Evaluation of assimilable organic carbon in the Yodo River and the effect of coagulation on its control

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Abstract Biodegradable organic compounds in Yodo River water, the coagulated water and those ozonated waters were characterized on the concentration of assimilable organic carbon (AOC), the apparent molecular size and the sensitivity to E260 (UV absorbance at the wavelength of 260 nm). High molecular (>5,000 Da) organic compounds which were insensitive to E260 were contained with relatively high concentration in Yodo River, whereas they were not detectable as AOC. The AOC concentration in Yodo River was reduced by coagulation because the phosphorus concentration sharply decreased. The AOC concentration rapidly increased with the reduction of E260 and slowly with the depolymerization of high molecular organic compounds during the ozonation. However, 40–50% of AOC increasing with the ozonation was reduced by the coagulation. Furthermore, batch incubations of native bacteria living in the Yodo River were conducted in order to examine the validity of the apparent AOC concentration as an indicator of the regrowth potential. The AOC concentration calculated as acetate carbon equivalent would not exactly represent the concentration of the available carbon for the assimilation. However, maximum cell numbers for the incubation period were generally proportional to apparent AOC concentrations even if phosphorus limitation. In conclusion, it is suggested that coagulation is an effective process in controlling the concentration of AOC and is a useful indicator to evaluate the regrowth potential of heterotrophic bacteria in Yodo River.

Keywords Assimilable organic carbon (AOC); bacterial regrowth; coagulation; ozonation; phosphorus

Introduction

It is well known that a bacterial regrowth in drinking water distribution system causes various deteriorations of tap water, such as growth of pathogens, corrosion of pipes, tastes and odors. At water purification plants, it is important to control a concentration of assimilable organic carbon (AOC) which is a significant indicator of a regrowth potential of heterotrophic bacteria in drinking water (Huck, 1990; LeChevallier *et al*., 1991; Van der Koij, 1992; Van der Koij *et al*., 1982). However, it is difficult to predict the behavior of AOC and bacterial regrowth potential during water treatment processes because of the following reasons: (1) AOC is a minor fraction of biodegradable organic compounds in natural water (Hem and Efaimsen, 2001; Volk and LeChevallier, 2000); (2) it is possible that a concentration of an (apparent) AOC and regrowth potential of heterotrophic bacteria are influenced from concentrations of inorganic substances such as phosphorus (Miettinen *et al*., 1997, 1999; Sathasivan and Ohgaki, 1999). Therefore, a case study for investigating the behavior of AOC during water treatment processes and the validity of the apparent AOC concentration as an indicator of the regrowth potential is necessary.

In this study, the surface water at the downstream area of Yodo River, the representative water resource of Osaka prefecture in Japan, is focused. Potable water produced from the surface water is supplied to a very wide area. In addition, the water quality is influenced by effluents from some sewage water treatment plants located upstream of the basin.

In this paper, it is described that: (1) characteristics of biodegradable organic compounds contained in Yodo River water, based on the molecular size distribution, the sensitivity to E260 (UV absorbance at the wavelength of 260 nm) and the AOC concentration; (2) the

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effect of coagulation on the reduction of AOC in the river water and the control of AOC increasing with ozonation process; and (3) the relationship between apparent AOC concentrations and regrowth potential of heterotrophic bacteria living in the Yodo River.

Experimental procedure

Experiment of coagulation and ozonation

Yodo River water (raw water) has been sampled near the Osaka Institute of Technology (Asahi-ku, Osaka City, Japan) from June 1999 to November 2000. After the sampled waters had been transported to the laboratory, water quality measurements and coagulation and ozonation experiments were conducted immediately.

For the coagulation experiment, polyaluminium chloride with the dosage of 3 mg Al/L was added to 20 L of the raw water. After the addition of the coagulant, the sample water was rapidly mixed, followed by slow mixing, and then settled until turbidity of the supernatant in the settled water would be below 0.5 TU (coagulated water). The pH of the coagulated water was around 7.

