Turk J Chem
35 (2011) , 245 – 253.

doi:10.3906/kim-0910-242

Assimilable organic carbon generation from algogenic organic matter in drinking water

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Received 26.10.2009

A study of assimilable organic carbon (AOC) derived from algogenic organic matter in different drinking water resources was conducted. Seasonal changes in the concentration of AOC at Kamafusa Dam were dependent on the algal cell numbers. Variations in AOC concentrations were found to be more reliant on the AOC-P17 component than the AOC-NOX component. The algal culture experiment showed that extracellular organic matter (EOM) released during the growth of *Phormidium tenue* in M-11 culture medium led to a significant increase in AOC concentration; however, no significant variations of AOC concentration were observed in CT culture medium containing high amounts of dissolved organic matter. The AOC included in EOM was not easily removed by chlorination; however, the AOC included in intercellular organic matter (IOM), which was released from algal cells, was easily removed under conditions where residual chlorine was detected.

Key Words: Dissolved organic compounds, bacterial regrowth, chlorination, algal material

Introduction

Maintenance of drinking water quality in water supply systems beyond water treatment plants is very important. In addition to other factors, the quality of drinking water is primarily affected by bacterial regrowth in the water supply piping network. To avoid this problem, residual chlorine has been used in water to suppress the multiplication of bacteria. However, it was learned that heterotrophic bacteria adhere to the inner surface of the

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pipes, proliferating and forming a biofilm under conditions with residual chlorine.[1−2] Regrowth of bacteria and detachment of the biofilm continue over time, causing deterioration of water quality in the water distribution system.[3−4] In general, bacterial regrowth is associated with the presence of assimilable organic carbon (AOC). Van der Kooij[5−7] suggested that AOC should be less than 10 μg/L to limit the growth of heterotrophic plate counts (HPCs) of bacteria in unchlorinated systems. Therefore, reduction of AOC is also an important measure for controlling bacterial regrowth. In Japan, interest has not been turned toward reduction of AOC, since the maintenance of residual chlorine has successfully controlled bacterial regrowth. However, reduction of AOC may be effective both in reducing chlorine doses and controlling bacterial regrowth.

The amount of AOC is difficult to control by conventional water treatment techniques.[8−10] Therefore, the behavior of AOC in water resources needs to be studied. In particular, the effects of eutrophication on the behavior of AOC are not well determined, even though eutrophication significantly affects the water purification process. Algogenic organic material (AOM) is not easily degraded, chemically or biologically. Algal blooms of blue-green algae or diatoms cause difficulties in controlling dissolved organic carbon (DOC) because of the release of extracellular organic matter (EOM) and intracellular organic matter (IOM) into the water by cell lysis. Algae and extracellular organic matter can be precursors of disinfection byproducts (DBPs). The existence of AOM composed of such substances as glycolic acid, carbohydrate, amino acids, and organic phosphorous might be able to cause an increase in AOC concentration.[11−14]

This study was conducted in order to examine variations in the AOC concentrations of drinking water and to learn characteristics of AOC derived from algae. The seasonal changes in the AOC concentration of Kamafusa Dam, the main reservoir of Sendai, Japan, and of 3 inflow rivers were investigated. A series of experiments was conducted to see the influence of algae on AOC concentrations. Generation of AOC derived from the growth of blue-green alga, *Phormidium tenue*, in different culture medium conditions was studied. Assessment of the formation and removal of AOC derived from EOM and IOM in the course of chlorination was also conducted.

Materials and methods

Study sites

Samples of water were taken from Kamafusa Dam and 3 rivers, the Taro River, Kita River and Mae River. Kamafusa Dam is a main drinking water resource in Sendai, Japan and the 3 rivers flow into the dam. Water from Kamafusa Dam treated at the water treatment plant was also sampled. Sampling was carried out once bimonthly for a period of 14 months.

