treatment. It should be emphasized that this phenomenon is very similar to the formation of THMs in the drinking water treatment process; that is, NOMs are major precursors for both the estrogenic effect and THMs.

On the other hand, the estrogenic effects of most micropollutants decrease after chlorination as shown in Figure 5(a). The effects of chlorination of bisphenol A (BPA), 4-nonylphenol (4-NP), estrone (E_1), 17β-estradiol (E_2), estriol (E_3), and 17α-ethynylestradiol (EE_2) on the estrogenic effect have been reported (Hu *et al.* 2002; Kuruto-Niwa *et al.* 2002, 2007; Lenz *et al.* 2003; Tabata *et al.* 2003; Deborde *et al.* 2004; García-Reyero *et al.* 2004; Lee *et al.* 2004; Nakamura *et al.* 2006). In fact, some chlorinated derivatives or intermediates during chlorination of BPA and 4-NP show stronger estrogenic effect than parent compounds; however, the estrogenic effect of these compounds eventually decreases after chlorination with chlorine dosage typically used in practice.

Different results have been reported about the effect of chlorination on the estrogenic effect of river water and treated wastewater. The estrogenic effect decreased by chlorination in some studies (Takigami *et al.* 1998; Akatsuka *et al.* 2000); however, it increased in another study (Yakou *et al.* 2000). Figure 5(a) indicates that organic matter of which the estrogenic effect increases or decreases after chlorination is present in raw water. The findings demonstrate that the overall estrogenic effects in chlorinated drinking water are the sum of the increased and decreased activities of individual constituents after chlorination. The effect of chlorination depends on the quantity of the estrogenic effect that increases and decreases by chlorination.

In addition, the estrogenic effect originated from NOMs shown in Figure 5(a) following chlorination increased gradually over time, even in the absence of residual chlorine (Itoh *et al.* 2009). It is known that the concentration of THMs and HAAs increases while in the distribution system. The obtained result suggests that some part of the estrogenic effect in drinking water also increases over time after chlorination. The increase in estrogenic effect is faster at a higher pH than at a neutral pH, which is reasonable because the hydrolysis rate increases as the pH increases. Based on this finding, Figure 5(b) illustrates the components of the estrogenic effect originated from NOMs. It shows that the components, which form the 'estrogenic effect formation potential' and 'estrogenic effect intermediates', can be defined. The estrogenic substances formed just after chlorination are part of the chlorinated by-products. The 'estrogenic effect intermediates' change into estrogenic substances over time, explaining why the increased estrogenic effect shown in Figure 5(a) continues to increase over time after chlorination.

This phenomenon is similar to the formation of THMs because NOMs are major precursors of both estrogenic effect and THMs. The 'THM formation potential' and the 'THM intermediates' in the formation process of THMs have definitions that are similar to those illustrated in Figure 5(b) (Xie 2004). To decrease the estrogenic effects of drinking water, NOMs in addition to suspected EDCs should be removed before chlorination. Furthermore, it is important to assess the reproductive and developmental toxicity of mixtures of by-products that originated from NOMs.

ATTEMPTS TO ESTIMATE THE OVERALL TOXICITY OF DISINFECTED WATER

In vitro mutagenicity testing

As discussed above, we have to pay much attention to numerous other DBPs in addition to typical ones formed by disinfection. It has been emphasized for many years that it is important to measure and evaluate the toxicity of complex DBP mixtures in chlorinated water. *In vitro* short-term bioassays such as the Ames test can evaluate the combined action of DBPs. Many studies have investigated the mutagenicity of organic extract in disinfected water, including chlorinated water (Loper *et al.* 1978; Donald *et al.* 1989). As a mutagenicity test, the Ames test was mainly carried out until the 1980s; however, various kinds of *in vitro* bioassay such as assays using cultured mammalian cells have been performed since then.

Our review of studies that compared the mutagenicity of water treated with different disinfectants (Zoeteman *et al.* 1982; Backlund 1985; Meier & Bull 1985; Cognet *et al.* 1986; Kamei *et al.* 1989; Anderson *et al.* 1990; Sayato *et al.* 1991; DeMarini *et al.* 1995; Monarca *et al.* 1998; Guzzella *et al.* 2004; Maffei *et al.* 2005) found that a study by Meier &

Bull (1985) yielded typical results. This study showed that the mutagenicity of chlorinated water was the strongest and that chloramine-treated water was also mutagenic. The mutagenicity of chlorine dioxide-treated water was minimal, and ozonated water had no detected mutagenicity. DeMarini *et al.* (1995) showed that different types of disinfected water had mutagenicity in the following order: chlorination > ozonation plus chlorination > chloramination > ozonation plus chloramination > ozonation > raw water. There are additional findings on ozonation such as: ozone has the effect of reducing the mutagenicity of raw water (Zoeteman *et al.* 1982); and the mutagenicity of ozonated water is detected in some cases (Cognet *et al.* 1986) and not in others (Meier & Bull 1985; Anderson *et al.* 1990). In addition, these results may vary with raw water quality and sample preparation procedure. For example, Sayato *et al.* (1991) showed that chlorination reduces mutagenicity because the mutagenicity of raw water is strong.

Performing *in vitro* mutagenicity testing is not constrained by chemical analysis, which quantifies the concentration of individual chemicals, thus providing one of the indicators of the overall toxicity of water. As a matter of fact, epidemiological studies have reported associations between the mutagenicity of chlorinated drinking water and increased risk of cancers of the bladder, rectum, kidney, pancreas and lymphatic system (Koivusalo *et al.* 1995; Koivusalo *et al.* 1997; Koivusalo *et al.* 1998). The results of *in vitro* mutagenicity testing can be employed for reducing the risk of drinking water, and can contribute to the development of a better water treatment process. On the other hand, these tests have the limitation that toxicity to the human body cannot be assessed and a health-based value cannot be derived by extrapolating the results for humans.

In vivo testing

It is essential to estimate the overall toxicity of disinfected water with *in vivo* assays so that the toxicity of TOX (i.e. complex mixtures of chlorinated water) can be estimated. However, only a few carcinogenicity studies using experimental animals have been conducted.

Bull *et al.* (1982) showed an increased number of tumours when concentrations of US drinking water were applied to mouse skin as tumour initiators in initiation/promotion studies. The same study also showed that water disinfected by chlorine, ozone and chloramine resulted in a greater number of papillomas compared with non-disinfected water. Van Duuren *et al.* (1986) administered a chlorinated humic acid solution (1 g TOC/L) as drinking water to mice for two years. There were no increases in tumours. Similarly no adverse effects relevant to carcinogenicity have been detected in other studies (Kool *et al.* 1985; Miller *et al.* 1986; Condie *et al.* 1994). Condie *et al.* (1985) carried out a sub-chronic toxicity test administering chlorinated humic acid solution in drinking water for 90 days. NOAEL (no-observed adverse effect level) was derived as 0.5 g TOC/L. Daniel *et al.* (1991) conducted a sub-chronic toxicity test in male and female rats. A provisional NOAEL of untreated humic acid solution, ozonated water and ozonated/chlorinated water was set to be 1.0 g TOC/L.

In summary, no studies have shown evidence of the carcinogenic effects of complex DBP mixtures via drinking water consumed by rodents. There have been many epidemiological studies of associations between consumption of chlorinated drinking water and increased risk of various cancers (International Agency for Research on Cancer 2004; US Environmental Protection Agency 2006). The US EPA has concluded that the available data indicates a potential association between consumption of drinking water and bladder cancer, and it also suggests a potential association between consumption of drinking water and rectal and colon cancers. Although an epidemiological study is useful as a means to observe adverse effects on human health, there is no attempt to date to derive health-based values of DBPs based on epidemiological evidence. *In vivo* assays using experimental animals should be given a higher priority to derive a health-based value of a DBP mixture.