For the ozonation experiment, the ozonized air was contacted to 5 L of the raw water and/or the coagulated water in a column with a volume of 13 L. The concentration and the flow rate of the ozonized air were 5 mg O_3/L and 1.5–5 L/min, respectively.

Measurements of water quality

DOC, E260, AOC, molecular size distribution of organic matters, T-N, NH_4^+-N and PO_4^{3-}-P were measured. All sample waters were filtered by membrane filters with a pore size of 0.5 μm and the material of PTFE (DOC, E260, AOC, molecular size distribution, T-N, NH_4^+-N and PO_4^{3-}-P) or mixed cellose ester (E260). At the measurement of AOC, the filtered water was sterilized at 60°C for 30 min. The sterilized water was incubated at 15°C after *P. fluorescence* strain P17 and *Spirillum sp.* strain NOX had been simultaneously inoculated. Inorganic substances were not added to the sample waters for measuring AOC concentration (apparent AOC concentration). AOC concentration was calculated as acetate carbon equivalent on bacterial numbers at 5, 6, 7, 8 and 9 days after the inoculation. Yield factors of 4.1×10^6 CFU/μg of C for strain P17 and 1.2×10^7 CFU/μg of C for strain NOX were employed for the calculation, respectively (*Standard Methods for the Examination of Water and Wastewater*, 1998). At the measurement of molecular size distribution, the technique of gel permeation chromatography with the conditions of Table 1 was applied. Samples for injecting into the gel column were evaporated to a volume of 1/40 and filtered again before injection by PTFE membrane filter (0.45 μm).

Batch incubation of native bacteria in the Yodo River

In order to evaluate the relationship between apparent AOC concentrations and bacterial regrowth potential, batch incubations of native bacteria living in Yodo River were carried out with measurement of AOC concentrations. Conditions of the experiment are listed in Table 2. Sample waters for this experiment were prepared by the ozonation or the addition of KH_2PO_4 because only AOC or PO_4^{3-}-P concentration which have possibilities for

Table 1 Conditions of gel permeation chromatography

Gel	Sephadex G-25 fine (< 5,000 Da)
Bed size	I.D. 2.6 * 90 cm
Elluent	Distilled water
Flow rate	97 mL/min
Injection volume	10 mL
Volume of 1 fraction	10 mL

I.D.: Inside diameter

Table 2 Experimental conditions for the analysis of the relationship between apparent AOC concentrations and bacterial regrowth potential

	Preparation of sample water	
Phase 1	Raw water	Before ozonation
		After ozonation[1]
	Coagulated water	Before ozonation
		After ozonation[1]
Phase 2	Raw water	No addition of KH_2PO_4
		Addition of KH_2PO_4[2]
	Coagulated water	No addition of KH_2PO_4
		Addition of KH_2PO_4[2]

[1] The flow rate of the ozonized air and the contact time were 1.5 L/min and 10 min, respectively
[2] Final concentration of PO_4^{3-}-P in a sample water was 0.05 mg P/L

limiting growth of bacteria in the raw water and the coagulated water was increased. Sample waters for the batch incubations were filtered by membrane filters with a pore size of 0.5 μm and a material of PTFE. The filtered waters were sterilized at 60°C for 30 min. The sterilized water was incubated at 15°C after 1 mL of the unfiltered Yodo River water had been added. Numbers of bacteria for the batch incubation were periodically enumerated by the total cell direct count method (stained with DAPI) and the heterotrophic plate count method (spread onto R2A agar and incubated at 25°C for 5 days).

