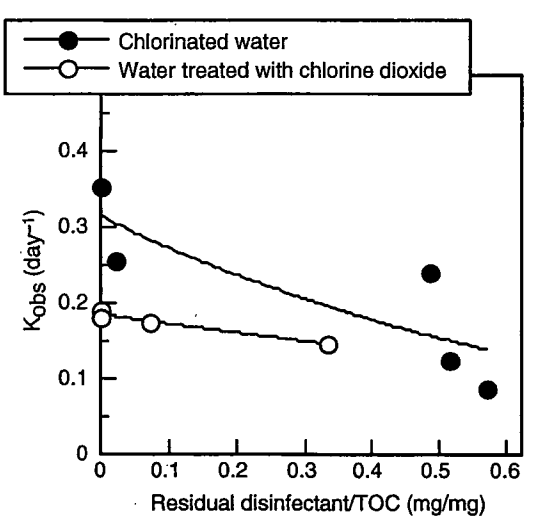

Figure 10 | Effects of residual disinfectant on the decreasing rate constant of the activity that induced chromosomal aberrations.

from the data in Figures 1 and 5. The decreasing rate constant K_{obs} was plotted against residual disinfectant concentration (residual disinfectant/TOC) in the treated water. K_{obs} was then obtained as a function of the concentration of residual disinfectant as follows:

Chlorinated water :

$$K_{obs} = 0.32\exp\{-1.4\times(Cl_2/TOC)\} \quad (2)$$

Water treated with chlorine dioxide :

$$K_{obs} = 0.17\exp\{-0.40\times(ClO_2/TOC)\} \quad (3)$$

The K_{obs} of chlorinated water was estimated to be 1.4 to 1.9 times greater than that of water treated with chlorine dioxide. It is also evident that the decreasing rate constant is smaller, as the residual disinfectant concentration is higher. For example, the K_{obs} of waters treated with chlorine or chlorine dioxide without residual disinfectants were estimated to be 0.32 day^{-1} and 0.18 day^{-1}, respectively, and the half-lives were calculated to be 2.2 days and 4.1 days, respectively. The activity that induced chromosomal aberrations in chlorinated water is greater than that of water treated with chlorine dioxide, as shown in Figures 1 and 5; however, it is noteworthy that this difference decreases over time after the treatment.

Next, we tried to estimate changes in the activity that induced chromosomal aberrations in distribution systems. The difficulty here is that the experiments in this study were carried out using commercial humic acid at a high concentration (910 mg/L of TOC as a final concentration). However, it has been confirmed that there was not a large difference in the time to reach the maximum activity and the decreasing rate between humic acid and natural water (Itoh *et al.* 2006). Thus, we could suppose that there is not a large error when the change in tap water is estimated using the results obtained with humic acid solution in this study.

When the disinfection efficiency is estimated, there are two ways to compare the efficiency by the weight of a disinfectant and by the equivalent weight of a disinfectant. Since the purpose of this study is to estimate the change in the mutagenicity of actual drinking water in distribution systems, a comparison by the weight would be more desirable from the practical point of view. In addition, it seems that the injection dose (disinfectant/TOC) of chlorine dioxide in actual water disinfection to achieve the sufficient disinfection efficiency is almost the same as the injection dose of chlorine (Ozawa *et al.* 1991; Inoue *et al.* 2005), although there may be a case in that the injection dose of chlorine dioxide has to be smaller because of the levels of formed chlorite and chlorate ions. Thus, the mutagenicity formed by chlorine and chlorine dioxide was compared by the weight of a disinfectant and with similar concentrations. It is also possible, however, to compare by the equivalent weight of a disinfectant.

Table 1 shows the assumed conditions of supplied tap water in Japan. In the case of polluted raw water, however, it might be difficult for chlorine dioxide to be used because the target values of chlorine dioxide, chlorite ion, and chlorate ion have been set at 0.6 mg/L in the treated water.

The results estimated for a typical case are shown in Figure 11. 1.0 on the vertical axis indicates the maximum activity that induces chromosomal aberrations observed in chlorinated water, and the relative activity is plotted. The time to reach the maximum activity that induces chromosomal aberrations observed in chlorinated water or water treated with chlorine dioxide was set at 24 hours or 10 hours, respectively, based on the data in Figures 1 and 5. The results clearly show that the activity that induces

Table 1 | Conditions of supplied water. The values in the first row are for typical tap water in Japan, and those in the second row are for cases in which the raw water is somewhat polluted

DOC (mg/L)				
Raw water	**Rapid sand filtered water**	**Disinfectant added (mg/L)**	**Disinfectant/DOC**	**Residual disinfectant (mg/L)**
2.0	1.1	1.1	1	0.1, 0.4
3.5	1.65	3.3	2	0.4, 0.7

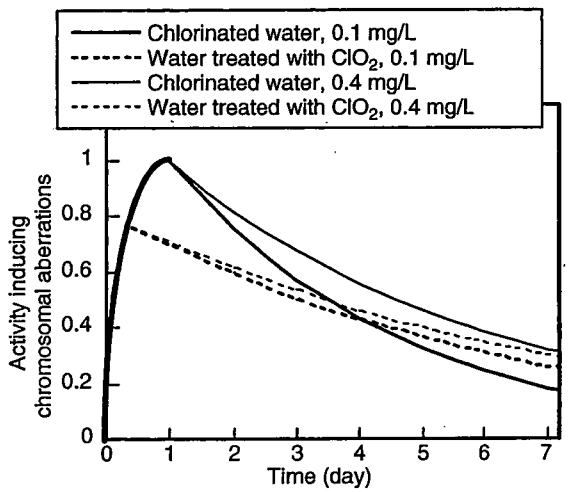

Figure 11 | Estimated changes in the activity that induced chromosomal aberrations in drinking water. DOC of raw water, 2.0 mg/L; Added disinfectant, 1.1 mg/L (disinfectant/DOC = 1).

chromosomal aberrations in water treated with chlorine dioxide is weaker than that in chlorinated water; however, this difference decreases over time after the treatment. In the case of 0.1 mg/L of residual disinfectant, the activity that induced chromosomal aberrations in water treated with chlorine dioxide becomes equal to that in chlorinated water at approximately four days.