Toxicity estimation project initiated by the US EPA

Available evidence suggests that it will be essential to perform *in vivo* toxicity tests on disinfected water to obtain results that can be used to derive water quality standards for TOX (μg Cl/L). In the future, monitoring and managing drinking water quality using a standard value of TOX should be implemented in the case of chlorinated water. The US EPA has initiated the Integrated Disinfection Byproducts

Mixture Research Project for this purpose (Simmons *et al.* 2002, 2004).

In this project, the following *in vivo* toxicology tests will be performed: reproductive and developmental toxicity, mutagenicity, carcinogenicity, immunogenicity, hepatic/renal toxicity, neurotoxicity, developmental neurotoxicity, and kinetics/metabolism. *In vitro* bioassays on similar types of toxicity have also been designed to be performed. It is very valuable that, in addition to *in vitro* bioassays, *in vivo* toxicity studies that are associated not only with carcinogenicity but also with several other types of toxicity have been planned.

This project confronts challenging technical issues such as the development of a concentration procedure using a reverse osmosis membrane (Speth *et al.* 2008), preparation of water concentrates that are drinkable by laboratory animals (Narotsky *et al.* 2008), and ensuring the chemical stability of water concentrates (McDonald *et al.* 2010). As multi-disciplinarity is needed to tackle these technical issues, specialists from different fields have designed and initiated this huge project.

The reproductive and developmental endpoints are being given first priority in this project. The results obtained to date showed that 130-fold concentrates of both chlorinated and ozonated/post-chlorinated water appeared to exert no adverse developmental effects (Narotsky *et al.* 2008). Cancer endpoints, however, were assigned a lower priority because of the difficulty in obtaining enough water concentrate for a two-year cancer bioassay. In addition, water is disinfected either by chlorination or by ozonation/post-chlorination, and there is no plan to research adverse effects of water that has been treated with chlorine dioxide or chloramines (Simmons *et al.* 2008). Future research progress is highly encouraged.

Since obtaining useful information for actual regulation depends on the progress and success of *in vivo* bioassays, they should be given a higher international priority.

CONCLUSIONS AND RECOMMENDATIONS

The regulation of DBPs has played a great role in producing safe drinking water; however, there are numerous limitations with the current system. Only a few of the 600–700 chlorinated by-products are regulated, accounting for only a small portion of the overall toxicity represented by DBPs.

Water suppliers typically focus their water quality management efforts to comply with defined maximum concentration standards for individual regulated parameters. As a result, toxicity from causes other than regulated by-products is overlooked, leading to potentially inappropriate and counterproductive treatment measures. The contribution of bromate ion to overall water toxicity (Figure 1) and the toxicity and changes in chlorine dioxide-treated water (Figure 4) are good examples. Standard values are never sufficient as golden rules as far as DBPs are concerned. Instead, they should serve as important points of reference for water quality management.

We recommend a paradigm shift towards preventive and holistic DBP management based on a comprehensive health-based risk assessment that takes into account the overall toxicity. This approach is recommended in the WHO Guidelines for Drinking-water Quality as 'Water Safety Plans' (WSPs). WSPs require assessment of risks from catchment to consumer, and implementation of control measures that are validated to effectively mitigate risks. Moreover, the WSP approach puts more emphasis on monitoring of control measures rather than on monitoring at end-of-pipe against an ever-growing list of standards. The implication for DBP management is to focus efforts on the implementation and monitoring of preventive control measures such as removal of DBP precursor compounds, the consideration of the costs and benefits of using alternative or non-chemical disinfection processes, and if appropriate, to establish and validate removal of DBPs prior to distribution. Care must be taken to not compromise disinfection efficacy in efforts to reduce DBP toxicity, and this should also be demonstrated in the WSP risk management plan.

Other than a progressive shift to promotion of WSPs, there may be a limited number of immediately implementable policy or regulatory actions. One step would be to keep standard values that have been derived with sufficiently large safety (uncertainty) factors. For example, first, an alternative approach such as the benchmark dose method has been introduced to derive tolerable daily intakes (TDIs). This method may give a new health-based value that differs from a previous value, even when the same toxicity data are analysed. Second, it has been emphasized that a standard value should be set using an appropriate allocation of the TDI to drinking water. An actual measurement of the proportion of intake from drinking water may give a new allocation of intake instead of the default value, ultimately resulting in a new health-based value. Even in these cases, however, any changes in the present standard values should be considered carefully and the overall toxicity of water should be considered.

International organizations and authorities charged with reviewing and revising national drinking water standards should collect information on the overall toxicity of disinfected water. Obtaining useful information for actual regulation depends on the progress and success of *in vivo* bioassays that can be used to derive health-based values. Therefore, *in vivo* assays with experimental animals should be given a higher international priority.

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A survey on levels and seasonal changes of assimilable organic carbon (AOC) and its precursors in drinking water

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In Japan, customers' concerns about chlorinous odour in drinking water have been increasing. One promising approach for reducing chlorinous odour is the minimization of residual chlorine in water distribution, which requires stricter control of organics to maintain biological stability in water supply systems. In this investigation, the levels and seasonal changes of assimilable organic carbon (AOC) and its precursors in drinking water were surveyed to accumulate information on organics in terms of biological stability. In tap water samples purified through rapid sand filtration processes, the average AOC concentration was 174 μgC/L in winter and 60 μgC/L in summer. This difference seemed to reflect the seasonal changes of AOC in the natural aquatic environment. On the other hand, very little or no AOC could be removed after use of an ozonation–biological activated carbon (BAC) process. Especially in winter, waterworks should pay attention to BAC operating conditions to improve AOC removal. The storage of BAC effluent with residual chlorine at 0.05–0.15 $mgCl_2/L$ increased AOC drastically. This result indicated the possibility that abundant AOC precursors remaining in the finished water could contribute to newly AOC formation during water distribution with minimized residual chlorine. Combined amino acids, which remained at roughly equivalent to AOC in finished water, were identified as major AOC precursors. Prior to minimization of residual chlorine, enhancement of the removal abilities for both AOC and its precursors would be necessary.

Keywords: assimilable organic carbon (AOC); AOC precursors; amino acids; drinking water; advanced water treatment

1. Introduction

Waterworks have been working to improve drinking water quality to reduce health risks caused by man-made pollutants, natural toxins and disinfection by-products. The installation of advanced water treatment processes is one of these efforts and it has been working effectively to supply safe water. However, customers' complaints about drinking water have been increasing even after advanced water treatment processes had been in operation. A questionnaire survey conducted in a distribution area supplied with drinking water treated by advanced treatment processes indicated that more than 40% people who avoided direct drinking of tap water could perceive chlorinous odour [1]. Thus, chlorinous odour has attracted increasing attention as one of important factors to increase customers' satisfaction for drinking water.

Chlorinous odour is produced by the reaction between chlorine and precursors such as nitrogen compounds [2]. Therefore, two types of approach for reducing chlorinous odour may be effective. One is improving water quality by removing its precursors still left even after the application of current advanced processes. Another promising approach is minimization of the chlorine disinfectant itself. However, the latter approach can cause deterioration of microbiological safety of drinking water by allowing bacterial regrowth within the distribution system. In Japan, a residual chlorine level of 0.1 $mgCl_2/L$ should be maintained at each tap, though many waterworks have been trying to keep a residual chlorine level of 0.3–0.4 $mgCl_2/L$ at each tap [3]. This residual chlorine level has worked effectively to inactivate bacteria within distribution systems and to provide a 'biologically stable' state. With lesser disinfection power, bacteria can regrow using a small amount of organics as substrate; therefore, stricter control of organics is needed at the same time with this approach.

The organic fraction, which can be consumed by bacteria, is called biodegradable organic matter (BOM). Assimilable organic carbon (AOC) and biodegradable organic carbon (BDOC) are often used as indicators for

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BOM. Each indicator has advantages and disadvantages. AOC is a very sensitive indicator for bacterial regrowth potential of drinking water, especially distributed drinking water, while BDOC is considered to be linked to chlorine demand or the formation of disinfection by-products [4].