Results and discussions

Biodegradable fraction of organic matters and AOC in Yodo River

Measurements of apparent AOC, DOC and E260 in the raw water and the coagulated water are listed in Table 3. In the raw water, DOC/E260 ratios as an indicator of biodegradability of organic compounds and (apparent) AOC concentrations as an indicator of biological stability were relatively high and varied with DOC concentration. These results suggest that the Yodo River water contains relatively high concentrations of organic compounds which are insensitive to E260 (biodegradable), and these variations are larger than non-biodegradable organic compounds such as humic and fulvic substances. In addition, Table 3 also indicates that AOC/DOC ratios in the raw water were not related to DOC/E260 ratios although similar indicators. Figure 1 shows typical molecular size distributions of organic compounds in the raw water and the coagulated water. Organic compounds in Yodo River

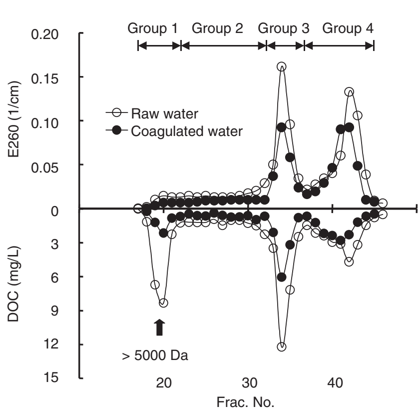

Figure 1 Typical molecular size distributions of organic compounds in the raw water and the coagulated water (1999.10.6)

consisted of three molecular groups (>5,000 Da, 1,000–2,000 Da, and <1,000 Da), and DOC which were insensitive to E260 were unevenly distributed toward high molecular size. Tambo and Kamei (1978) reported that decomposition rates of these compounds are slow although biodegradable. It is considered that these compounds are proteins or polysaccharides contaminated from effluents from sewage water treatment plants, and would not be detectable as AOC.

Change in apparent AOC concentration by coagulation

According to Table 3, apparent AOC concentrations in the raw water and the coagulated water were about 80–600 and about 90–200 μg ac-C eq/L, respectively. 7–76% of apparent AOC concentrations were reduced by the coagulation, and the variation became smaller. For clearing the influence of phosphorus on the reduction of AOC by the coagulation, AOC concentrations in the raw water and the coagulated water were measured with and without adding KH_2PO_4 solution. The KH_2PO_4 solution was added for adjusting the final concentration of PO_4^{3-}-P in the sample waters to 50 μg P/L. As shown in Table 4, AOC concentrations, which were directly measured, always decreased with coagulation, whereas they increased by the addition of KH_2PO_4. In addition, about 70% of high molecular (>5,000 Da) organic compounds which were insensitive to E260 were removed by the coagulation on 6 October 1999 (Figure 1), whereas the AOC concentration measured with adding KH_2PO_4 unchanged (Table 4). These results suggest that most biodegradable organic compounds which were removed by coagulation were not detectable as AOC. Volk *et al*. (2000) reported that removal of AOC by coagulation was difficult because there were low molecular organic compounds in general. Therefore, it is considered that the reason for the reduction of the apparent AOC concentrations by coagulation would not be the removal of biodegradable organic carbon, but the decrease of phosphorus concentration. Moreover, Table 3 and Table 4 also suggest that more concentrations of AOC-P17 were reduced by coagulation and increased by the addition of KH_2PO_4 than AOC-NOX. It is considered that a growth of strain P17 would be easier limited by phosphorus than strain NOX.

Change of organic matters and increase of AOC by ozonation

Figure 2 shows molecular size distributions of organic compounds before and after the ozonation. When the raw water and the coagulated water were ozonated for 10 min, E260 in all groups were reduced and DOC in Group 1 was degraded. As a result, DOC/E260 ratios in Group 3 and Group 4 became about 2 times higher by the ozonation. These phenomena

Table 3 Measurements of apparent AOC, DOC and E260 in raw water and coagulated water