The results estimated for polluted water are shown in Figure 12. The time to reach the maximum activity that induces chromosomal aberrations of chlorinated water or water treated with chlorine dioxide was set at 36 hours or 10 hours, respectively, based on the data in Figures 1 and 5. These results show tendencies similar to those observed in typical water, as shown in Figure 11, although the relative activity that induces chromosomal aberrations in water treated with chlorine dioxide was slightly weaker than that shown in Figure 11.

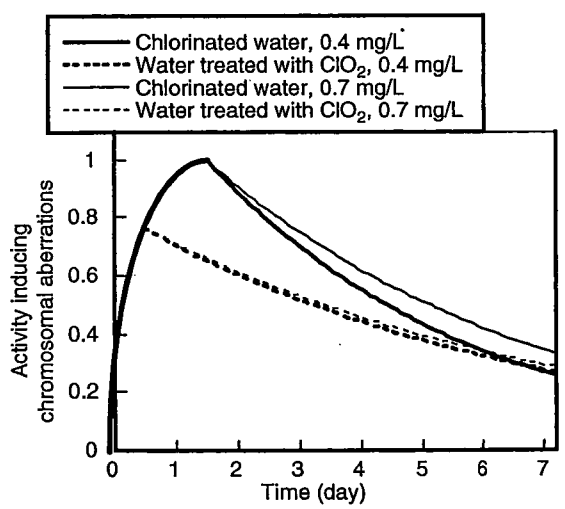

Figure 12 | Estimated changes in the activity that induced chromosomal aberrations in drinking water. DOC of raw water, 3.5 mg/L; Added disinfectant, 3.3 mg/L (disinfectant/DOC = 2).

Assuming that the typical retention time of typical drinking water in the distribution system is within two days, Figure 11 shows that the mutagenicity of drinking water treated with chlorine dioxide would be 70–80% of that of chlorinated water. In the case of polluted water, Figure 12 shows that the mutagenicity of chlorine dioxide treated water would be 65–70%. This decreased mutagenicity is an advantage of chlorine dioxide. However, the difference in mutagenicity is small when drinking water remains in distribution systems for a long period.

The use of chlorine dioxide instead of chlorine can solve the THMs problem. Judging from the findings of this study, however, it should be noted that chlorine dioxide does not have much advantage over chlorine in terms of the mutagenicity of drinking water.

CONCLUSIONS

The change in the mutagenicity of water treated with chlorine dioxide was compared with that of chlorinated water to estimate the mutagenicity of drinking water in distribution systems. Major findings of this study are as follows.

The levels of chloroform and TOX produced by chlorine dioxidation were approximately 1% and 5–7%, respectively, of those produced by chlorination. However, it was revealed that the activity that induces chromosomal aberrations in water treated with chlorine dioxide is stronger than would be expected based on the quantity of by-products produced.

The observed decreasing rate constant of the activity that induced chromosomal aberrations in chlorinated water was 1.4 to 1.9 times greater than that of water treated with chlorine dioxide. This indicates that the mutagenicity of water treated with chlorine dioxide is more stable than that of chlorinated water.

The mutagenicity of drinking water treated with chlorine dioxide was estimated to be 70–80% of that of

chlorinated drinking water. This is an advantage of using chlorine dioxide. However, the difference in mutagenicity would be small when drinking water remains in distribution systems for long periods. The use of chlorine dioxide instead of chlorine can solve the THMs problem. The findings of this study, however, demonstrate that chlorine dioxide does not have much advantage in terms of the mutagenicity of drinking water.

There were no disinfection by-products that demonstrated similar tendencies of change compared to the changes in the activity that induced chromosomal aberrations.

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Influence of backwashing on the microbial community in a biofilm developed on biological activated carbon used in a drinking water treatment plant

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Abstract The influence of backwashing on the biofilm community developed on biological activated carbon (BAC) used in a drinking water treatment plant was investigated by means of bacterial cell enumeration and terminal-restriction fragment length polymorphism (T-RFLP) fingerprinting analysis of bacterial and eukaryotic ribosomal RNA genes (rDNA). After backwashing, the attached bacterial abundance in the top layer of the BAC bed decreased to 64% of that before backwashing. The community level changes caused by backwashing were examined through the T-RFLP profiles. In the bacterial 16S rDNA analysis, the relative abundances of some terminal-restriction fragments (T-RFs) including the *Planctomycetes*-derived fragment increased; however, the relative abundances of some T-RFs including the *Betaproteobacteria*-derived fragments decreased. In the eukaryotic 18S rDNA analysis, the relative abundances of some T-RFs including the protozoan Cercozoa-derived fragments increased; however, the relative abundances of some T-RFs including the metazoan *Chaetonotus*- and *Paratripyla*-derived fragments decreased. The T-RFLP analysis suggests that backwashing can cause changes in the relative compositions of microorganisms in a BAC biofilm in the top layer of the bed.

Keywords Backwashing; biological activated carbon; microbial community; ribosomal RNA gene; terminal-restriction fragment length polymorphism

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Introduction

Ozonation followed by granular activated carbon (GAC) adsorption has been widely used as one of the promising advanced drinking water treatments. This hybrid method is originally based on chemical oxidation and physical adsorption. However, it has been found that various microorganisms form biofilms on the surface of GAC and contribute to the treatment by biological oxidation of organic pollutants and ammonia (LeChevallier *et al.*, 1992; Kim *et al.*, 1997; Takeuchi *et al.*, 1997; Lehtola *et al.*, 2002; Liu *et al.*, 2002; Volk and LeChevallier, 2002). Such a biologically enhanced GAC system is known as "biological activated carbon" (BAC) (Wilcox *et al.*, 1983; Suzuki, 1997).