One type of compound of most concern in BOM is amino acids, which contain carbon and nitrogen, and can become good energy sources and nutrients for heterotrophic bacteria. The amino acids have also become a target of researchers' attention because of their high reactivity with chlorine disinfectant [5]. Inorganic or organic chloramines can be formed by the reaction of amino acids with chlorine disinfectant, and it is commonly believed that these products can cause not only a decrease of disinfection efficiency but also an increase of chlorinous odour. Thus, there has been a recent focus on amino acids as key compounds in both chlorinated and chlorine-free water supply systems.

In this investigation, AOC levels in drinking water samples distributed from two different water treatment plants with different treatment processes were determined. AOC removal during advanced water treatment processes was then evaluated. The possibility of AOC increases during water distribution was also examined. Finally, the amino acids as BOM components were determined by analyses of free and combined amino acids, and their contribution to BOM is discussed. This information would be useful in allowing us to improve the microbiological stability of water for water supply systems with minimized residual chlorine.

2. Materials and methods

2.1. *Water sampling in distribution systems*

Two different distribution areas were chosen.

One is a distribution area (System-A) supplied from a conventional water treatment plant (WTP-A), which includes coagulation–sedimentation, rapid sand filtration (RSF), and chlorination. The raw water of WTP-A is piped from Lake Biwa.

Another distribution area (System-B) is supplied from a water treatment plant (WTP-B), which has advanced processes consisting of coagulation–sedimentation, intermediate ozonation, rapid sand filtration, post-ozonation, biological activated carbon (BAC) and chlorination. The WTP-B purifies surface water taken from the Yodo River, which has a source in Lake Biwa.

Forty water samples and six water samples were taken from each tap in System-A in different seasons, May/June 2007 and January 2008, respectively. Six water samples were also taken from each tap in System-B in January 2008. The water samples after 5 min flashing were collected in carbon-free glass bottles prepared by thermal treatment at 550°C for four hours. The samples for analyses were transported to the laboratory under refrigerated conditions and processed within four hours of sampling.

2.2. *Water sampling at a treatment plant*

Two samplings were conducted at the above-mentioned advanced water treatment plant (WTP-B) in December 2008/January 2009 (winter season) and June/July 2009 (summer season), respectively. The effluent samples after each treatment process and the water intake were collected and transported to the laboratory under refrigerated conditions. AOC, total organic carbon (TOC) and heterotrophic plate count (HPC) in the samples were analysed within four hours. Those samples such as water intake and BAC effluent that were expected to contain not negligible amounts of microorganism cells were filtrated through an Anodisc 47 membrane filter (GE Healthcare Japan, Tokyo, Japan) to reduce the effect of indigenous bacteria on tested bacterial strains for AOC measurement.

Dissolved amino acids were determined only in samples taken during summer. For determination of dissolved free amino acids (DFAAs), benzalkonium chloride was added immediately to each sample at a final concentration of 0.1% to prevent unintentional biodegradation during transportation [6].

2.3. *AOC formation under non-chlorinated and chlorinated conditions*

The effluent samples of BAC process at WTP-B were collected in January 2009 as previously described and were stored without any additional treatment at 4°C after being divided into three different carbon-free bottles. The same BAC effluent samples were also stored at 20°C after adding sodium hypochlorite at final concentrations of 0.05 and 0.15 mgCl$_2$/L. After four days storage, the AOC content in each sample was determined after filtration using an Anodisc 47 membrane filter.

2.4. *Analytical methods*

TOC was analysed using a TOC-5000A or TOC-V$_{CSH}$ analyser (Shimadzu, Kyoto, Japan). HPC bacteria were enumerated using a pour plating procedure with R2A agar (Nippon Pharmaceuticals Co., Ltd, Tokyo, Japan) after seven days incubation at 20°C [7].

AOC was measured according to the standard methods proposed by the Japan Water Works Association [7]. Water samples were pasteurized for 30 minutes at 75°C in a water bath. To make sure that carbon was the only limiting nutrient, nutrients including nitrogen, phosphorus and other trace elements were supplemented by adding 2 mL mineral solution. Samples were inoculated simultaneously with *Pseudomonas fluorescens* strain P17 (ATCC 49642) and *Aquaspirillum* sp. strain NOX (ATCC 49643) and incubated at 20°C; the growth of each strain was monitored every two or three days during 14–20 days by pour plating on R2A agar at 20°C. AOC was calculated using the average (CV $\leq$ 20%) or maximum numbers of colonies at steady state and the yield factors determined with sodium acetate as a carbon source. The yield factors in this investigation were determined as 4.53×10^6 and 1.56×10^7 colony-forming units (CFU)/µg of acetate-C for P17 and NOX, respectively.

Free and combined chlorine were analysed using a DPD-ferrous titration method according to standard methods [8].

Amino acids were determined using a liquid chromatography system for amino acids analysis after post-column derivatization with *o*-phtalaldehyde (OPA). This system was equipped with a trapping column for ammonium ion (Shim Pack ISC-30, Shimadzu, Kyoto, Japan) and a cation exchange column (Shim-Pack AMINO Li, Shimadzu, Kyoto, Japan) for separation. The derivatized compounds with OPA were detected using a fluorescence detector (RF-10A, Shimadzu, Kyoto, Japan) at an excitation wavelength of 350 nm and an emission wavelength of 450 nm. For DFAAs analyses, 1/10 volume of dilution buffer (2.5 mol Li/L lithium citrate buffer (pH 2.1)) was added to each sample and filtrated through a 0.22 µm filter (Millex-GP, Nihon Millipore, Tokyo, Japan). For dissolved combined amino acids (DCAAs) analyses, the samples without an antiseptic agent were dried under a stream of N_2 gas and hydrolyzed in 6N hydrochloric acid containing 11.36 mM ascorbic acid at 110°C for 20 hours, according to the method reported by Robertson et al. [9]. Milli-Q water samples treated by thermal hydrolysis in the same way were also analysed as blank samples.

2.5. *Statistical analysis*

Statistical analyses were performed with GraphPad Prism version 4.0 for Macintosh (GraphPad software Inc., San Diego, CA, USA). 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.

3. Results and discussion

3.1. *AOC levels in tap water samples*

Average AOC levels in two different distribution systems in winter are compared in Figure 1(a). The average AOC in System-B was slightly lower than that in System-A, but the difference was not significant, while the average TOC content in samples taken from System-B (1.3 mg/L) was considerably lower than that in System-A (1.8 mg/L).

This result suggested that the AOC removal through advanced water treatment processes consisting of ozonation and BAC adsorption achieved only a limited improvement. It is well-known that ozonation increases the AOC fraction by converting high molecular weight organics to low molecular weight and polar compounds, particularly carboxylic acids [6]. Some parts of AOC