Date	Raw water							Coagulated water						
	AOC (μg ac-C eq/L)			DOC	E260	D/E[1]	A/D[2]	AOC (μg ac-C eq/L)			DOC	E260	D/E[1]	A/D[2]
	P17	NOX	Total	(mg/L)	(1/cm)			P17	NOX	Total	(mg/L)	(1/cm)		
99.6.16	84	2	86	3.0	0.034	88	2.9							
99.7.29	72	9	81	1.6	0.040	40	5.1							
99.10.6	90	43	133	1.8	0.036	50	7.4	41	52	93	1.0	0.021	47	9.3
99.11.4	238	5	243	1.8	0.032	56	13.5							
99.12.8	112	24	136	2.0	0.031	65	6.8							
00.1.7	209	12	221	2.0	0.026	77	11.1	191	15	206	0.9	0.017	53	22.9
00.7.28	193	95	288	4.2	0.048	88	6.9	N.D.	105	105	2.4	0.025	96	4.4
00.9.1	563	43	606	4.1	0.048	85	14.8	N.D.	146	146	–	0.032	–	–
00.9.20	487	46	533	4.8	0.048	100	11.1	N.D.	179	179	3.1	0.024	129	5.8
00.10.26	353	53	406	3.2	0.051	63	12.7	N.D.	98	98	2.4	0.021	114	4.1
00.11.19	175	55	230	3.7	0.042	88	6.2	N.D.	149	149	2.3	0.018	128	6.5

[1] DOC/E260 (–); [2] AOC-Total/DOC (%)
N.D. = not detected

Table 4 AOC concentrations measured with and without adding KH_2PO_4 solution

	Raw water		Coagulated water	
KH_2PO_4	No addition	Addition	No addition	addition
(1999.10.6)				
PO_4^{3-}-P	0.07	0.12	N.D.	0.04
AOC-P17	90	80	40	75
AOC-NOX	43	50	53	55
AOC-Total	133	130	93	130
(2000.11.19)				
PO_4^{3-}-P	0.09	0.14	0.02	0.07
AOC-P17	175	402	0	223
AOC-NOX	55	35	149	122
AOC-Total	230	436	149	345

PO_4^{3-}-P: mg P/L, AOC: μg ac-C eq/L

indicate that biodegradability of low molecular (below 1,000–2,000 Da) organic compounds particularly increased by the ozonation. Changes in E260, DOC and AOC (measured with adding KH_2PO_4) with the ozonation in the raw water and the coagulated water are listed in Table 5. Increases of AOC for 1 min in the raw water and the coagulated water were 71 and 35 μg ac-C eq/L, and those for 10 min were 166 and 95 μg ac-C eq/L, respectively. From about 40% of AOC increasing with the ozonation had increased within 1 min, it is obvious that AOC concentration rapidly increased at the beginning of the ozonation, and then slowly increased. According to Figure 2 and Table 5, it is suggested that reductions of E260 were dominantly for 0–1 min and depolymerizations of high molecular organic compounds for 1–10 min, respectively. Therefore, it is considered that an easily assimilable organic carbon was rapidly produced with degradation of unsaturated combination of organic compounds, and slowly produced with depolymerization of high molecular organic compounds.

Effect of coagulation on the reduction of AOC increasing with ozonation

Table 5 also indicates that an increase of AOC with the ozonation was reduced by the coagulation. The reduction efficiencies of increases of AOC at the contact time of 1 and 10 min were 50 and 40%, respectively. This result suggests that organic compounds which were removed by the coagulation would be precursors of AOC produced by the ozonation. According to Figure 1, most of high molecular organic compounds with a molecular size

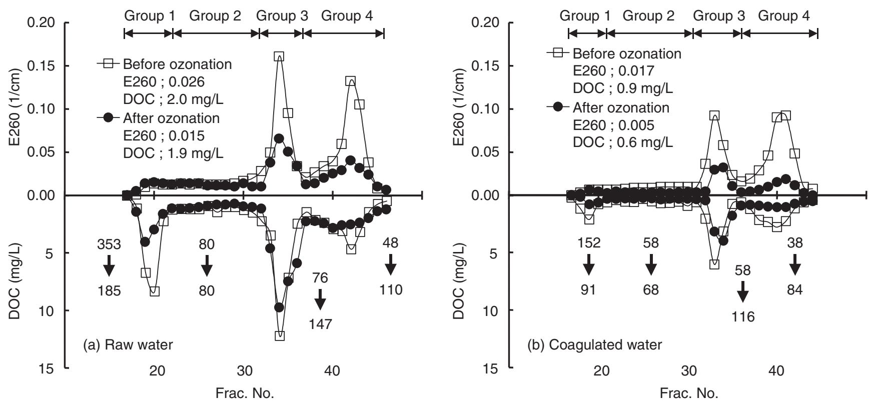

Figure 2 Molecular size distributions of organic compounds before and after the ozonation: (a) the raw water; (b) the coagulated water. The flow rate of the ozonized air and the contact time were 5 L/min and 10 min, respectively. Numbers represent DOC/E260 ratio of each group