Usually, backwashing is periodically carried out during BAC treatment by blows of water and air to remove accumulated pollutants and to control biofilm abundance. If the biofilm abundance exceeds an optimal level, there is a concern that some microorganisms in the biofilm may be stripped off from BAC into the following distribution systems (Morin and Camper, 1997). On the other hand, an extensive loss of the biofilm would lead to deterioration in the performance of the biological treatment. Recently, a simulation model was developed for drinking water biofilters that remove biodegradable organic matter by taking the periodic backwashing frequency into account (Hozalski and Bouwer, 2001). In the conventional BAC operation, however, the frequency of backwashing has been most often determined empirically without sufficient knowledge of the biofilm associated with BAC. In order to fully understand the effect of backwashing on BAC treatment, it is necessary to investigate the manner in which the microbial

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community in a BAC biofilm is influenced by backwashing. It is possible that the community structure consisting of bacteria and eukaryotes would be different before and after backwashing. Some microorganisms that reside in the inner layer of the biofilm or have a high affinity of attachment to GAC are likely to remain after backwashing.

In this study, we applied terminal-restriction fragment length polymorphism (T-RFLP) analysis to evaluate the effects of backwashing on biofilm community of BAC samples in a drinking water treatment plant in Japan. The T-RFLP analysis of ribosomal RNA genes (rDNA) is one of the effective fingerprinting tools for investigating microbial community structures (Liu *et al.*, 1997). Here, we compared the bacterial and eukaryotic T-RFLP profiles obtained from the BAC samples before and after backwashing by targeting 16S rDNA for the bacterial community and 18S rDNA for the eukaryotic community.

Methods

Drinking water treatment plant

A drinking water treatment plant in Japan was investigated in this study. Approximately 1 million m^3 day^{-1} of purified water is produced from river surface water in this plant. The plant employs an advanced treatment process of coagulation, followed by sedimentation, rapid sand filtration, ozonation, BAC treatment, chlorination and secondary rapid sand filtration. Additional chlorination is conducted before coagulation (pre-chlorination) or after the secondary sand filtration (post-chlorination) depending on various situations. In most cases in Japan, an ozonation-BAC system is installed before or after a rapid sand filtration. However, the ozonation-BAC unit of this plant is installed between two rapid sand filtration steps to decrease inflowing pollutant loads and to remove activated carbon particles released from the BAC bed. The activated carbon used in the plant is made of coal with an average diameter of 0.7 mm. Water is introduced downwards into the fixed BAC bed of 2.5-m depth. The contact time is approximately 15 min. The BAC bed is backwashed with air for 5 min followed by drinking water produced for 17 min every 4 days. The bed expansion during the backwashing is kept at 30%.

Sampling

In August 2005, BAC samples were collected from the top layer of BAC bed that had been consecutively used for 9 months. Residual free chlorine was not detected in the influent water of the ozonation-BAC system. At the time of sampling, 4 days had passed since the last backwashing (i.e. immediately before the next backwashing). First, the "BAC sample before backwashing" was collected. After the backwashing operation, the "BAC sample after backwashing" was collected from the same top layer of the BAC bed. These samples were kept in sterilised plastic tubes containing the on-site water. They were kept in a cold box, transported to the laboratory within 1 h, and treated immediately.

Enumeration of total bacterial cells

The wet BAC samples (4.0 g) were placed in sterilised plastic tubes containing 40 mL of Milli-Q water (Millipore, Billerica, MA). After vortex treatment for 1 min, sonication was carried out for 2 min twice to detach and disperse bacterial cells. After allowing them to settle for 10 min, an appropriate portion of the suspended bacterial cells in the supernatant was stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), and the cells were enumerated by using a BX60 epifluorescence microscope (Olympus, Tokyo, Japan).

DNA extraction from BAC samples

DNA was separately extracted from three 0.5-g portions of the BAC sample by using an UltraClean™ Soil DNA Kit (Mo Bio Laboratories, Inc., Carlsbad, CA). All three DNA extracts were combined to obtain a composite DNA extract.

PCR amplification of 16S and 18S rDNA fragments

For 16S rDNA amplification, the universal bacterial primer set with 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) primers was used (Lane, 1991). For 18S rDNA amplification, the universal eukaryotic primer set with 26F (5′-CTGGTTGATYCTGCCAGT-3′) and 1514R (5′-GATCCTTCCGCAGGTTCACC-3′) primers was used (Winnepenninckx *et al.*, 1995; Strumberg *et al.*, 2000). The 5′ ends of the forward primers (27F and 26F) were labelled with a 6-carboxyfluorescein-derived phosphoramidite fluorochrome (6-FAM). PCR amplification was conducted in triplicate by using an AmpliTaq Gold DNA Polymerase kit (Applied Biosystems, Foster City, CA). The thermal cycling conditions for both amplifications consisted of initial heat denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min. A final extension was then performed at 72°C for 10 min.

T-RFLP analysis

The triplicate PCR products were mixed and purified using a MinElute PCR Purification Kit (QIAGEN, Hilden, Germany). The DNA concentration was quantified using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Restriction enzyme digestion was conducted in triplicate. The PCR product (100 ng) was digested with 10 U of *Hha*I (TaKaRa BIO Inc., Otsu, Japan) in a 20-μL volume according to the manufacturer's instructions. The triplicate digested products were purified using a QIAquick Nucleotide Removal Kit (QIAGEN) and combined into a single tube. The 6-FAM-labeled fragments were analysed with an ABI Prism® 310 Genetic Analyzer (Applied Biosystems). Fragment analysis was carried out by using GeneMapper™ v3.0 software (Applied Biosystems). The detection threshold for terminal-restriction fragments (T-RFs) was set to 100 relative fluorescent units (RFU) for the software. Relative abundances of T-RF peaks were calculated based on their peak areas.