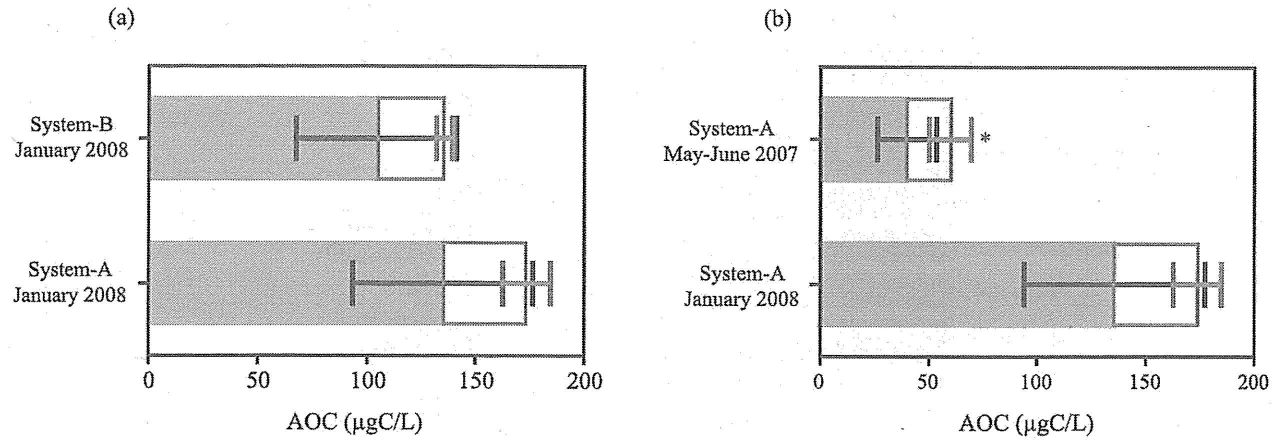

Figure 1. Comparison of AOC levels in tap water samples. Two components of AOC, P17 component (grey solid bar) and NOX component (white bar), were determined by the van der Kooij method [11]. (a) AOC in tap water samples ($n = 6$) purified through different treatment processes, WTP-A (conventional processes) and WTP-B (advanced processes) in winter season. (b) AOC in tap water samples taken from System-A in summer ($n = 40$) and winter ($n = 6$) seasons. The data represented the mean ± the standard deviations. $*p<0.01$.

should be removed during the BAC process by microbial activity on the surface of activated carbon, but in our case the total efficiency of AOC removal was not improved significantly.

On the other hand, as shown in Figure 1(b), the AOC data in the samples taken from the same distribution system (System-A) showed a clear difference between the seasons. The average AOC in summer (*n* = 40) was 59.8 μgC/L, while the average AOC in winter (*n* = 6) was 174 μgC/L. It is widely recognized that the raw water quality of WTP-A has been affected by seasonal changes of water quality in Lake Biwa. Mitamura and Saijo [10] have reported that TOC in the summer season was slightly higher in southern basin of Lake Biwa and that the fluctuation might be caused by phytoplankton blooms. This report suggested that:

- constituents of organics might shift to aquagenic or autochthonous organic matter with higher molecular weight by microbial conversion in the summer;
- the observed seasonal changes in AOC might directly reflect the seasonal changes in quantity and quality of organic fraction in the lake water.

The obtained AOC values were much higher than the AOC level of 10 μgC/L proposed by van der Kooij [11] for biologically stable water in non-chlorinated systems. In the winter season, the AOC levels were higher than 100 μgC/L, which was proposed by LeChevallier *et al.* [12] as a criterion to prevent regrowth of coliforms in chlorinated systems.

The impact of raw water quality and the AOC removal during advanced water processes in WTP-B on AOC levels in finished water are more fully discussed in the next section.

3.2. AOC removal during advanced water treatment processes

3.2.1. Comparison of organic carbon in raw water and finished water

The averages of AOC concentrations obtained by two samplings in the winter and summer season at WTP-B are shown in Figure 2. The average TOC values and proportion of AOC to TOC are shown in Table 1.

The average AOC levels in raw water were 148 and 32.2 μgC/L in winter and summer, respectively. The AOC level in the winter season was five-times higher than that in summer season, although Huck *et al.* [13] have reported that AOC concentrations in raw water during the winter-fall period were significantly lower than those during summer, and that a similar trend was observed with non-volatile organic compounds.

One of the characteristics of the surveyed WTP-B was that the raw water quality depended on the water qualities of the river water, which has a source in Lake Biwa, and the discharged water from wastewater treatment facilities located upstream of the basin. It is recognized that phytoplankton growth in the summer season can contribute to an increase in TOC in the Yodo River water, because 70% of the water in the river comes from Lake Biwa, where phytoplankton blooms were observed during the season when the water temperature was high. A rough seasonal fluctuation in BOD (higher in winter and lower in summer) was also observed in discharges from wastewater treatment facilities in this basin [14].

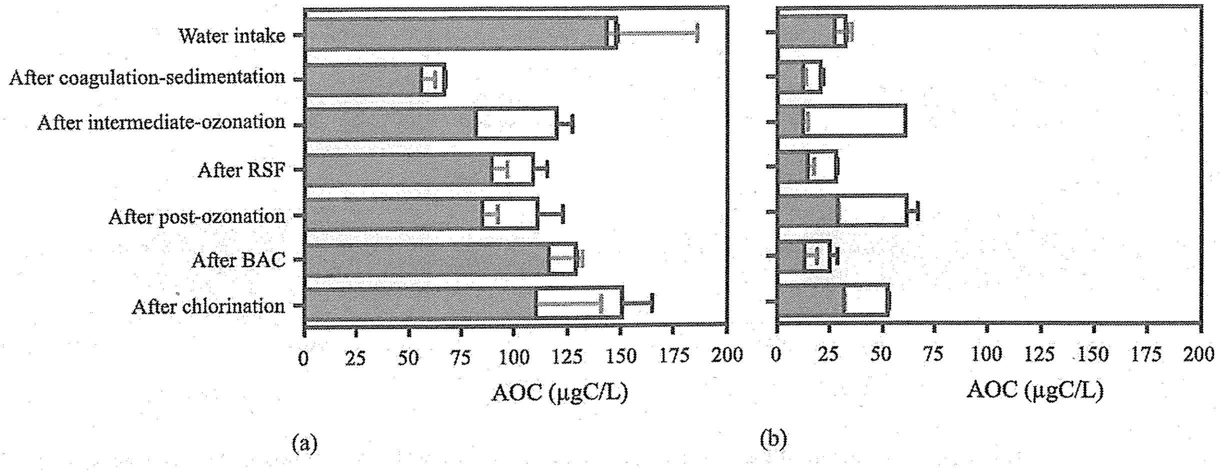

Figure 2. Comparison of AOC changes during advanced water treatment process in (a) winter and (b) summer. Two components of AOC, P17 component (grey solid bar) and NOX component (white bar), were determined. The data represented the mean ± the range of two samplings. BAC = biological activated carbon; RSF = rapid sand filtration.

Table 1. Changes in TOC and AOC/TOC proportions during water treatment processes.

	Winter 2008		Summer 2009	
	TOC (mg/L)	AOC/TOC (%)	TOC (mg/L)	AOC/TOC(%)
Water intake	2.0	6.5	2.0	1.4
After coagulation-sedimentation	1.7	3.5	1.4	1.3
After intermediate-ozonation	1.7	5.8	1.4	4.3
After RSF	1.5	6.4	1.1	2.5
After post-ozonation	1.5	7.6	1.0	6.0
After BAC	1.2	11.9	0.9	3.0
After chlorination	1.1	12.3	0.8	6.7

However, there was no difference in our limited TOC data between the two seasons. This information from the literature supports our assumption (presented in the previous section) that the decrease in AOC in the summer season might reflect the changes in the organic constituents in the raw water. The organic fraction that can be easily consumed by heterotrophic bacteria could, in summer, be converted into biomass or extracellular components with a higher molecular weight such as proteins or polysaccharides, with higher bacterial activity in the natural aquatic environment. The decrease in the proportions of AOC to TOC also indicated that the organic constituents shifted in summer to those considered refractory to biodegradation. In both seasons, the AOC component consumed by the P17 strain preferentially accounted for over 80% of AOC in surface water samples, and the NOX component did not contribute to the seasonal changes in AOC.

After whole treatment processes, the AOC levels in the finished water increased slightly to 151 μgC/L in winter season and 52.7 μgC/L in summer season, respectively. The seasonal difference in AOC levels detected in the finished water was consistent with the results of AOC in tap water samples taken from System-A and AOC in raw water samples as mentioned previously. Therefore, these seasonal changes of organic constituents in raw water could directly affect the AOC levels in finished water.