Table 5 Changes in E260, DOC and AOC with the ozonation process. The flow rate of the ozonized air was 5 L/min

	Raw water			Coagulated water		
Contact time (min)	**0**	**1**	**10**	**0**	**1**	**10**
E260 (1/cm)	0.026	0.017	0.015	0.017	0.006	0.005
DOC (mg/L)	2.0	2.1	1.9	0.9	1.1	0.6
AOC-P17	209	216	287	191	173	220
AOC-NOX	12	76	100	15	68	81
AOC-Total	221	292	387	206	241	301

AOC: μg ac-C eq/L

above 5,000 Da (Group 1) and part of organic compounds with a molecular size of 1,000–2,000 Da (Group 3) which were sensitive to E260 were removed by coagulation. These phenomena would be the reason for the reduction of AOC which increased with the ozonation.

Relationship between apparent AOC concentration and regrowth potential of native bacteria in the Yodo River

Qualities of each sample water and maximum cell numbers for the batch incubation are shown in Table 6 and Figure 3, respectively. Apparent AOC concentrations and maximum cell numbers for the batch incubation were increased by both ozonation and the addition of KH_2PO_4. These results indicate that a growth of bacteria in the raw water and the coagulated water were limited by both organic carbon and phosphorus. In addition, about 200 μg ac-C eq/L of apparent AOC (especially AOC-P17) concentrations were increased when about 50 μg P/L of KH_2PO_4 were added to the raw water and the coagulated water (Phase 2). Moreover, C/P ratios without the addition of KH_2PO_4 in the raw water and the coagulated water were 2.6 and 7.5, respectively. These ratios were obviously lower than a consumption ratio of substances for an assimilation of heterotrophic bacteria (50) and a composition ratio of a bacterial cell (23). However, growths of bacteria in both sample waters were limited by phosphorus. These results suggest that the concentration of the available carbon for the assimilation in this experiment would be underestimated when AOC concentrations were calculated as acetate carbon equivalent. However, as shown in Figure 4, the maximum cell numbers for the incubation period enumerated by the total cell direct count method and the heterotrophic plate count method were generally proportional

Table 6 Measurements of water qualities

	Phase 1				Phase 2			
	Raw water		Coagulated water		Raw water		Coagulated water	
Ozonation	**Before**	**After**	**Before**	**After**	–	–	–	–
KH_2PO_4	–	–	–	–	**No addition**	**Addition**	**No addition**	**Addition**
E260 (1/cm)	0.051	0.025	0.021	0.04	0.042	–	0.018	–
DOC (mg C/L)	3.2	–	2.4	–	3.7	–	2.3	–
AOC-P17[1]	222	489	N.D.	N.D.	175	402	N.D.	223
AOC-NOX[1]	42	131	91	127	55	35	149	122
AOC-Total[1]	264	621	91	127	230	436	149	345
T-N (mg N/L)	1.85	–	1.89	–	1.66	–	1.52	–
NH_4^+-N (mg N/L)	0.14	–	0.18	–	0.08	–	0.11	–
PO_4^{3-}-P (mg P/L)	0.13	–	0.03	–	0.09	0.14	0.02	0.07
C/P[2]	2.0	4.8	3.0	4.2	2.6	3.1	7.5	4.9

