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Effects of bacterial counts and temperature on the biodegradation of bisphenol A in river water

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Abstract

Total 15 surface river waters were collected from thirteen different rivers to investigate a relationship of bacterial counts and temperature to the degradation of bisphenol A (BPA). Autoclaved and non-autoclaved river water samples were spiked with 0.2 mg/l BPA. The spiked samples were placed at temperatures of 4, 20, and 30 °C and analyzed by high performance liquid chromatography. BPA was degraded at all temperatures in the non-autoclaved samples. However, BPA in the autoclaved samples was not changed at all temperatures for 20 d. These results show that the primary factor of BPA degradation in river water is bacteria. Moreover, three groups [group A (>10 000 CFU/ml), group B (2000–10 000 CFU/ml), and group C (<2000 CFU/ml)], were made on the basis of bacterial counts of the samples. Half-lives for BPA degradation in groups A, B, and C were 2, 3, and 6 d at 30 °C and were 4, 5, and 7 d at 20 °C, respectively. But at 4 °C, the loss of BPA was about 40%, 20%, and 10% in groups A, B, and C for 20 d, respectively. Bacterial counts exerted an influence on BPA degradation in river water with temperature. Our results also show that BPA-degrading bacteria are widely distributed in river waters.
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Keywords: Bisphenol A; Degradation; Aquatic environment; Half-life; HPLC

1. Introduction

Bisphenol A (BPA; 4,4′-isopropylidenediphenol; CAS Registry no. 80-05-7) has an estrogenic activity. Its estrogenic activity and binding affinity with the estrogen receptor in vitro are 1000–5000-fold lower than those of 17β-estradiol (Dodge et al., 1996; Steinmetz et al., 1997). BPA also has an acute toxicity in the range of about 1–10 mg/l for a number of freshwater and marine species (Alexander et al., 1988). Yokota et al. (2000) reported effects on growth in the medaka fish at 1.82 mg/l, but not at 0.355 mg/l in a 60 day chronic assay.

BPA, made by combining acetone and phenol, is used to make epoxy resins and polycarbonate plastics. Epoxy resins are used to coat can surfaces that are in contact with foods and beverages (Staples et al., 1998). BPA can be leached from the can coating when canned products are heated at a high temperature (Brotons et al., 1995). Leaching of BPA from polycarbonate baby bottle (Takao et al., 1999) and reusable container (Biles et al., 1997) has also been reported. Moreover, BPA was detected in plastic waste (Yamamoto and Yasuhara, 1999), in plasma stored in polycarbonate tube (Sajiki et al., 1999), in aquatic environment and air (Staples et al., 1998), and on land (Staples et al., 1998).

The water solubility of BPA ranges from 120 to 300 mg/l (Dorn et al., 1987; Staples et al., 1998). BPA can be contained in wastewater from its production factories because it is partially removed during wastewater treatment. The wastewater containing BPA can be the source of contamination in aquatic environment (Staples et al., 1998; Fürhacker et al., 2000). On the other hand, several

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bacteria distributed in river water can degrade BPA. Many studies reported that BPA had a short half-life and was rapidly biodegraded in river water (Dorn et al., 1987; Jin et al., 1996; Staples et al., 2000; Klecka et al., 2001; West et al., 2001). In addition, BPA is readily biodegraded in waste treatment using bacteria (Staples et al., 1998).

Despite several studies concerned with BPA biodegradation, there are few data on a relationship of bacterial counts and temperature to BPA degradation in river water. Manzano et al. (1999) found that temperature played an important role in the biodegradation of alkylphenol ethoxylates in river water. Jin et al. (1996) also reported that even if there were differences in the levels of BPA degradation, most bacteria (about 90%) isolated from river waters had BPA biodegradability. Therefore, temperature and bacterial counts can have an effect on BPA degradation.

The purpose of our study is to identify the effects of temperature and bacterial counts to BPA degradation.

2. Materials and methods

2.1. Sample collection

Surface water samples were taken from the Kiyotake River and its tributaries (the Mizunashi, the Kubo, the Tagami, and the Kumano River), the Yae River and its tributaries (the Yamauchi and the Kojo River), the Kaeda River and its tributary (the Fukata River), the Oyodo River and its tributary (the Aoyagi River), and the Kakihara River of the Miyazaki from September to November 2000 (Fig. 1). The sampling site in all tributaries was 50–100 m away from the main stream. The samples in the Kiyotake and the Kaeda River were collected from the upstream and downstream (about 2 km from the estuary). Water samples were gathered from the riverbank using a sampler with a glass bottle at the water depth of 0.5–1 m. After sampling, sample bottles were rinsed two or three times using the collected waters. About 4 l water samples were gathered in the rinsed bottles and stored at <4 °C (no freezing). Water samples for bacteriological examination were collected in sterile glass flasks, were kept at <4 °C (no freezing), and then were tested within 4 h.