Cloning and sequencing

The 16S rDNA and 18S rDNA fragments were amplified from the BAC sample before backwashing under the same thermal cycling conditions except that the forward primers were unlabelled. The PCR products were cloned using a QIAGEN PCR Cloning^{plus} Kit (QIAGEN). The sequences of the positive clones of 16S rDNA and 18S rDNA were determined using a BigDye Terminator Cycle Sequencing Kit ver 3.1 (Applied Biosystems) with an ABI 3100 Avant sequencer (Applied Biosystems). Clones with different sequences were reamplified with the labelled primer sets and analysed by T-RFLP using the same procedure as the one described above to confirm their T-RFs. Sequence homology searches were performed at DNA Data Bank of Japan (DDBJ) using the BLAST network service. Suspected chimeric sequences were screened by using the "Bellerophon" online analysis (Huber *et al.*, 2004).

Nucleotide sequence accession numbers

The 16S and 18S rDNA sequences determined in this study have been submitted to the DDBJ/EMBL/GenBank databases under accession numbers AB237962–AB238010 and AB238109–AB238159, respectively.

Results and discussion

Attached bacterial abundance in BAC biofilms before and after backwashing

The average of the total bacterial cells attached to the BAC before backwashing was estimated as 1.3×10^9 cells g^{-1} (wet weight) (S.D. $= 8.1 \times 10^7$ cells g^{-1}), while 8.3×10^8 cells g^{-1} (wet weight) (S.D. $= 8.4 \times 10^7$ cells g^{-1}) remained after backwashing. The total bacterial cell count decreased to 64% by the backwashing treatment. This result shows that backwashing causes a considerable reduction in the bacterial abundance in the top layer of the BAC bed. Hozalski and Bouwer reported that 20–40% of the attached bacteria were removed from a laboratory-scale biofiltration apparatus, where sand and glass beads were used as filter media, by a 10-min backwashing with bed expansions ranging from 15–50% (Hozalski and Bouwer, 1998). Taking the periodic backwashing operation of the plant (every 4 days) into consideration, it is possible that the decreased BAC biofilm would return to its previous level by the time the next operation is performed.

Interpretation of T-RFLP profiles

The T-RFs in T-RFLP profiles reflect the compositional fingerprints of the bacterial or eukaryotic community in the top layer of the BAC bed. In this study, DNA extraction, PCR amplification, and restriction enzyme digestion were conducted in triplicate for each BAC sample to obtain representative and reproducible results. In addition, the amount of the PCR products that were digested was set to 100 ng, and the equal amounts of the digested products were loaded to electrophoresis for T-RFLP analysis. Thus, the T-RFLP profiles before and after backwashing were obtained under the same conditions. It is also reasonable to assume that biases inherent in the experimental procedures affect the results to the same degree. Therefore, a semi-quantitative change found in the relative abundance of a specific T-RF peak before and after backwashing indicates that backwashing influences the relative dominances of the microorganisms corresponding to the T-RF.

T-RFLP analysis of 16S rDNA fragments

Figure 1 shows the electropherograms of the bacterial 16S rDNA T-RFLP profiles before and after backwashing. Both of the profiles mainly consisted of the identical T-RFs; however, some of the T-RFs had different peak areas between the two profiles. According to the clone analysis, T-RF4, T-RF11, and T-RF12 were derived from some

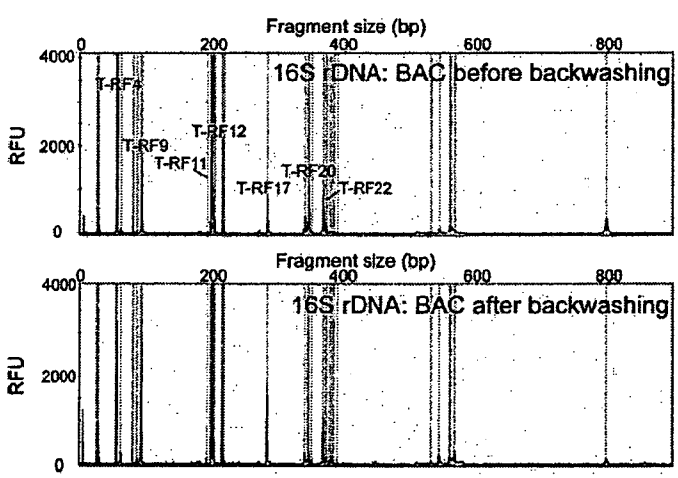

Figure 1 Electropherograms showing the bacterial 16S rDNA T-RFLP profiles of the BAC samples before and after backwashing

Burkholderiales-related bacteria in the *Betaproteobacteria* subclass. These T-RFs were dominant in the T-RFLP profile before backwashing. The *Burkholderiales*-related group has been reported to survive a conventional water treatment process (Hoefel *et al.*, 2005). The *Planctomycetes*-related clone corresponded to T-RF17. Although the *Planctomycetes* group is distributed in many habitats including biofilms in rivers, its ecological function is not well understood (Brummer *et al.*, 2004).