This result also indicated that the whole treatment processes of WTP-B (including ozonation and BAC adsorption) could not remove AOC at all, whereas more than 40% TOC could be removed constantly in both seasons. The increases in the NOX component were observed during the treatment processes in both seasons. The effect of each treatment process on AOC changes is discussed in the next section.

3.2.2. *Effect of each treatment process on AOC*

Based on Figure 2, the first treatment process, coagulation–sedimentation, was effective for AOC reduction, especially for reduction of the P17 component. The facility uses aluminium sulphate as a coagulant.

There have been conflicting arguments about an efficiency of AOC removal during a coagulation process. Huck *et al.* [13] have pointed out that polyaluminium chloride prolonged the lag phase in the growth of *P. fluorescence* P17 because of its toxicity, but did not impact on maximum colony numbers. However, Lehtola *et al.* [15] have confirmed the inhibitory effect on colony formation of *P. fluorescence* P17 in their surveys at water treatment process plants using aluminium salt. Therefore, there is a possibility of the growth inhibition of *P. fluorescence* P17 being misidentified as AOC removal. On the other hand, Kasahara *et al.* [16] demonstrated that the P17 component of AOC could be reduced by a coagulation process based on their jar test results using polyaluminium chloride as a coagulant. Liang *et al.* [17] also reported that coagulation processes using aluminium sulphate and ferric chloride as coagulants could remove total AOC effectively, especially under the conditions for enhanced coagulation. In general, coagulation is widely recognized as a process suitable for removal of hydrophobic organic fraction with high molecular weight. Those results should be re-evaluated more carefully in terms of an inhibitory effect of alum salt on bacterial strains for AOC determination.

The next step, an intermediate-ozonation, increased a NOX component of AOC significantly. This phenomenon was also observed after the post-ozonation process. It is known that molecular ozone reacts with unsaturated bonds of organic compounds, converting them to lower molecular weight compounds with carbonyl- or carboxyl groups [6]. These newly formed carboxylic acids were measured as NOX components by AOC determination. However, our result indicated that the increased NOX component could be removed quickly during the following rapid sand filtration (RSF) process.

The most noteworthy point in our results was the efficiency of the BAC process for AOC removal.

Although many studies have demonstrated relatively high efficiency (37–86%) in AOC removal during biological filtration processes [18–21] such as BAC or biofiltration, our results suggested that BAC operated in this facility did not remove AOC at all in the winter season although it could remove 50% of AOC in the summer season.

One reason for the low efficiency of the BAC process in the winter season could be an immature bacterial community on the surface of activated carbon because of lower water temperature. In fact, the heterotrophic plate counts (HPC) in the BAC effluent during the winter season were less than 10 CFU/mL, while the HPC in the summer season averaged 105 CFU/mL. In addition, the bacterial community on the activated carbon might have lower activity to assimilate organic carbon in the winter than that in the summer.

Persson *et al.* [22] reported that the specific respiratory activity of biofilter biomass was dependent on water temperature. Their results suggested that there was a crucial point between 13°C and 8°C for the respiratory activity of bacteria. The average water temperature during the period of our winter survey was reported as 8–12°C; such a low water temperature had a significant impact on bacterial activity in the BAC process. Therefore, some improvement in operation such as the longer contact time for BAC treatment would be needed to improve AOC removal efficiency during the winter season.

The final step, chlorination, also increased AOC slightly in the summer season and drastically in the winter season. This suggested that the finished water in the winter season contained a larger fraction of AOC precursors even after BAC treatment, which could be readily converted into the AOC fraction by chlorination.

3.3. *AOC formation under non-chlorinated and chlorinated conditions*

AOC levels after storage, with and without residual chlorine, are compared in Figure 3. The BAC effluent samples at WTP-B (AOC 149 µgC/L) taken in winter season were stored at 4°C for four days. AOC levels determined after storage were 269–289 µgC/L and an average increase of 87% was observed. This result proved that AOC precursors, which could easily release AOC into aqueous phase not only through a chemical reaction but also by microbial activity, remained abundant in the winter season even after ozonation–BAC treatment processes.

A small count of bacteria, which was detected in BAC effluent samples and did not increase during storage in every sample, might contribute to the newly formed AOC in these samples despite no intentional inoculation. Although the samples without residual

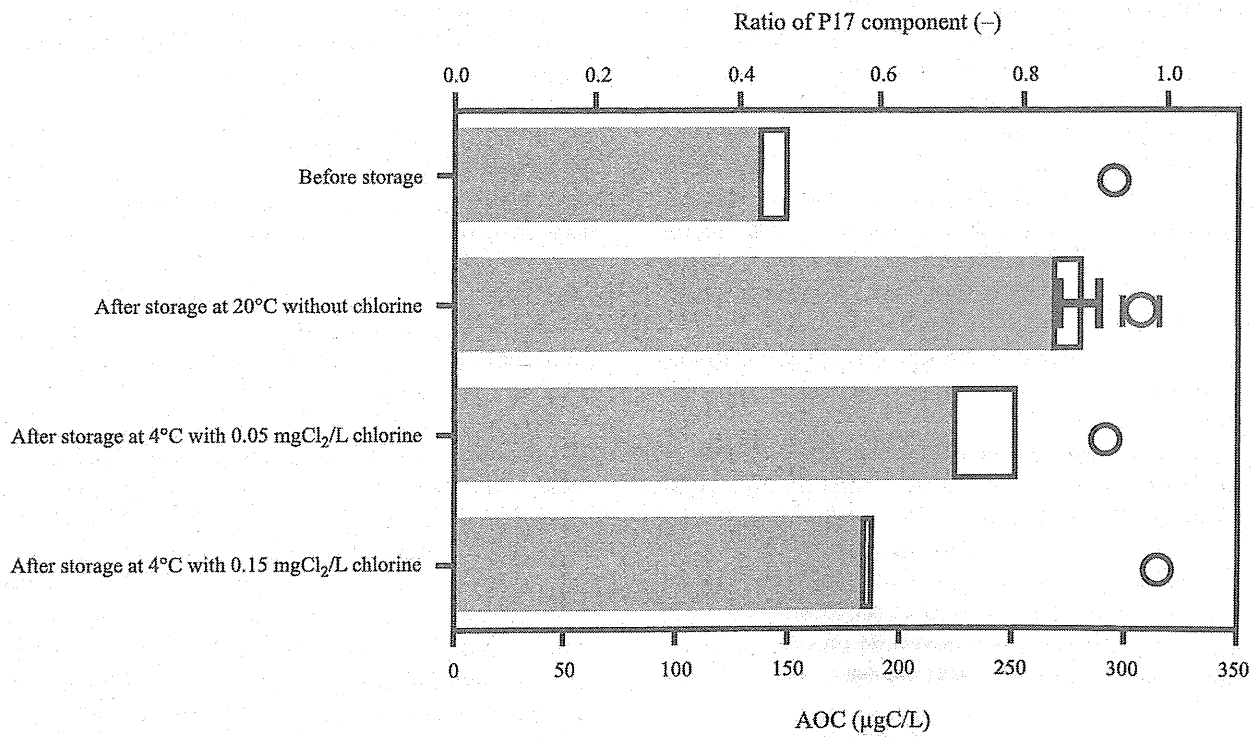

Figure 3. Effect of residual chlorine on AOC changes during sample storage. AOC concentrations after storage (P17 component, grey solid bar; NOX component, white bar) and the ratios of P17 component (open circle) are shown. Only stored samples without residual chlorine were measured in triplicate and represented the mean ± the standard deviation.

chlorine were kept in a refrigerator and no bacterial regrowth was detected under this storage condition, surprisingly, the hydrolysis of organics by microbial activity progressed very quickly. This result strongly suggested that the AOC increase was caused by enzymatic hydrolysis of bacteria remaining in the BAC effluent even at 4°C. Based on the AOC increment during storage, the hydrolysis rate of BOM to produce AOC was estimated to be much greater than the microbial assimilation rate of organic carbon at 4°C.