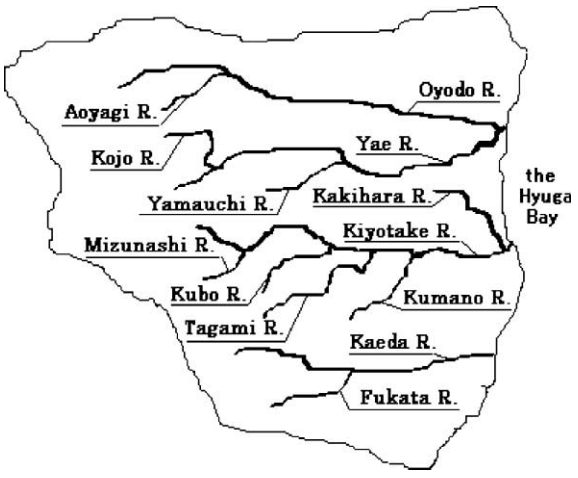

Fig. 1. The thirteen sampling rivers.

2.2. Bacteriological examination

The pour plate method was used for the bacteriological examination of the water samples in accordance with the Standard methods for the examination of water and wastewater (American Public Health Association, 1998). The plate count agar (Difco Laboratories, Detroit, MI, USA) containing tryptone (5.0 g), yeast extract (2.5 g), glucose (1.0 g), and agar (15.0 g) was prepared according to the recommendation of the manufactures. The samples were diluted with 0.1% peptone by 1:10 serial dilutions and added to each of the two replicate petri dishes. The prepared agar was poured to each of the petri dishes, mixed, and incubated at 35 °C for 48 h. The petri dishes having 30–300 colonies were counted and results were reported as the colony forming units (CFU) per milliliter.

2.3. Chemicals and reagents

All solvents used were analytical grade and were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Bisphenol A (99+%) was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Water was distilled and then purified by a Milli-Q water purification system (Nihon Millipore, Yonezawa, Japan). A stock solution of BPA (1 g/l) was prepared in acetonitrile.

2.4. HPLC analytical condition

The HPLC system was Waters 600E (Millipore Corporation, Milford, MA, USA). The fluorescence detection (Model F-1050, Hitachi, Japan) was used with excitation at 235 nm and emission at 310 nm. A column was symmetry C_{18} 3.5 μm (4.6 × 150 mm) (Millipore Corporation, Milford, MA, USA). The mobile phase consisted of acetonitrile–water (50:50, v/v) and pumped at a flow rate of 1 ml/min under isocratic conditions. The column temperature was 40 °C and the injection volume was 20 μl.

2.5. Analysis of water samples

The analysis of river water samples was performed in duplicate. A 2 l aliquot of each water sample was autoclaved at 121 °C for 15 min. A 40 mg/l BPA solution

was prepared by diluting a stock solution with water. A 1 ml aliquot of 40 mg/l BPA solution was diluted to 200 ml with autoclaved or non-autoclaved water sample, resulting in a 0.2 mg/l water sample of BPA. The spiked sample bottles were covered with the hydrophobic wool and placed in the dark at temperatures of 4, 20, and 30 °C. The wool and sample bottles were sterilized at 121 °C for 15 min before use. Exactly 10 ml samples were taken from each of the bottles and filtered through a filter of 0.45 μm pore size (Millipore Corporation, Bedford, MA, USA) (Dorn et al., 1987). A 20 μl volume of the sample was injected for HPLC analysis.

2.6. Calibration curve and recovery test

Calibration curves were constructed using at least five concentrations in the range of 0–0.5 mg/l for BPA. The curves were obtained from a linear regression program by concentrations versus detector responses. The correlation coefficients of peak height to concentration were better than 0.998. In addition, the retention times were 5.74 ± 0.45 min and 5.78 ± 0.38 min, respectively, when the BPA standard was consecutively measured for 0.05 and 0.1 mg/l ($n = 6$). For the recovery test of BPA, river water samples ($n = 5$) were spiked with 0.05 and 0.1 mg/l of BPA and analyzed as described above. The results of recovery were $92.5 \pm 9.7\%$ at the added amount of 0.05 mg/l and $98.5 \pm 3.5\%$ at 0.1 mg/l. The limit of detection for BPA analysis was 0.005 mg/l.