[1] μg ac-C eq/L, [2] AOC-Total/PO_4^{3-}-P
N.D. = not detected

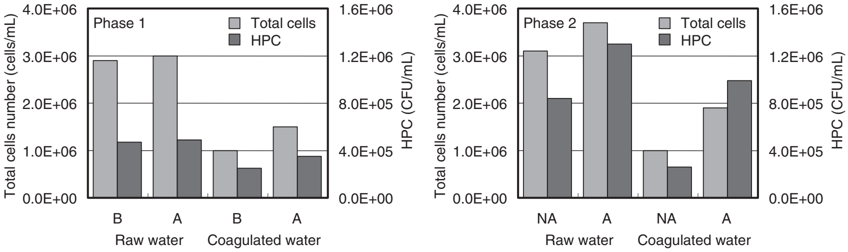

Figure 3 Maximum cell numbers for the batch incubations (symbols in Phase 1, B: before ozonation, A: after ozonation; Phase 2, NA: no addition of KH_2PO_4, A: addition of KH_2PO_4). In all sample waters, total cell numbers and HPC have reached to the maximum at 3 days and 2 days after the start of the incubation, respectively

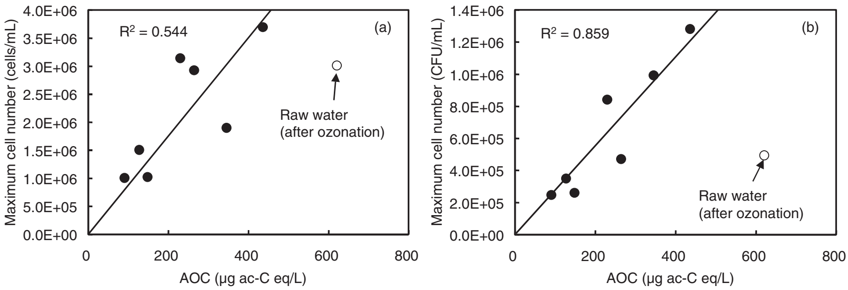

Figure 4 Relationship between apparent AOC concentrations and maximum cell numbers for the batch incubation. The cell numbers were enumerated by: (a) the total cell direct count method; and (b) the heterotrophic plate count method

to apparent AOC concentrations except the raw water after the ozonation. Therefore, it is considered that apparent AOC concentrations in the raw water, the coagulated water and those ozonated waters would represent the regrowth potential of native bacteria living in the Yodo River at the range of low AOC concentrations even in phosphorus limitation. According to the relationship between apparent AOC concentrations and maximum cell numbers, apparent growth yields of native bacteria living in the Yodo River were estimated at 9.8×10^6 cells/μg of AOC (as total cell numbers) and at 2.6×10^6 CFU/μg of AOC (as HPC), respectively.

Conclusions

In this study, for clearing behaviors of AOC during water treatment processes, characteristics of biodegradable organic compounds in Yodo River water, the coagulated water and those ozonated waters were evaluated by molecular size distribution, the sensitivity to E260 and the AOC concentration. Furthermore, batch incubations of native bacteria living in the Yodo River were conducted with measurements of AOC concentrations in order to examine the validity of the apparent AOC concentration as an indicator of regrowth potential of heterotrophic bacteria. Experimental results are summarized as follows.

- Organic compounds which were insensitive to E260 with a molecular size above 5,000 Da were contained with relatively high concentration in Yodo River, whereas they were not detectable as AOC.
- Apparent AOC concentrations in the raw water and the coagulated water were 80–600 and 90–200 μg ac-C eq/L, respectively. Maximum 75% of apparent AOC concentrations in the raw water were reduced by the coagulation. The variation of AOC also became smaller.

- Organic compounds which were detectable as AOC were not removed by the coagulation in this experiment. The reduction of the apparent AOC concentration by the coagulation was caused by not the removal of biodegradable organic carbon, but the decrease of phosphorus concentration.
- When the raw water and/or the coagulated water were ozonated, AOC concentration rapidly increased with the reduction of E260 and slowly increased with the depolymerization of high molecular organic compounds. However, 40–50% of AOC increasing with ozonation were reduced by the coagulation.
- It was suggested that the concentration of the available carbon for assimilation in this experiment would be underestimated when AOC concentrations were calculated as acetate carbon equivalent. However, apparent AOC concentrations could represent the regrowth potential of native bacteria living in the Yodo River at the range of low AOC concentrations even in phosphorus limitation.

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