In contrast, smaller increases in AOC were observed in the samples stored at 20°C for four days with residual chlorine, and the increments were smaller at higher residual chlorine. This result also could be evidence that bacteria in the status of 'resting cells' played an important role in enzymatic hydrolysis of BOM remaining in the BAC effluent to AOC even under unfavourable conditions for bacterial regrowth such as chlorinated conditions or at lower water temperature.

According to the comparison of AOC levels in finished water at WTP-B with those in tap water taken from System-B presented in previous sections, significant AOC increases were not however observed in an actual distribution system (System-B). This discrepancy was attributed to different levels of residual chlorine because the waterworks had been maintaining a minimum residual chlorine concentration of 0.4 mgCl_2/L during water distribution in System-B. But our data shown in Figure 3 suggested the possibility that the AOC increase could be occurring during water distribution at 20°C if lower residual chlorine than the present level applied. As mentioned in the Introduction, minimization of residual chlorine is a promising option for reducing chlorinous odour to an acceptable level for customers and it should be widely applied to water distribution systems in the near future. Under these circumstances, the waterworks should pay much more attention not only to AOC levels in finished water but also to levels of AOC precursors.

The component that contributed to the increase in AOC was identified as the P17 component. In all samples, only P17 components increased dramatically while NOX components did not change significantly. This result was completely different from the specific increase in NOX components (including acetate, formate and oxalate) observed during oxidation processes such as ozonation and chlorination [6,23]. While organics containing unsaturated bonds can become predominant AOC precursors during these oxidation processes, our results suggest that hydrophilic AOC precursors such as poly- or oligosaccharides, peptides or proteins, which can be converted into P17 components by microbiological hydrolysis, could cause a drastic increase in AOC during water distribution with minimized residual chlorine. Considering that Jo [24] reported that hydrophilic fractions accounted for approximately 50% of DOM in the Yodo River water and that their percentage was increased after an ozonation–BAC process, the removal of hydrophilic organics during water treatment could be key to the production of biologically stable water. Thus, organic constituents that could increase AOC during water distribution should be also identified in order to improve biological stability of the finished water.

3.4. Determination of amino acids in processed water samples

Dissolved free amino acids (DFAAs) and dissolved combined amino acids (DCAAs) were analysed separately in order to determine the contribution of amino acids, peptides and proteins to AOC precursors. Their concentrations in processed water samples at each sampling point are shown in Table 2.

DCAAs were detected in both samples during water treatment processes. However, the concentrations of DFAAs were less than detection limits in both samplings. These results are consistent with our assumption that the AOC fraction could be easily converted into organics with a relatively higher molecular weight by microorganisms in this basin.

In our analysis, the samples to which an antiseptic agent had been added immediately after sampling were provided for free amino acids analysis, while the samples without antiseptic agent were provided for combined amino acids analysis. It was expected that DFAAs in the samples without an antiseptic agent would be readily degraded by bacteria during transportation to our laboratory and therefore overlap of DFAAs and DCAAs was considered to be negligible in our analysis.

The interesting finding is that the concentration of DCAAs rose drastically on occasion. We have not yet been able to identify the causes or sources for these spikes in the concentration of amino acids.

DCAAs were removed during water treatment processes efficiently, even though we observed a large difference between the two samplings of raw water. The concentrations of total DCAAs in finished water were quite stable at around 1 μM in both samplings and were equivalent to 24.6–65.0 μgC/L. Their percentages in TOC in finished water were not so large at less than 8%. However, if the concentrations of combined amino acids were compared to AOC levels, their fractions were relatively large (approximately 50–100%).

Needless to say, direct comparison of organic carbon levels derived from amino acids with AOC levels is difficult because the AOC was calculated as the acetate-based amount of organic carbon from biomass. However, these results indicated that the combined amino acids could be one of the major AOC precursors,

Table 2. Changes in concentration of amino acids during water treatment processes.

	June 25, 2009		July 6, 2009	
Sampling points	DFAA (μM) <(μgC/L)>	DCAA (μM) < (μgC/L)>	DFAA (μM) < (μgC/L)>	DCAA (μM) <(μgC/L)>
Water intake	N.D.	87.2 <4332.2>	N.D.	3.60 <165.5>
After coagulation–sedimentation	N.D.	3.01 <137.7>	N.D.	1.44 <54.0>
After intermediate–ozonation	N.D.	5.17 <237.0>	N.D.	1.11 <35.9>
After RSF	N.D.	2.48 <111.4>	N.D.	1.32 <48.2>
After post-ozonation	N.D.	1.34 <50.0>	N.D.	1.79 <69.8>
After BAC	N.D.	1.95 <82.2>	N.D.	3.24 <149.1>
After chlorination	N.D.	0.76 <24.6>	N.D.	1.39 <65.0>

N.D. = not detected.

which could be converted by *P. fluorescens* P17 as observed in Figure 3. Our data was limited in the summer season in that AOC was relatively low. Further data in other seasons should be accumulated because information on the constituents of AOC and its precursors could be crucial in optimizing water treatment processes in terms of biological stability.

4. Conclusions

A survey of AOC levels in drinking water samples was conducted in two different distribution areas. In tap water samples purified through rapid sand filtration processes, the average AOC was 174 μgC/L in the winter season and around 60 μgC/L in the summer season. The limited effect of advanced water treatment processes on AOC removal was also noted. Removal of AOC and combined amino acids during advanced water treatment processes was also evaluated. We concluded that very little or no AOC was removed throughout the whole advanced water treatment processes and that AOC levels in the finished water reflected the seasonal fluctuation of AOC in the natural aquatic environment. Especially in winter, waterworks should pay attention to BAC operation to improve AOC removal.

The AOC increase during storage with minimized residual chlorine implied that AOC precursors remained abundant in the finished water and that they could contribute to a drastic AOC increase during water distribution. Analysis of amino acids identified combined amino acids, which remained at roughly equivalent to the AOC level in finished water, as major AOC precursors. These results indicated that the removal abilities of water treatment processes for hydrophilic organics, not only AOC but also its precursors, should be largely enhanced prior to minimization of residual chlorine.

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(8-56)ナノろ過処理を適用した浄水の微生物学的安定性に関する研究

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1. はじめに

水道事業体は原水水質の状況に合わせて高度浄水処理を導入し，科学的な観点から十分に安全な水を供給しているにも関わらず，利用者の満足度は必ずしも高いとは言えない。その一つの理由として，4割弱の利用者が感知すると回答している“カルキ臭”の存在が挙げられ，満足度の高い水供給システムを確立するために優先的に取り組むべき課題と言える[1]。カルキ臭を低減するためには，浄水中に残存する前駆物質の除去と並んで残留塩素濃度の低減が有効な手段となりうる。著者らは，カルキ臭抑制の一手法として残留塩素濃度を低減した水道システム構築を想定し，残留塩素濃度低減環境においても微生物学的安定性を維持するための同化可能有機炭素(AOC)要求水準を決定した[2]。本研究では，高い有機物除去効果が確認されているナノろ過(NF)処理をオゾン(O_3)-活性炭(GAC)処理後段に適用した場合のAOC除去特性を明らかにするとともに，処理水の微生物学的安定性を評価する。

2. 実験方法

(1) 膜処理による有機物除去特性の評価：淀川表流水を原水とするA浄水場で稼働している実験処理施設を用いて，NF処理による有機物除去特性の評価を行った。使用した膜処理ユニットの概要を表1に示す。流入水には実施設のO_3-GAC処理水を用いた。採水は2010年5月～12月の期間に行い，処理前後におけるTOC，AOC，従属栄養細菌数(HPC)の変化を調べた。運転期間中，膜ファウリングによる圧力上昇が見られたため，上記期間内に計5回の薬品洗浄（クエン酸洗浄＋苛性ソーダ洗浄）を実施した。試料中のAOC濃度は上水試験方法解説編[3]に従い，*Pseudomonas fluorescens* P17株と*Aquaspirillum* sp. NOX株を用いて測定した。