3. Results

The Oyodo River is the largest river running through the central part of the Miyazaki. The Yae, the Yamauchi, and the Aoyagi River go through a residential area and the others through an agricultural area. Moreover, the downstream of the Kiyotake and the Kaeda River is tidal environment. Bacterial counts in the water samples ranged from 9.0×10^2 to 1.3×10^5 CFU/ml. The samples taken from the rivers running through a residential area and the downstream of the Kaeda River showed high bacterial counts, compared with those of other rivers (Table 1). BPA was not detected (<0.005 mg/l) in all samples.

All samples were divided into two groups, autoclaved and non-autoclaved sample group, and spiked with BPA (0.2 mg/l). BPA biodegradation was tested at temperatures of 4, 20, and 30 °C. BPA in the non-autoclaved samples was degraded at all temperatures and was not detected (<0.005 mg/l) on the fifteenth day at 20 and 30 °C. About 20% of BPA was degraded at 4 °C for 20 d (Fig. 2(A)). However, BPA in the autoclaved water samples was not degraded at all temperatures for 20 d (Fig. 2(B)). These results mean that the primary factor

Table 1
Bacterial counts in river water samples used in this study

Classification	Ranges of bacterial counts	Sampling sites	Bacterial counts (CFU/ml)
Group A ($n = 5$)	>10 000	Yamauchi	6.4×10^4
		Kakihara	2.4×10^4
		Aoyagi	2.0×10^4
		Yae	1.3×10^5
		Kaeda downstream	4.6×10^4
Group B ($n = 7$)	2000–10 000	Fukata	4.3×10^3
		Oyodo	4.9×10^3
		Kumano	6.1×10^3
		Mizunashi	3.1×10^3
		Tagami	6.5×10^3
		Kubo	3.8×10^3
		Kiyotake downstream	5.3×10^3
Group C ($n = 3$)	<2000	Kojo	1.8×10^3
		Kaeda upstream	9.0×10^2
		Kiyotake upstream	1.7×10^3

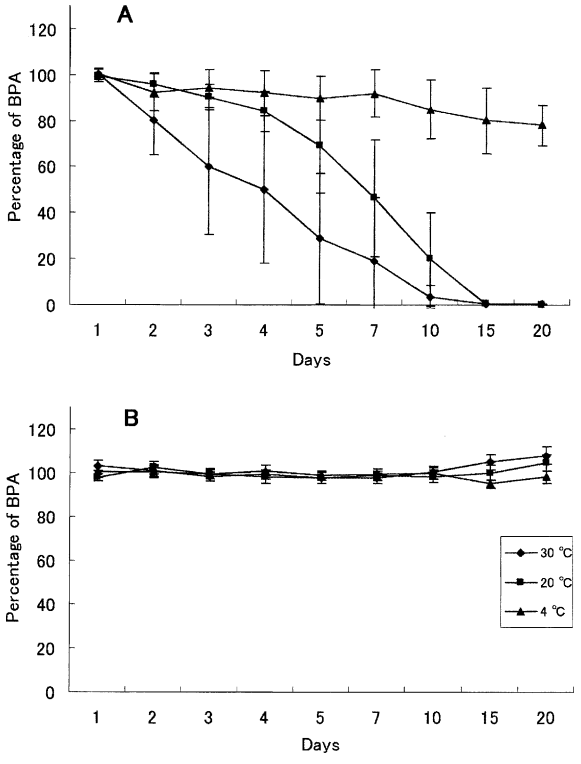

Fig. 2. BPA degradation in non-autoclaved water samples (A) and in autoclaved samples (B). The result is mean BPA degradation of 15 river water samples at temperatures of 4, 20, and 30 °C for 20 d.

of BPA degradation in river water is bacteria. On the other hand, the level of BPA in the autoclaved samples slightly increased at 20 and 30 °C for 20 d. This may be due to the evaporation of water in samples.

Group A (>10 000 CFU/ml), group B (2000–10 000 CFU/ml), and group C (<2000 CFU/ml) were made on the basis of bacterial counts of the samples to identify a relationship of temperature and bacterial counts to BPA degradation (Table 1). At 30 °C, half-lives for BPA in the groups A, B, and C were 2, 3, and 6 d, respectively, while BPA was below the detection limit (<0.005 mg/l) on the seventh day in groups A and B and on the fifteenth day in the group C. Half-lives for BPA were 4, 5, and 7 d at 20 °C in groups A, B, and C, respectively. At 20 °C, BPA in groups A, B, and C was also not detected at 10, 15, and 20 d, respectively. At 4 °C, the loss of BPA in groups A, B and C was about 40%, 20%, and 10% for 20 d, respectively. The more bacterial counts in same temperature increased, the more a rate of BPA degradation increased (Fig. 3). These data mean that bacterial counts have an important influence on BPA degradation in river water with temperature.