AOC determination

AOC determination is based on the measurement of the maximum extent of growth of a selected pure bacterial culture in representative water samples, in which indigenous bacteria have been killed or inactivated by heat treatment.[15−16] Colony counts are used for determining bacterial densities. The analytical method developed by van der Kooij was adopted in the present study. The method employs *Pseudomonas fluorescens* strain P17 and *Spirillum* strain NOX. These 2 microorganisms prefer different groups of compounds. P17 has a great

nutritional versatility and may grow on a variety of carbohydrates, aromatic acids, and amino acids. It can also grow on carboxylic acids, with the exception of formic, glyoxylic, and oxalic acids as typical byproducts of ozonation. Therefore, the growth of strain P17 was used for the determination of the concentration of aromatic and amino acids, etc. Meanwhile, NOX cannot utilize carbohydrates, alcohols, or aromatic acids, but rather a wide range of carboxylic acids. NOX can also utilize a few amino acids; however, this organism cannot assimilate amino acids when growing on mixtures of compounds. Therefore, the growth of strain NOX was used for the determination of the concentration of carboxylic acids in water. All glassware was thoroughly cleaned and rendered free of organic carbon by combustion at 550 $^\circ$C for 1 h. Samples were incubated at 15 $\pm$ 0.5 $^\circ$C and the number of colonies was measured with the plate count technique after 72 $\pm$ 3 h of incubation at 28 $\pm$ 1 $^\circ$C on R2A agar. The total AOC from microorganisms per liter of equivalent acetate-carbon is the sum of AOC-P17 and AOC-NOX. In the present study, the yield coefficients of P17 and NOX were measured as 4.1 $\times$ 10^7 μg acetate-C/L and 1.2 $\times$ 10^7 μg acetate-C/L, respectively.

Algal culture

The blue-green algae *Phormidium tenue* (*P. tenue*, ATCC 49642) was selected as test algae to form AOM. It was cultivated under an illumination condition of 3000 lx with a 12:12 photoperiod in an incubator. The temperature was maintained at 22 $^\circ$C *P. tenue* was cultured with 2 different types of culture media, M-11 and CT. Table 1 shows the components of each culture medium.

Table 1. Components of culture media.

M-11 culture medium		CT culture medium	
$NaNO_3$	10 mg	$Ca(NO_3)_24H_2O$	15 mg
K_2HPO_4	1.0 mg	KNO_3	10 mg
$MgSO_47H_2O$	7.5 mg	Na_2 glycerophosphate$5H_2O$	5.0 mg
$CaCl_22H_2O$	4.0 mg	$MgSO_47H_2O$	4.0 mg
Na_2CO_3	3.0 mg	Vitamin B_{12}	0.01 μg
$FeSO_47H_2O$	0.1mg	Biotin	0.01 μg
$Na_2EDTA2H_2O$	0.1 mg	Thiamine HCl	1.0 μg
Distilled water	100 mL	PIV metals	0.3 mL
pH	8.0	TAPS	40 mg
		Distilled water	99.7 mL
		pH	8.2

Chlorine oxidation

Sodium hypochlorite (NaOCl) solution was standardized by titration and then diluted with distilled water. Chlorine doses were set at 0, 2, 5, 10, 20, and 30 mg/L. Sealed amber glass bottles, each 3 L, were used for the chlorine oxidation experiment. The amber glass bottles were also covered with aluminum foil to prevent photodegradation of the solutions. The contents of the bottles were stirred continuously with a magnetic

stirrer. After chlorine oxidation, the sample was withdrawn from each bottle, then filtered with a GF/C filter and terminated by addition of sodium thiosulfate immediately after collection.

Ultrafiltration

The sample was initially filtered through Whatman GF/F filters and then submitted to ultrafiltration (regenerated cellulose PL, ultrafiltration membranes, Millipore). The sample was fractionated into 4 fractions by molecular weight: lower than 1000 Da, 10005000 Da, 500010,000 Da and higher than 10,000 Da.