Figure 2 shows the differences in the relative abundances of the major 16S rDNA T-RFs before and after backwashing. After backwashing, the relative abundances of T-RF9 (unidentified) and T-RF17 (the Planctomycetes-derived fragment) increased by nearly twice; this implied that the relative dominance of the bacteria corresponding to these T-RFs increased in the community after backwashing. Complementary to this, the relative abundances of the other major T-RFs decreased after backwashing, suggesting that the relative dominances of the bacteria corresponding to these T-RFs decreased due to backwashing. Particularly, the relative abundances of the *Betaproteobacteria*-derived T-RFs decreased substantially from 15.1 to 11.0 for T-RF4, from 8.2 to 3.3 for T-RF11, and from 14.7 to 4.0% for T-RF12. It is evident from the enumeration of total bacterial cells and the T-RFLP profiles before and after backwashing that the abundance and relative composition of bacterial community in the top layer of BAC bed changed after backwashing. We can infer that direct exposure of the BAC in the top layer to fluid shear stress causes such changes. Some microorganisms may be easily washed out, while others may remain, resulting in the reduction of bacterial abundance and changes in relative dominances of microorganisms in the biofilm. It is possible that the T-RFs whose relative abundances decreased after backwashing are derived from the microorganisms that are easily stripped off from BAC by backwashing.

T-RFLP analysis of 18S rDNA fragments

Figure 3 shows the electropherograms of the eukaryotic 18S rDNA T-RFLP profiles before and after backwashing. As well as the bacterial T-RFLP profiles, the eukaryotic profiles were mainly composed of the same T-RFs of the identical sizes; however, some of the T-RFs had different peak areas before and after backwashing. Clone analysis showed that T-RF6 was derived from the protozoan *Cryothecomonas*-related clone. T-RF14 and T-RF24 were associated with the protozoan *Cercomonas*-related clones. *Cryothecomonas* and *Cercomonas* belong to the phylum Cercozoa, which includes many types of organisms that exhibit various ecological and morphological features (Keeling, 2001). T-RF25 was found to be derived from clones related to the phylogenetically different protozoans *Echinamoeba* and *Lecythium*. The protozoan *Gymnophrys*-related

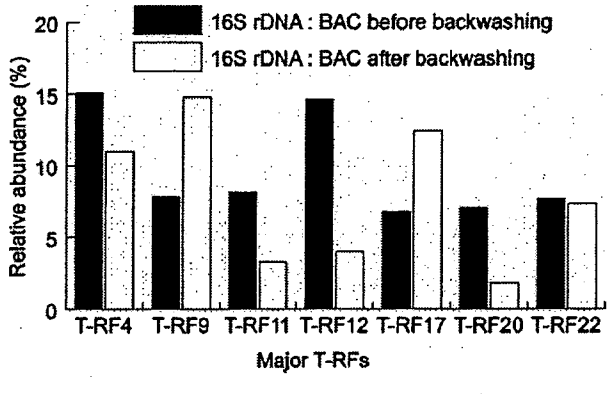

Figure 2 Differences in the relative abundances of the major 16S rDNA T-RFs before and after backwashing

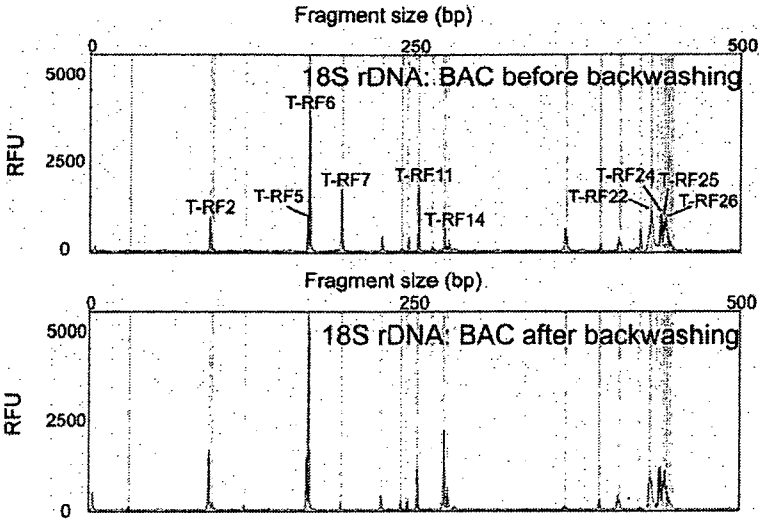

Figure 3 Electropherograms showing the eukaryotic 18S rDNA T-RFLP profiles of the BAC samples before and after backwashing

clone corresponded to T-RF26. It has been shown that both *Lecythium* and *Gymnophrys* also belong to the Cercozoa group (Nikolaev *et al.*, 2003). On the other hand, the metazoan clones related to *Chaetonotus* (phylum Gastrotricha) and *Paratripyla* (phylum Nematoda) produced T-RFs of the same length as T-RF7. Clones corresponding to the other major T-RFs were not found.

Figure 4 shows the differences in the relative abundances of the major 18S rDNA T-RFs before and after backwashing. The relative abundances of T-RF7 (the metazoan *Chaetonotus*- and *Paratripyla*-derived fragement) and T-RF22 (unidentified) decreased from 7.6 to 0.9 and from 15.9 to 7.1%, respectively. On the other hand, the relative abundances of T-RF6 and T-RF14 derived from the protozoan *Cryothecomonas*- and *Cercomonas*-related clones increased from 15.6 to 21.0% and from 3.0 to 11.0%, respectively. No large changes were observed in the relative abundances of the other identified T-RFs (T-RF24, T-RF25, and T-RF26). The increases or decreases in relative abundances of the eukaryotic T-RFs suggest that backwashing also causes changes in relative composition of the eukaryotic community in the top layer of the BAC bed as found in the bacterial community. The eukaryotes corresponding to the T-RFs whose relative abundances decreased after backwashing are possibly susceptible to backwashing.