表1　膜処理ユニットの概要

膜形式	スパイラル型NF膜
大きさ	φ 99.5 mm×L 1016 mm
本数	3本
型式	ESNA1-4040型（日東電工社製）
除塩率	90.0 %
膜材質	合成高分子系複合膜

(2) 残留塩素濃度低減環境における処理水の微生物学的安定性：2段階の残留塩素濃度(0, 0.05 mg/L)制御条件下で20 °Cにおける微生物の再増殖を調べた。あらかじめ採取し微生物再増殖量が定常に達するまで培養したNF処理水を無機塩類を添加した試験水に植種し，次亜塩素酸ナトリウム溶液を間欠添加することで残留塩素濃度を制御した。実験は回分培養系で行い，経時的に残留塩素濃度を確認するとともにHPCの測定を行った。また，連続通水条件下における処理水の微生物学的安定性を調べるため，アニュラーリアクター(AR)に残留塩素濃度を0および0.05 mg/Lに調整したNF処理水を通水し，ポリカーボネート切片上に形成された生物膜を回収してHPC密度を測定するとともに，流出水中のHPCの経時変化を調べた。

3. 結果と考察

(1) 膜処理による有機物除去特性の評価：NF処理前後のAOCおよびTOCの経時変化を図1に示す。TOCに関しては一部の採水日にファウリングに伴う除去率の低下が確認されたものの，運転期間を通して約90%と安定した除去効果が得られた。一方，流入水AOC平均68.7 ± 25.4 μgC/Lに対して処理水AOC平均は33.4 ± 23.6 μgC/Lと約50%の平均除去率が得られたものの，その除去率は大きく変動することがわかった。親水性かつ低分子量有機物群から構成されるAOCのNF処理による除去性能は，流入するAOCの構成成分にも大きく左右されると考えられる。

図2に示したように流入水（GAC処理水）のAOCは弱いながらも水温との相関があり，低水温期にはAOCが顕著に上昇する傾向が確認されている。著者らは，残留塩素濃度0.05 mg/Lの条件下で微生物学的安定性の維持に要求されるAOC水準を約11 μgC/Lと報告した[2]が，流入水

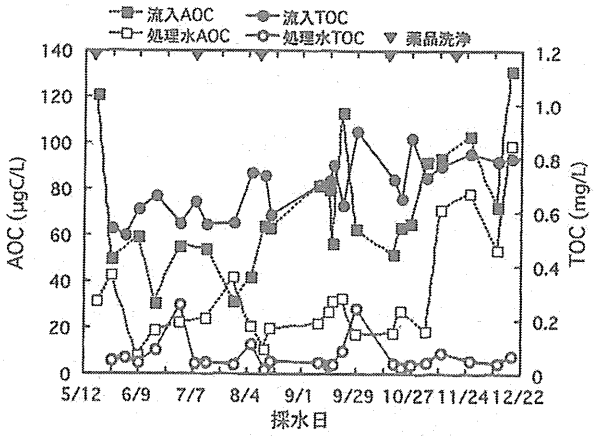

図１　NF 処理前後の有機物濃度経時変化

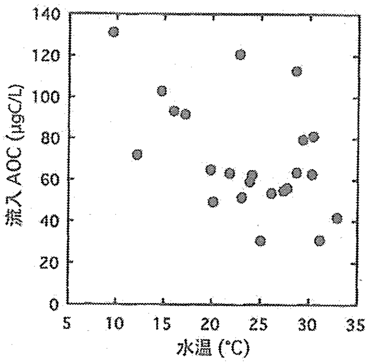

図２　GAC 流出水の AOC と水温の関係

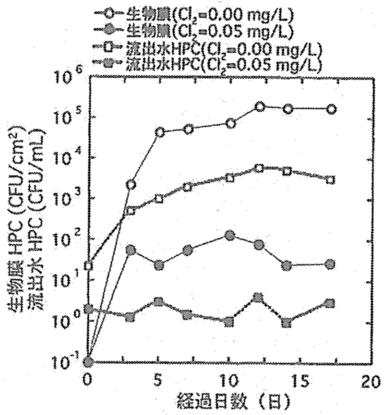

図３　NF 処理水を連続通水した場合の微生物再増殖

AOC が比較的低い夏季においても安定的にこの水準を達成することはできなかった。また，冬季には流入水 AOC の上昇に伴い，処理水 AOC 濃度も大幅に上昇した。

(2) 残留塩素濃度低減環境における処理水の微生物学的安定性

(A) 回分培養試験：AOC 濃度の異なる NF 処理水（17.1 - 31.2 μgC/L）を用いて残留塩素濃度 0.05 mg/L における HPC 再増殖を調べたが，検討した AOC 濃度範囲では再増殖は観察されなかった。一方，残留塩素なしの条件では全ての試料で再増殖が確認された。

(B) 連続通水試験：実験処理施設に設置した AR を用いた連続通水試験の結果を図３に示す。なお，実験期間中の平均水温は 13.2°C, 2 回測定した流入 AOC 濃度は約 53 μgC/L であった。残留塩素なしの条件では定常状態の生物膜密度が 1.2 × 10^5 CFU/cm^2 に達したのに対して，残留塩素 0.05 mg/L の条件では 3.4 × 10^3 CFU/cm^2 と大幅に抑制された。AR 流出水中の浮遊 HPC についても，残留塩素なしの場合には生物膜形成に伴い最大 6.0 × 10^3 CFU/mL まで増加したが，残留塩素ありの場合には 3 ～ 4 CFU/mL とほとんど増加せず推移した。今回の AR 運転は微生物再増殖が起こりにくいとされる水温 15°C 以下で行われたが，NF 処理水の AOC 濃度が高かったこともあり，残留塩素が 0.05 mg/L 存在する場合においても浮遊細菌濃度増加は抑制された一方で，配管内の生物膜形成は進行することがわかった。これらの結果は，現存の高度浄水処理プロセスに NF 処理を増設するのみでは浄水の微生物学的安定性の十分な確保は困難であり，微生物学的視点から処理プロセスの再構築を行う必要があることを示している。

4. 本研究のまとめ

本研究では，カルキ臭抑制の一手法として残留塩素濃度を低減した水道システム構築を想定し，現存の高度浄水処理プロセスに NF 処理を適用した場合の AOC 除去特性および微生物学的安定性改善効果を調べた。その結果，NF 処理は AOC 除去に一定の効果を示すもののその処理性は大きく変動し，継続的に浄水の微生物学的安定性を十分に確保することは困難であることがわかった。今後，微生物学的安定性といった視点から処理プロセスを再構築する必要があると考えられる。

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血清学的調査による *Campylobacter jejuni* の感染−発症割合推定に基づいた障害調整生存年数の定量化

Quantification of Disability Adjusted Life Years based on the estimation of the illness-to-infection ratio with *Campylobacter jejuni* by serological survey

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1. 緒言

現在の日本の水道システムは、消毒副生成物による健康影響問題のほか、カルキ臭をはじめとする水道水の異臭味による水道離れが問題視されており、将来は塩素注入量を低減し満足度の高い水道システムを構築する必要がある。しかし、残留塩素濃度の低減は微生物リスクの増大に直結しうることから、同時に微生物リスク管理手法の高度化を進めることが重要である。