Results and discussion

Seasonal variations of AOC in dam and river water

Concentrations of AOC at each sampling point throughout the year were conducted (Table 2). At Kamafusa Dam, the concentration ranged from 111 to 305 μg/L. For the 3 rivers flowing into the dam, it ranged from 34 to 293 μg/L. The average AOC concentration of Kamafusa Dam, 178 μg/L, was approximately 1.6 times higher than that of the rivers. This indicated that the AOC concentration of Kamafusa Dam tended to be higher than that of each river (t-test, $P < 0.01$). Treated water had an average AOC concentration of 107 μg/L. Unexpectedly, the AOC concentration of the treated water on 28 April 2007 was higher than that of Kamafusa Dam. It was thought that the sand in the rapid sand filtration unit was contaminated by organic substances. Except for the sample taken on 28 April 2007, the AOC concentration of the treated water was lower than that of Kamafusa Dam averaging about 50 μg/L. A maximum AOC concentration was observed in the spring season in March and April, at all survey points. This might be due to the increased growth of aquatic plants in spring.

Table 2. Concentration of AOC at various sampling points throughout the year.

Sampling period	AOC (μg/L)				
	Taro River	Kita River	Mae River	Kamafusa Dam	Treated water
25.04.2006	63	109	80	193	31
14.06.2006	67	75	44	174	53
29.07.2006	103	89	94	133	54
10.10.2006	54	115	34	111	44
16.01.2007	39	173	219	142	48
13.03.2007	293	46	226	305	192
28.04.2007	101	240	171	188	325
11.06.2007	165	65	50	167	64
Average	103	121	124	178	107

Figure 1 shows the average ratio of AOC-P17 and AOC-NOX at each survey point. In the case of untreated water from the rivers and dam, the AOC-P17 component accounted for 82%-93% of the total AOC

concentration, while in the treated water, the AOC-P17 component was 57%. It was obvious that the increase of the AOC-NOX component was due to the production of AOC-NOX by chlorine oxidation.

Relationship between AOC and algal cell number

In order to develop a relationship between increased concentrations of AOC and the algal cell numbers at Kamafusa Dam, the seasonal variations in algal cell numbers at the dam were studied during 2002-2006 (Figure 2). Data revealed that during the spring season (FebruaryApril), algal cell numbers were higher than in the rest of the months of the year. This tendency was similar with that of increased concentrations of AOC at Kamafusa Dam in the spring (Table 2); in other words, the algal cell numbers would increase in spring and decrease in autumn. The average cell number of about 4700 cells/mL in spring was twice that of other seasons. The results suggested that an increase in algal growth could cause an increase in the AOC concentration of the water.

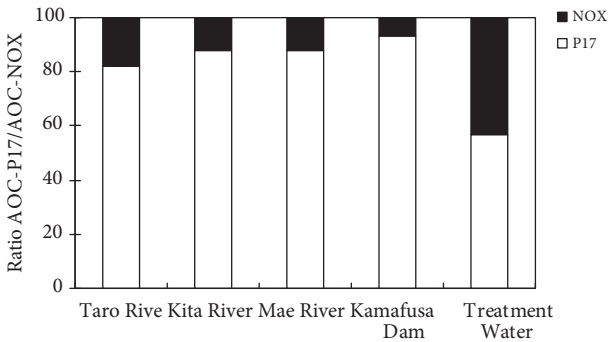

Figure 1. Ratio of AOC-P17/AOC-NOX at different survey points.

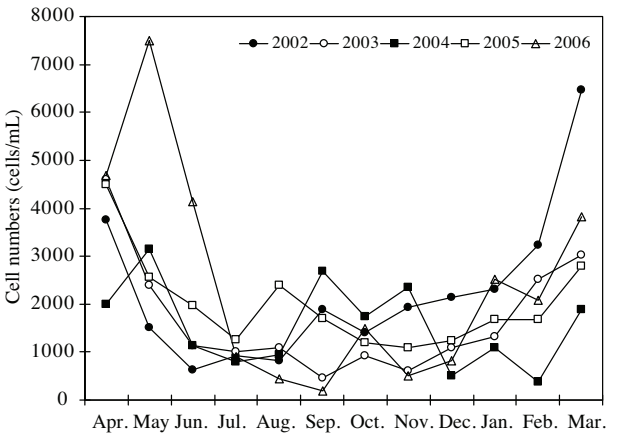

Figure 2. Seasonal variations in algal cell numbers at Kamafusa Dam.