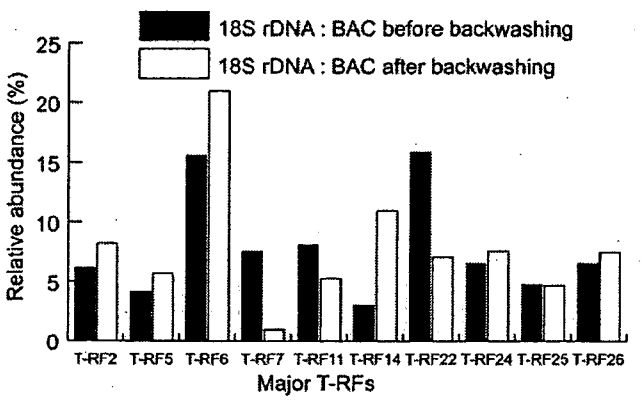

Figure 4 Differences in the relative abundances of the major 18S rDNA T-RFs before and after backwashing

Conclusions

In this study, we evaluated the influence of backwashing on the biofilm community developed on BAC used in a drinking water treatment plant in Japan. The enumeration of the bacterial cells attached to the BAC before and after backwashing revealed that 36% of the bacterial cells were lost after backwashing. The T-RFLP analysis of the bacterial and eukaryotic communities associated with the BAC demonstrated that the relative abundances of some T-RFs changed significantly after backwashing. The increases or decreases in the relative abundances of these T-RFs suggest that backwashing can provide impacts on the relative dominances of microorganisms in the BAC biofilm. Examination of the effects of backwashing on the biofilm community under more controlled conditions and monitoring of the biofilm in the recovery process after backwashing would be useful for further explanation of the results obtained in this study.

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(30) 水環境におけるエンドトキシンの変動要因と浄水処理過程におけるエンドトキシン除去特性

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微生物由来化学物質であるエンドトキシンに着目し，濃度変動に寄与する因子の検討を行った．グラム陰性細菌およびシアノバクテリアに由来するエンドトキシン量を比較した結果，*Synechococcus* sp. の季節変動に伴って原水中のエンドトキシン量が増減することが示唆された．また，琵琶湖・淀川水系におけるエンドトキシン調査の結果から，下水処理施設からの放流水がエンドトキシン増大に影響を与えること，また環境微生物群に由来する一細胞当たりのエンドトキシンは比較的高いことを示した．さらに，浄水処理過程におけるエンドトキシンの除去特性を調べ，活性炭処理後に濃度が増大するものの，高度浄水処理水中の残存エンドトキシンは十分に低濃度であること，またオゾン処理や塩素消毒により遊離エンドトキシン比率が増大することを示した．

Key Words : *total endotoxin, free endotoxin, environmental bacteria, aquatic environment, water treatment process*

1. はじめに

現行の水道水質基準では，微生物指標として一般細菌数および大腸菌数が設定されており，塩素消毒剤を注入・残留させることで病原性細菌の増殖能を抑制し，感染リスクを低下させている．しかしながら，原水中に混入する多種多様な微生物は，浄水処理によりある程度除去されるものの，一部は不活化された状態や増殖能が低下した状態 (Viable but nonculturable; VBNC 状態) に移行して浄水中に残存しており，配水過程における再増殖やバイオフィルム形成などの問題を引き起こすことが指摘されている [1),2)]．また，微生物は増殖・代謝に伴い，多種類の毒素を生産する．これらの毒素は物理的・化学的特性が多様性に富んでおり，顕れる健康影響も多岐にわたることが知られている．微生物に由来する毒素のうち，シアノトキシンの一種，microcystin-LR に関して知見の集積が進められ，暫定的な飲料水質ガイドライン値が提示されている [3)] 一方で，他の多くの毒素に関する知見は大幅に不足しているのが現状である．

こうした背景を踏まえて，本研究では微生物，特にグラム陰性細菌・シアノバクテリアの細胞外膜構成物質 (リポ多糖 ; LPS) に起因する生理活性物質 (以下，エンドトキシン) に着目する．エンドトキシンは強い免疫反応を惹起することが知られている．本来，生体は微量のエンドトキシン曝露により自然免疫を獲得すると考えられるが，年々アレルギー疾患が増加している現代においては，攪乱された免疫システムが新たなアレルギー症状を誘発する危険をはらんでおり，日常生活におけるエンドトキシン曝露とアレルギー反応との関連 [4),5)] に強い関心が持たれている．ここで，エンドトキシンは microcystin や anatoxin 等に代表される他のシアノトキシンと比較して幅広い微生物種で普遍的に存在すること，また分子量が 10 ～ 20 kDa と非常に大きいこと [6)] から，水環境中あるいは浄水処理過程における挙動も大きく異なると予想される．

そこで本研究では，シアノバクテリアを含む数種類の微生物を対象として一細胞当たりのエンドトキシン量を把握することで，水道水源のエンドトキシン増大に寄与する微生物種を探索するとともに，水道水源におけるエンドトキシン活性量および存在形態を明らかにする．また，浄水処理過程におけるエンドトキシン除去特性ならびに浄水中での存在形態を詳細に検討する．