A relationship between BPA degradation and the change of bacterial counts in the water sample of the Oyodo River are presented in Fig. 4. Similar results were found in other river water samples (data not shown).

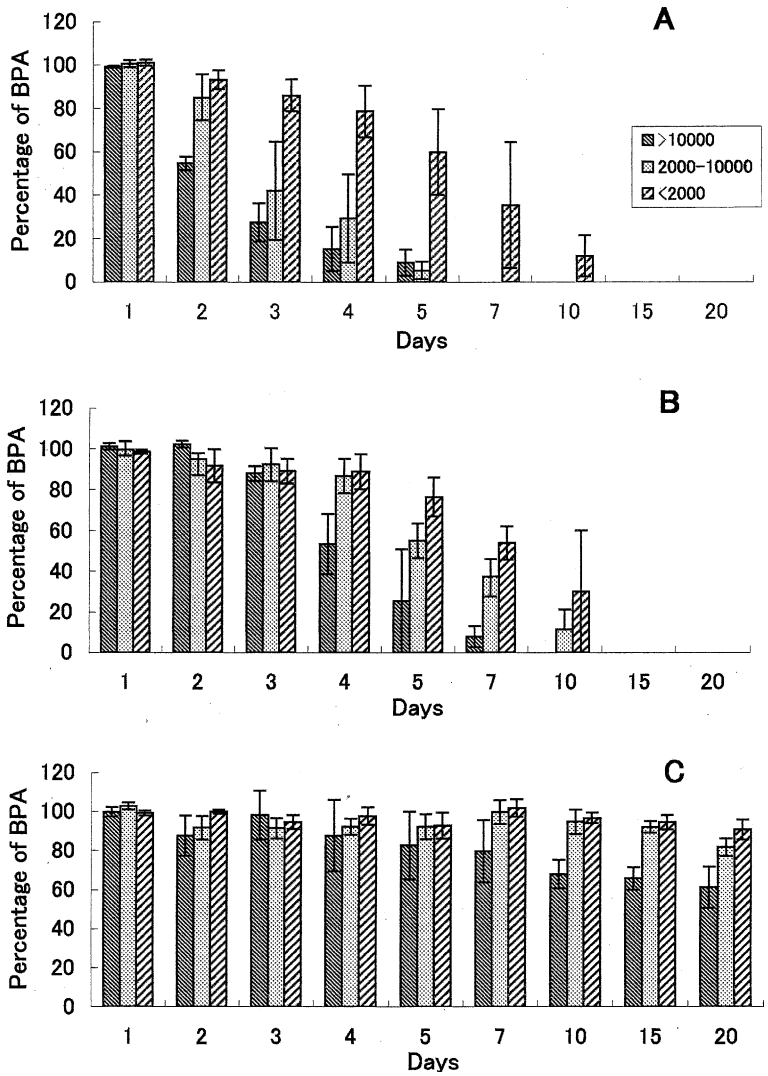

Fig. 3. A relationship of temperature and bacterial counts to BPA degradation. The samples spiked with BPA (0.2 μg/ml) were kept at temperatures of 30 (A), 20 (B), and 4 °C (C) for 20 d and analyzed by HPLC. Bacterial counts in river water samples were presented in Table 1.

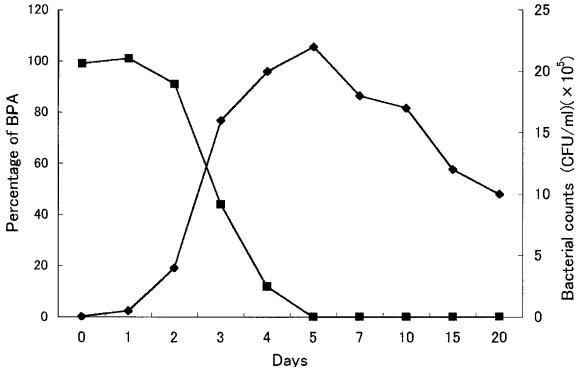

Fig. 4. Change of BPA and bacterial counts in the water sample of the Oyodo River at 30 °C for 20 d.