一般に微生物の種類によって疾患の深刻さや死亡率が大きく異なるため、多種多様な健康影響を評価する指標が微生物リスク定量には必要である。ここで、疾病要因による多種多様な健康影響の大きさを表す指標として障害調整生存年数(Disability Adjusted Life Years: DALYs)がある。飲料水の安全評価の分野では、WHO(世界保健機関)が数種類の微生物と化学物質を取り上げてDALYsの定量化作業を実施している[1]。しかし、水道水中の病原微生物に感染しても実際には不顕性感染で終わるケースが多い。そのため微生物リスクをDALYsを用いて定量する場合、微生物感染が成立した人のうち発症した人の割合(感染一発症割合)がDALYs推定値に影響を与える因子の一つであると考えられる。そこで対象とする微生物ごとに、感染一発症割合を推定する必要がある。

飲料水による水系感染症の主要原因菌のひとつに *Campylobacter jejuni* がある。*C. jejuni* 感染による感染一発症割合に関する有用な情報は乏しく、安全側サイドの考え方や既往研究[2]に基づき、感染一発症割合が1.2~100%と広範囲で設定されているのが現状である。そのため感染一発症割合はDALYs計算する上で、大きな不確実性を持っていると考えられる。このような背景に基づき本研究では *C. jejuni* を対象とし、初めに血清学的調査に基づいて *C. jejuni* 感染による感染一発症割合の推定を行った。そして、推定した感染一発症割合を用いて *C. jejuni* 感染者1人あたりのDALYsの定量を試みた。

2. 実験方法

2.1. 血清中の *C. jejuni* に対する抗体価の測定

国立感染症研究所に保管されている血清の中で、京都府で2006年~2008年に採取された血清140サンプルを使用した。なお、各年齢区分ごと(5~9歳、10~19歳、20~29歳、30~39歳、40~49歳、50~59歳、60歳以上)に20検体、男女比が1：1になるように設定した。

次に、血清中の *C. jejuni* に対する抗体価を定量した。対象とする抗体クラスは、immunoglobulin A (IgA)、IgG、IgMとした。なお抗体価測定には、SERION ELISA classic *Campylobacter jejuni* IgG/IgA/IgMキットを使用した。

2.2. 血清学的調査に基づいた感染−発症割合の推定

感染一発症割合の推定には、一般集団における不顕性感染者(感染者)数と顕性感染者(発症者)数の把握が必要である。感染者数については、2.1.により得られた抗体価結果に基づいて推定を行った。既往研究では、顕性感染者集団、不顕性感染者集団、健常者集団の順に抗体価レベルが低くなる傾向にあると報告している[3,4]。そこで得られた抗体価分布に対して感染者と健常者を判別する陽性カットオフ値を設定し、抗体価陽性と判断された集団を感染者の集団と定義した。そして、140サンプル中の感染者サンプル数割合を一般集団における感染者割合とみなした。

C. jejuni 感染は、主要な汚染源の一つである養鶏場などの畜産業が盛んな地域で多いと予想される。そのため発症者数の推定には、京都府と産業形態の違いが小さいと考えられ、*C. jejuni* 菌体分離数の報告率が100%である神戸市を選択した。しかし、神戸市の市内定点病院検査室ならびに神戸市環境保健研究所、

神戸市中央市民病院で分離された *C. jejuni* 報告数の集計データのみでは、実際の被害実態を把握できない。そこで、図1に示す宮城県で実施された急性下痢症疾患の実被害者数推定研究の考え方[5]に従い、神戸市での *C. jejuni* 感染症実患者数を推定した。なお、ここでの *C. jejuni* 感染症実患者数を発症者数と設定した。そして、得られた一般集団における感染者数と発症者数から、感染－発症割合を推定した。

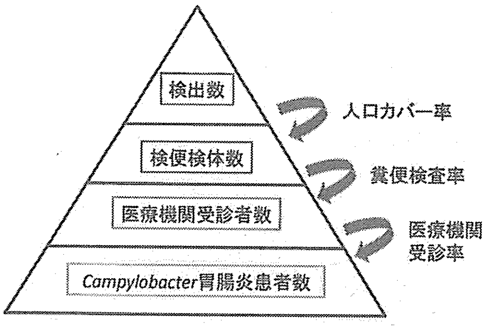

図1 *C. jejuni* 感染症実患者数推定の概念図[5]

2.3. *C. jejuni* 感染者1人当たりのDALYsの定量

DALYsは、早期死亡によって失われた生存年数(Years of Life Lost: YLL)と、障害をもって生存する年数(Years Lived with Disability: YLD)との和で表される。先進国における *Campylobacter* の感染によるDALYsは、1000人の胃腸炎がある場合に、YLDは3.2、YLLは1.4と推算されており、3.2 + 1.4 = 4.6 DALYsと求められている[1]。ここで感染者から発症者の発生割合は、推定した感染－発症割合 $P_{\mathrm{inf\text{-}ill}}$ を用いることとした。そして、以下の式により感染者1人当たりのDALYsを試算した。

$$\text{感染者1人当たりのDALYs} = 1(\text{人})\times P_{\mathrm{inf\text{-}ill}}\times 4.6\ (\text{DALYs}\cdot 1000\text{人})/1000\ (\text{人}) \qquad (1)$$

3. 実験結果と考察

3.1. 血清学的調査による一般集団における感染者割合の推定

得られた抗体価分布に対して、感染者と健常者のカットオフ値(陽性カットオフ値)を設定して感染者判定を行った。キットには、発症者を判定するカットオフ値が設定されている。このカットオフ値は、およそ400人の血清を分析した結果から得られた値である。そして急性胃腸炎の糞便検査判定との比較の結果、キットのカットオフ値が発症者を示すカットオフ値として妥当なものであることが示されている。一方で感染者は症状を示さないことから、キットにおける健常者集団にも感染者集団が含まれていると考えられる。そのためキットの発症者判定カットオフ値は、発症者と感染者のカットオフ値として用いることとした。次に感染者と健常者のカットオフ値(陽性カットオフ)の設定を行った。既往研究では、発症者、感染者、健常者に対してそれぞれ抗体価をELISA法を用いて吸光度で測定しており、各集団における吸光度上昇の差が確認された[3, 4]。そこで本研究では、各集団において *C. jejuni* 曝露後に最も高い上昇を示した時の吸光度平均値[3, 4]を用いて、その上昇比率からカットオフ値の設定を行った。カットオフ値設定方法の概念図を図2に示す。その結果、吸光度上昇率はIgA抗体で2.4~2.6倍、IgG抗体で1.7~1.8倍、IgM抗体で1.3~2.6倍であった。そして得られた各抗体クラスの吸光度上昇率とキットの発症者カットオフ吸光度値を用いて、感染者のカットオフ吸光度値を推定し、キットの説明書に記載してある吸光度と抗体価の関係式からカットオフ吸光度値を抗体価に変換した。その結果、陽性カットオフ値は、IgA抗体で8.2~9.0 U/mL、IgG抗体で13.2~14.9 U/mL、IgM抗体で21.9~47.3 U/mLの範囲となった。

次に、感染者を判定するために抗体クラスの選定を行った。各抗体クラスには、それぞれ特有の

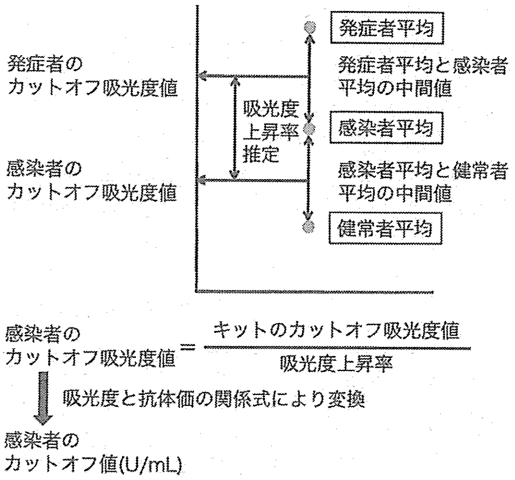

図2 感染者カットオフ値設定方法の概念図