Effects of algal growth on AOC

P. tenue, one of the dominant species at Kamafusa Dam was selected and cultured in M-11 and CT culture media. Variations in *P. tenue* cell numbers in each culture medium are shown in Figure 3. The initial cell numbers of *P. tenue* in M-11 and CT culture media were 0.8×10^4 and 1×10^4 cells/mL, respectively. The cell number increased rapidly in each culture medium, and on the 39th day, the cell numbers of the M-11 and CT media had increased to 67×10^4 and 115×10^4 cells/mL, respectively. On the 68th day, the cell number in each culture medium was 90×10^4 and 102×10^4 cells/mL, respectively.

Figure 4 shows variations in the DOC and AOC concentrations produced by *P. tenue* in M-11 culture medium. The DOC concentration of M-11 culture medium before inoculation of *P. tenue* was about 1.5 mg/L. The DOC concentration had a tendency to increase with elapsed time. In the stationary phase, after the 39th day, the DOC concentration had increased to 9.2 mg/L. The AOC concentration of M-11 culture medium before inoculation of *P. tenue* was 39 μg/L. The AOC concentration increased significantly in the latter half of the

logarithmic phase and reached about 4000 μg/L during the stationary phase. The AOC-P17 component was about 90% or more. In other words, variations in the AOC concentration depended on the AOC-P17 component.

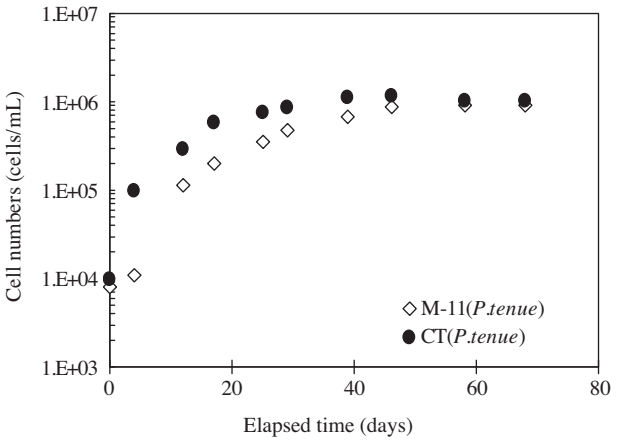

Figure 3. Profile of *P. tenue* cell numbers in different culture media with respect to time.

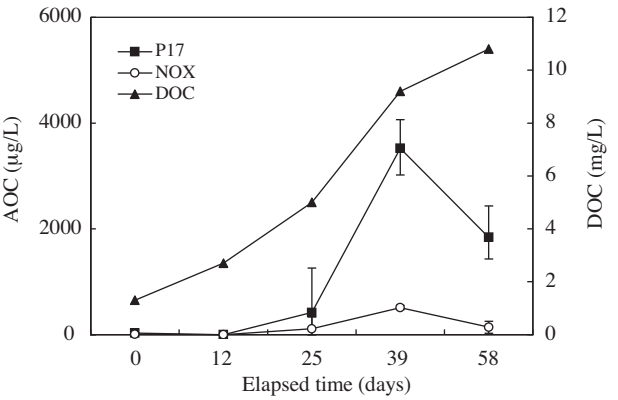

Figure 4. Profile of DOC and AOC concentrations produced by *P. tenue* in M-11 culture medium.

Figure 5 shows variations in DOC and AOC concentrations produced by *P. tenue* in CT culture medium. The DOC concentration of the CT culture medium was much higher than that of the M-11 culture medium. DOC concentration, which did not change throughout the culture period, averaged 152 mg/L. In contrast to M-11 culture medium, CT culture medium did not lead to a significant variation in AOC concentration. The maximum AOC concentration during the culture period was about 80 μg/L. These results suggest that the generation of AOC from algae was greatly influenced by culture medium conditions. It was concluded that the generation of AOC occurred actively, when the concentration of organic substances in algal water was relatively low.