4. Discussion

Biotic and abiotic methods are used for treating wastewater containing BPA. The significant amount (>90%) of BPA is removed during wastewater treatment (Staples et al., 1998; Fürhacker et al., 2000). Moreover, BPA cannot be persisted and be rapidly degraded in river water. When river water samples spiked with BPA (3 mg/l) were held at 22–25 °C for 8 d, BPA was reduced below detection limit (0.1 mg/l) on the fifth day and half-lives ranged from 2.5 to 4 d (Dorn et al., 1987). Klecka et al. (2001) reported that biodegradation half-lives for BPA were less than 2 d following lag phases ranging from 2 to 4 d in seven different surface river waters. In out study, a decrease of BPA was identified in 15 surface river water samples taken from thirteen different rivers at all temperatures of 4, 20, and 30 °C. This result agrees with previous studies that BPA cannot persist in the aquatic environment (Dorn et al., 1987; Staples et al., 2000; Klecka et al., 2001; West et al., 2001). The fact that BPA biodegradation was found in all samples taken from 13 different rivers also suggests that BPA-degrading bacteria are widely distributed in river water. Similar consequences have been identified in previous studies (Dorn et al., 1987; Jin et al., 1996; Klecka et al., 2001). Jin et al. (1996) reported that about 90% bacteria (40 out of 44 bacteria) isolated from multiple sites of seven rivers could degrade BPA to a certain degree, but only six samples in 40 bacterial samples were able to do complete BPA degradation. Moreover, the levels of BPA in river water were 8 μg/l or less according to results from Japan (Matsumoto et al., 1977; Matsumoto, 1982), the Netherlands (Hendriks et al., 1994), German (Heemken et al., 2001), and the United States (Staples et al., 2000).

BPA degradation in aquatic environment is influenced by several factors. BPA is effectively removed from wastewater using electrochemical oxidation in a high sodium chloride and pH (Boscoletto et al., 1994). Sajiki and Yonekubo (2002) reported that BPA was easy to degrade in the presence of reactive oxygen species, superoxide anion and hydroxyl radical, and that lipid and/or sodium chloride could accelerate BPA degradation. In the present study, bacterial counts had an important effect on BPA degradation. A rate of BPA degradation in the group A with high bacterial counts (>10 000 CFU/ml) was faster than that in the group B (2000–10 000 CFU/ml) and C (<2000 CFU/ml). However, Klecka et al. (2001) reported that BPA degradation did not correlate with bacterial counts. These differences may be due to the size of bacterial population that can do fast and complete BPA degradation or mineralization. Bacteria capable of doing complete BPA degradation or mineralization were isolated from the contaminated river waters with high bacterial counts, but bacteria incapable of degrading BPA were found in the fresh river water with low bacteria counts (Jin et al., 1996).

Our study also showed that the higher temperature, the faster BPA degradation. Half-lives for BPA degradation in groups A, B, and C were 2, 3, and 6 d at 30 °C and were 4, 5, and 7 d at 20 °C, respectively. In previous studies, significant increases in the degradation rate of alkylphenol ethoxylates were identified by rises in temperature (Manzano et al., 1999; Staples et al., 1999).

The biodegradation routes of BPA have been identified in the studies using a gram-negative bacterium, strain MV1, isolated from a sludge enrichment of BPA-wastewater treatment plant. The strain MV1 grows on BPA as a sole source of carbon and energy. Two primary metabolites of BPA degradation by the strain MV1 are 4-hydroxybenzoic acid and 4-hydroxyacetophenone in the major pathway, and are 2,2-bis(4-hydroxyphenyl)-1-propanol and 2,3-bis(4-hydroxyphenyl)-1,2-propanediol in the minor pathway (Lobos et al., 1992; Spivack et al., 1994). In addition, manganese peroxidase and laccase that are lignin-degrading enzymes produced by white rot basidiomycetes fungi can degrade BPA and remove its estrogenic activity (Hirano et al., 2000; Tsutsumi et al., 2001).

BPA is released from production facilities at high enough temperatures to be in the vapor phase. In the vapor phase only, BPA is subject to rapid photo-oxidation, mediated by hydroxyl radicals. Upon condensing, BPA can fall out or rain out of the atmosphere (Matsumoto and Hanya, 1980).

In conclusion, the present study shows (1) that BPA cannot persist in river waters, (2) that BPA-degrading bacteria are widely distributed in river waters, (3) and that the biodegradation of BPA is influenced by temperature and bacterial counts.

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