2. 実験方法

(1) 微生物細胞に由来するエンドトキシンの定量

微生物細胞に由来するエンドトキシン量を明らか

にするため，グラム陰性菌として *E. coli* NBRC 3301 を，またシアノバクテリアとして *Microcystis aeruginosa* NIES-44, *Synechococcus* sp. NIES-946（採取地：霞ケ浦）ならびに NIES-957（採取地：琵琶湖）を選定した．*E. coli* NBRC 3301 については，Nutrient broth を用いて 36°C・120 rpm の条件で振盪培養を行った．また，*M. aeruginosa* NIES-44 は CB 培地[7]を，*Synechococcus* sp. NIES-946 および NIES-957 は C 培地[7]をそれぞれ用いて，20°C·50 $\mu E/m^2sec$ の条件下で静置培養した．増殖期にある各微生物の培養液を適宜サンプリングして培養液中の細胞数を計数するとともに，総エンドトキシン・遊離エンドトキシンの定量を行った．なお，*E. coli* NBRC 3301 株および *Synechococcus* sp. では，0.2 μm 孔径の黒色フィルター上に細胞を捕集した後に，それぞれ DAPI (4',6-diamino-2-phenylindole, 同仁化学研究所) 蛍光染色後の UV 励起下顕微鏡観察（全菌数測定法），および直接 G 励起下顕微鏡観察を行うことにより，細胞計数を行った．一方，*M. aeruginosa* NIES-44 は上水試験方法で採用されているプランクトン計数盤（MPC-200, 松浪硝子工業）を用いて計数[8]を行った．エンドトキシン活性は，カブトガニ血球抽出成分 LAL（エンドスペシー ES-50M, 生化学工業）を使用したエンドポイント比色法（トキシカラー DIA-MP, 生化学工業）により測定し，*E. coli* O113:H10 由来のエンドトキシン標準品を用いて検量線を作成した．本研究では水中エンドトキシンの存在形態を反映した実測を目的として，総エンドトキシン活性は培養液全画分を使用していかなる抽出操作も加えず測定を行った．遊離エンドトキシン測定は培養液を 14,000 rpm で 10 分間遠心分離することにより，微生物細胞とともに結合エンドトキシンを沈降させた上清画分を使用した．測定には，全てエンドトキシンフリーのプラスチック器具を使用した．なお，エンドトキシン濃度は EU/mL で表記した．

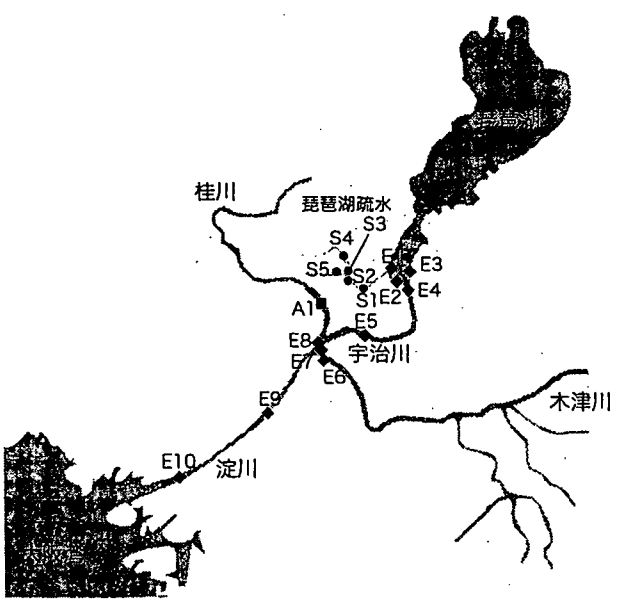

図 -1　採水地点（琵琶湖・淀川水系）

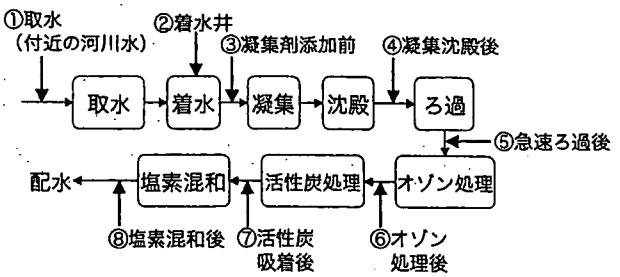

図 -2　A 浄水場の処理フローと採水場所

(2) 琵琶湖・淀川水系におけるエンドトキシン調査

図 -1 に示す琵琶湖淀川水系（琵琶湖疏水を含む）の各地点で採水を行い，試料中のエンドトキシン濃度と微生物量を測定した．採水は冬季（2005 年 12 月～2006 年 2 月）に実施し，降雨による影響を除外するため雨天当日および翌日を避けて採水日を決定した．採水瓶は予め 250°C，2 時間の熱処理によりエンドトキシンを不活化して使用し，採水後の試料は 4°C で運搬後，4 時間以内に下記の各項目について測定を行った．

1) 微生物試験：
   一般細菌数 (SPC/ 標準平板計数；37 °C, 24 時間)
   従属栄養細菌数 (HPC/R2A 平板計数；20 °C, 7 日間)
   全菌数 (DAPI 染色計数)
2) 一般水質項目：
   全有機炭素 (TOC-5000A, 島津製作所)
   紫外線吸光度 (MultiSpec-1500, 島津製作所)
   pH
   濁度 (O.D. 660)
3) 総エンドトキシン，遊離エンドトキシン

(3) 浄水処理過程におけるエンドトキシンの除去特性

調査対象施設として，琵琶湖・淀川水系から取水している A 浄水場を選定した．本施設は，急速ろ過後にオゾン - 活性炭処理を行う高度浄水処理施設である．採水は，2006 年 11 月および 12 月に計 3 回行った．浄水処理フローならびに採水場所を図 -2 に示す．採水試料は，4°C 保冷下で運搬し，採水後 4 時間以内に 2. (2) に記した各項目について測定を行った．

3. 実験結果と考察

(1) 微生物細胞に由来するエンドトキシンの定量

本研究で対象としたそれぞれの菌株について，細胞数と総エンドトキシン間に得られた関係を図 -3 に示す．いずれの菌株においても，総エンドトキシンの対数値は細胞数の対数値に比例して増大した．各菌株で求め

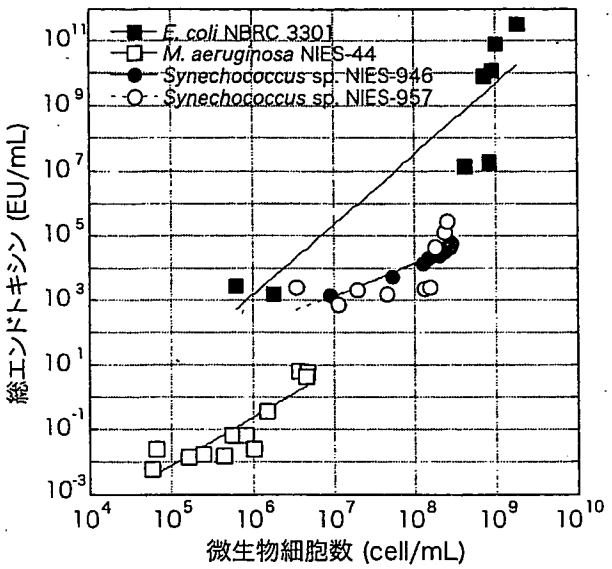

図-3 微生物細胞数と総エンドトキシン量の関係

られた回帰式を以下に示す.