To investigate the profile of AOC and DOC concentrations derived from EOM in the stationary phase, EOM was fractionated by ultrafiltration into 4 fractions of molecular weight (Figure 6). The DOC produced by EOM was detected mainly at molecular weights under 1000 Da. The percentage of DOC concentrations under 1000 Da was over 60% of the total DOC concentration. As shown in Figure 7, the AOC produced by *P tenue* was also detected mainly in the molecular weight fraction lower than 1000 Da. Although AOC-NOX occupied 20% of the total AOC at this fraction, AOC-NOX was not detected in the fraction for which molecular weight was over 10,000 Da.

Effects of chlorination on AOC included in AOM

Figure 8 shows the relationship between chlorine dose and residual chlorine for EOM and IOM. The demand of chlorine for the oxidation of both EOM and IOM (*P. tenue*, M-11 culture medium) was about 20 mg/L for the sample containing 67 $\times$ 10^4 cells/mL. In the case of fresh culture medium, chlorine was not consumed. Figures 9a and 9b show the variations of DOC and UV absorbance at 260 nm (E260) for the oxidation of EOM and IOM by chlorine. In the case of fresh culture medium, the DOC concentration and E260 were not significantly changed with the increase of chlorine doses. However, the E260 of EOM had a tendency to decrease with the

increase of chlorine doses. In the case of IOM, the DOC concentration and E260 increased with the increase of chlorine doses. This increase in DOC and E260 by chlorination was due to the release of dissolved AOM through the destruction of algal cells. Although E260 increased due to chlorine oxidation, the maximum value of IOM was not higher than that of EOM.

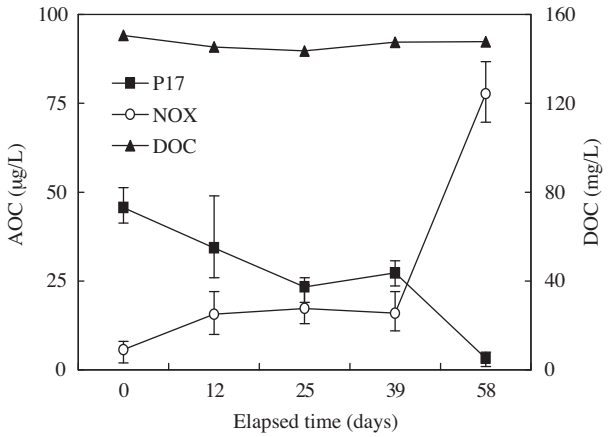

Figure 5. Profile of AOC and DOC concentrations produced by *P. tenue* in CT culture medium with respect to time.

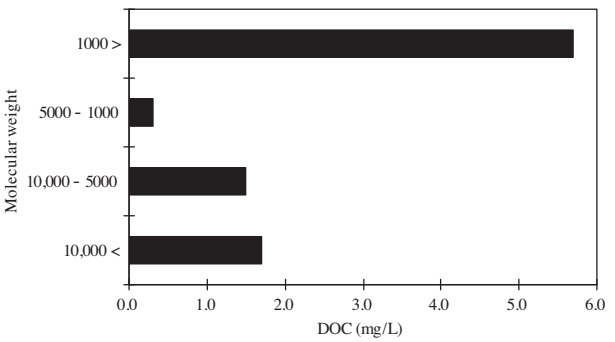

Figure 6. Fraction of DOC produced by EOM (M-11 culture medium).

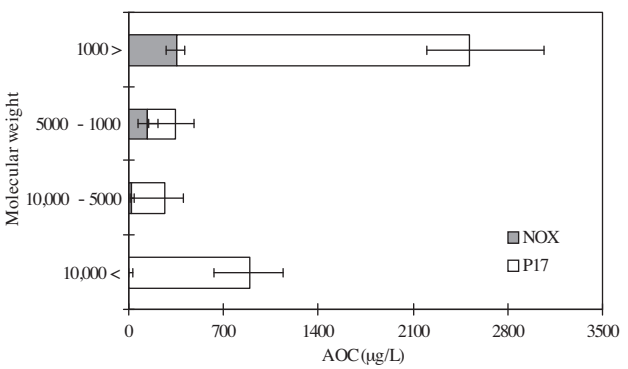

Figure 7. Fraction of AOC produced by EOM.

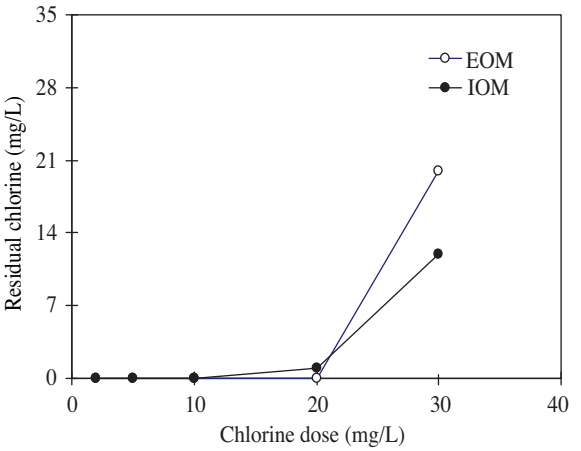

Figure 8. Relationship between chlorine dose and residual chlorine.

Figure 10 shows variations in AOC concentration during chlorination of EOM. Although AOC concentration increased 18% with a low chlorine dose (2 mg/L), it decreased with the increase of the chlorine dose to over 2 mg/L. A chlorine dose of 5 mg/L resulted in a decrease in AOC concentration, from 636 to 336 μg/L. In the case of 10 and 30 mg/L chlorine doses, the AOC concentration further decreased to 219 and 98 μg/L, respectively. The percentage of AOC-P17 and AOC-NOX for EOM before chlorine oxidation was about 50%. With the increase of chlorine dose, the AOC-P17 component decreased significantly and the ratio of the

AOC-NOX component increased. When residual chlorine was detected at a chlorine dose of 30 mg/L, an AOC concentration of 98 μg/L still remained. This value of chlorine seems insufficient to prevent bacterial regrowth. To treat the AOC included in EOM, a greater chlorine dose than usual was required.

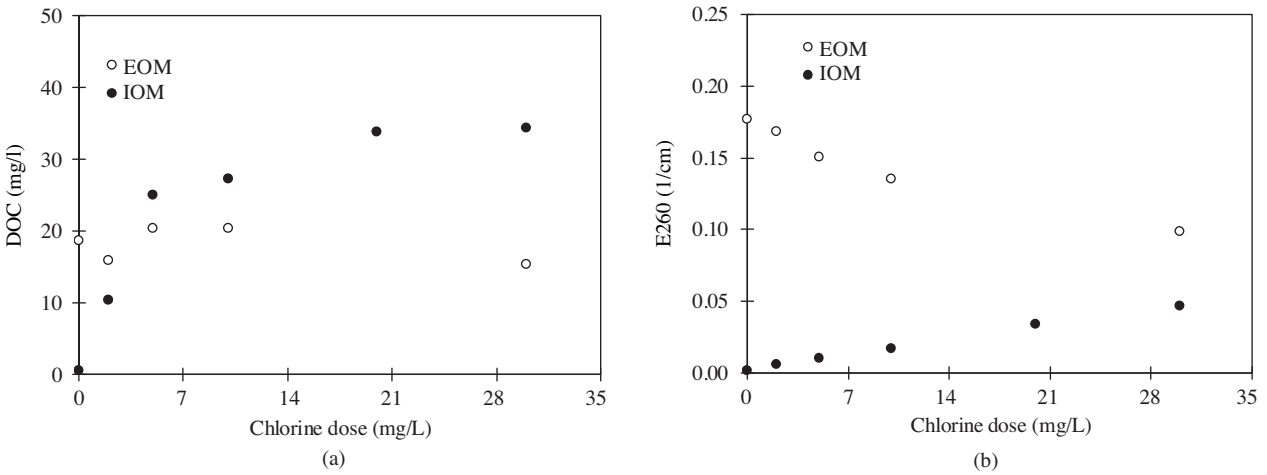

Figure 9. At each chlorine dose, profile of a) DOC and b) E260.