<*E. coli* NBRC3301>

$$y = 1.41\times10^{-10}\cdot x^{2.17} \qquad \left(R^2 = 0.835\right) \qquad (1a)$$

<*M. aeruginosa* NIES-44>

$$y = 3.08\times10^{-10}\cdot x^{1.48} \qquad \left(R^2 = 0.793\right) \qquad (1b)$$

<*Synechococcus* sp. NIES-946>

$$y = 4.13\times10^{-5}\cdot x^{1.07} \qquad \left(R^2 = 0.963\right) \qquad (1c)$$

<*Synechococcus* sp. NIES-957>

$$y = 2.89\times10^{-4}\cdot x^{0.956} \qquad \left(R^2 = 0.458\right) \qquad (1d)$$

ここで, x：1mL 中の各微生物細胞数 (cell/mL), y：総エンドトキシン濃度 (EU/mL) である.

これらの回帰式を用いて, 細胞数 10^7 cell の時の一細胞当たりエンドトキシン量を算出すると, *M. aeruginosa* NIES-44 では 7.06×10^{-7} EU/cell であるのに対して, *Synechococcus* sp. NIES-946 の場合には 1.28×10^{-4} EU/cell と約 180 倍高い値を, さらに *E. coli* NBRC 3301 の場合では 2.18×10^{-1} EU/cell と 300000 倍以上高い値をそれぞれ示した. *Synechococcus* sp. NIES-957 は, NIES-946 と同程度のエンドトキシン活性を示した. Rapala らは, 3 種類のグラム陰性細菌に加えて, *Microcystis* sp. 6 株, *Oscillatoria* sp. 5 株, *Anabaena* sp. 6 株, *Aphanizomenon* sp. 5 株, *Nodukaria* sp. 4 株を対象として細胞乾燥重量当たりのエンドトキシンを比較し, *Microcystis* sp. および *Nodukaria* sp. が相対的に高いエンドトキシン活性を示すものの, *E. coli* ATCC 11775 (1.4×10^5 EU/mg) との比較では, 非常に弱いエンドトキシン活性を示すこと, それ故にシアノバクテリアの大量発生現象はエンドトキシン活性の変動にほとんど影響しないと結論づけている[9]. しかしながら, 本研究の結果からは検討した微生物種のうち, *Synechococcus* sp. が環境水中のエンドトキシン変動に対して大きく寄与する可能性があると考えられる. その理由として, *Synechococcus* sp. に由来する一細胞当たりエンドトキシン含有量は *E. coli* NBRC 3301 の約 1/1700 と比較的小さいものの, *Microcystis* sp. との比較では, 相対的に高い活性を示したことが挙げられる. さらに, *Synechococcus* sp. は夏季の琵琶湖において繁殖することが知られているピコ植物プランクトンの代表株であり, ピーク時には 10^5 cell/mL を超える密度で検出される[10] など, 季節により細胞数の変動が非常に大きいことが第二の理由として挙げられる.

ここで, Rapala らの報告と同様, 本研究でも *E. coli* との比較で $1/10^5$ 倍未満の非常に低いエンドトキシン活性を示した *M. aeruginosa* であるが, LPS を精製することにより乾重量当たりのエンドトキシン活性が *E. coli* の約 1/60 程度まで増強するとの報告もある[11]. *M. aeruginosa* を始めとした多くのシアノバクテリアは, 寒天状基質によりその細胞膜が覆われているため, エンドトキシン活性がマスキングされている可能性が高い. すなわち, 塩素などの薬剤注入によりエンドトキシン活性の増強が起こりうると考えられるため, 別途詳細な検討を行う必要があるだろう.

(2) 環境水中のエンドトキシンの定量

各地点から採取した水試料中のエンドトキシン測定結果を表-1 に示す. 琵琶湖および河川表流水のエンドトキシン濃度は 3.11×10^2 ～ 2.43×10^3 EU/mL の濃度範囲で分布した. 一方, 下水処理施設放流水のエンドトキシン濃度は 1.08×10^4 EU/mL と一般水環境中と比べて非常に高い値を示した. ここで, 表流水のうち 2000 EU/mL 前後の高い値を示した地点, E3, E7 および E8 はいずれも下水処理放流水の影響を強く受けうる地点であった. これらの結果より, 取水口が下水処理放流口近くに存在する場合には, 原水中のエンドトキシン濃度が 1 オーダー程度増大する可能性があると考えられる.

Narita らは, 活性汚泥の自己酸化過程において汚泥構成微生物から 2-keto-3-deoxyoctulosonic acid (KDO) が放出され, 親水性有機物濃度が増大すると報告している[12]. KDO は種々の微生物に由来する LPS において保存的に存在するコア領域の成分で, Lipid A に隣接している化合物である[13]. さらに, バイオアッセイの結果から親水性有機物放出に伴って細胞毒性が増大することを示している[14]. これらの知見に基づくと, 下水処理施設からの放流水が水環境中のエンドトキシン濃度に与える影響は非常に大きいと考えられるため, 水資源