Furthermore, in order to study the effect of chlorination on AOC for IOM, chlorine oxidation of IOM was performed. IOM was collected by centrifugation of algae cells. The results are interpreted in Figure 11. When the chlorine dose was 0 mg/L, the AOC concentration in IOM was about 2 μg/L. However, AOC concentrations were increased with the increase of chlorine doses. For example, the AOC concentration increased to 92 μg/L at a chlorine dose of 2 mg/L. In the case of 20 and 30 mg/L of chlorine doses, however, AOC was not detected. The ratio of AOC-P17 to AOC-NOX for IOM differed from that of EOM. AOC-P17 was higher than AOC-NOX, at above 74% for each chlorine oxidation. From this, it was found that the AOC included in IOM could be removed sufficiently when chlorination was carried out as usual.

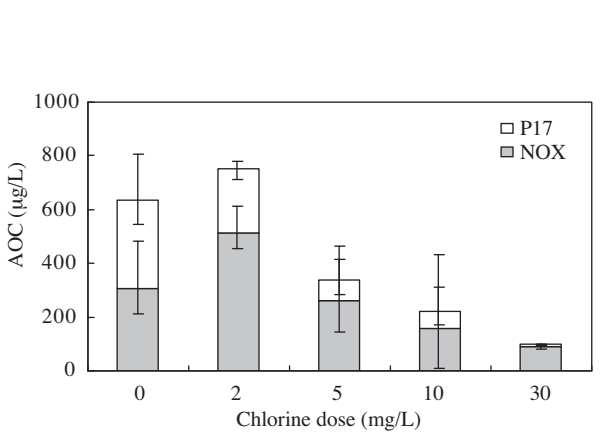

Figure 10. AOC concentration after chlorination (EOM).

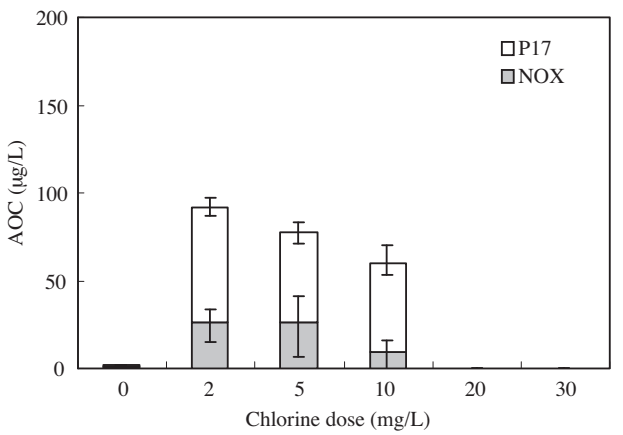

Figure 11. AOC concentration after chlorination (IOM).

Conclusions

Higher concentrations of AOC at Kamafusa Dam, as compared with those of the rivers, indicated that eutrophication could cause changes in the concentration of AOC. Algogenic materials seemed to be an important factor in AOC variation. The AOC included in EOM could be formed largely by algae grown in fresh culture media. Generation of AOC occurred actively when the concentration of organic substances in algal material was relatively low. EOM with molecular weights lower than 1000 Da produced by *P. tenue* would help the formation of AOC. Chlorination increased DOC and E260 absorbance due to the release of dissolved AOM. Chlorination of EOM and IOM revealed that the AOC included in EOM was difficult to remove; however, the AOC included in IOM could be removed under conditions where residual chlorine was detected.

Acknowledgement

This work was financially supported by the Korean Ministry of Environment (MOE) under the ET-Human Resource Development Project.

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