Biodegradation of Bisphenol A Used in Plastic Industries

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A bacterium strain T-1 capable of biodegrading bisphenol A (BPA) was isolated. Although strain T-1 could completely remove BPA (120 $mg \cdot l^{-1}$) at the first-order rate constant of 0.2 h^{-1}, approximately 20 $mg \cdot l^{-1}$ of dissolved organic carbon (DOC) remained in the culture medium of strain T-1. High performance liquid chromatography analysis of the culture medium of strain T-1 during BPA-biodegradation showed that BPA-biodegradation by strain T-1 produced mainly 4-hydroxy benzoic acid (4HBA) and that 4HBA in the culture medium was equivalent to 60% of the remaining DOC through BPA-biodegradation. Investigation of 4HBA-biodegradability by strain T-1 indicated that BPA-biodegradation by strain T-1 produced inhibitory compound(s) to strain T-1 itself.

Introduction

BPA used in factories producing plastics and resins is suspected of mimicking estrogen in organisms such as fish and humans. Karishnan et al. (1993) showed that BPA, which was leached out of polycarbonate flasks during its autoclave treatment, could bind to estrogen receptors from rat uterus [1]. Their further experiments using human mammary cancer cells (MCF-7) confirmed that BPA induced progesterone receptors in the cultured MCF-7 cells and increased their proliferation rate, thereby indicating that BPA mimicked estrogen [1]. Due to the suspicion, discharge of BPA to the environment must be reduced to protect human and the environment. In order to eliminate BPA in effluent of factories producing plastics and resins, bacteria capable of biodegrading BPA could be used.

Several studies was carried out to obtain BPA-biodegrading bacteria and to investigate their BPA-biodegradation ability [2-6]. Although these studies indicate the feasibility of eliminating BPA in effluent of factories producing plastics and resins by using BPA-biodegrading bacteria, much reserch must be carried out to establish this as a safe and effective method. Essential tasks are (i) to obtain bacteria excellent at biodegrading BPA and (ii) to reveal how the bacteria biodegrade BPA, however there is only limited available information about BPA-biodegrading bacteria and their BPA-biodegradation pathway. Toward this end, we isolate a BPA-biodegrading bacterium and attempt to identify BPA-biodegraded products produced by the bacterium.

Material and Methods

Isolation of a BPA-biodegrading bacterium Hundreds of soil and sediment samples taken from farms and river in Japan were cultivated in the medium (MBPA) containing BPA as a sole source of carbon (BPA, 120 $mg \cdot l^{-1}$; K_2HPO_4, 1 $g \cdot l^{-1}$; $(NH_4)_2SO_4$, 1 $g \cdot l^{-1}$; NaCl, 0.1 $g \cdot l^{-1}$;

$MgSO_4 \cdot 7H_2O$, 0.2 $g \cdot l^{-1}$; $FeCl_3$, 0.02 $g \cdot l^{-1}$; $CaCl_2$, 0.1 $g \cdot l^{-1}$) to acclimatize bacteria in soils and sediments to BPA solution. After separation of the acclimatized bacteria using the agar-medium obtained by adding agar (15 $g \cdot l^{-1}$) to the MBPA, finally, a BPA-degrading bacterium named as strain T-1 was obtained. Cultivation of strain T-1 in the aqueous mediums was performed at 25℃ using a rotation of 100 rpm.

BPA-biodegradation The MBPA in which strain T-1 had been cultivated for 2 d was centrifuged (15000×g, 15 min). After discarding the supernatant, cells of strain T-1 were washed twice with phosphate buffer (pH7) and inoculated into the new MBPA. In order to measure the BPA concentration of the MBPA and search for BPA-biodegraded products, the MBPA during BPA-biodegradation was subjected to high performance liquid chromatography (HPLC).

4HBA-biodegradation As described latter, it was confirmed that BPA-biodegradation by strain T-1 mainly produced 4HBA. Since the simple structure like 4HBA allowed us to expect 4HBA to be easily biodegraded, whether or not strain T-1 could biodegrade 4HBA was confirmed. Briefly, after inoculating the phosphate buffer-washed cells of strain T-1 into the medium (M4HBA) obtained by substituting 4HBA for BPA of the MBPA, the 4HBA concentration of the M4HBA was measured.

4HBA-biodegradation inhibition The 4HBA-biodegradation inhibition experiment was carried out to confirm whether or not BPA-biodegradation by strain T-1 produced inhibitory compound(s) to strain T-1 itself by using the culture medium (MMIX) containing both 30 $mg \cdot l^{-1}$ of 4HBA and other BPA–biodegraded products. The MMIX was obtained by diluting the MBPA where strain T-1 had been cultivated for 50 h with the M4HBA containing 30 $mg \cdot l^{-1}$ of 4HBA. After filtrating the MMIX through an autoclaved menbrane filter (0.22 μm), 4HBA-biodegradation by strain T-1 in the MMIX was performed by the same manner of the 4HBA-biodegradation experiment.

Analysis The BPA concentration of the MBPA was measured by subjecting the MBPA to HPLC on an ODS column (ODS 80TS, 250×4.6 mm, TOSOH) at 40℃ using two eluents: that is, eluent A (0.025% H_3PO_4 in water) and eluent B (0.025% H_3PO_4 in methanol) using an elution profile 0−20 min 40−100% B at a constant flow rate of 1 $ml \cdot min^{-1}$, while an elution profile 0−10 min 15−30% B, 10−20 min 30−100% B was used to analyze BPA-biodegraded products. A 1049A Hewlett Packard electrochemical detector (amperometry, glassy carbon, 0.9 V (vs. Ag/AgCl)) was used as the detection device. Qualitative confirmation of the BPA-biodegraded products detected in the MBPA was performed by HPLC using a spike test in which the MBPA was spiked with commercially available standard compounds. The DOC concentration of the culture medium of strain T-1 was measured by a TOC analyzer (TOC-5000, Shimadzu).

Results and Discussions

BPA-biodegradation As shown in Fig. 1, strain T-1 completely removed BPA at the first-order rate constant of 0.2 h^{-1}. This BPA-biodegradation rate constant is almost same as those of strains MV-1 [3] and FJ-4 [4].

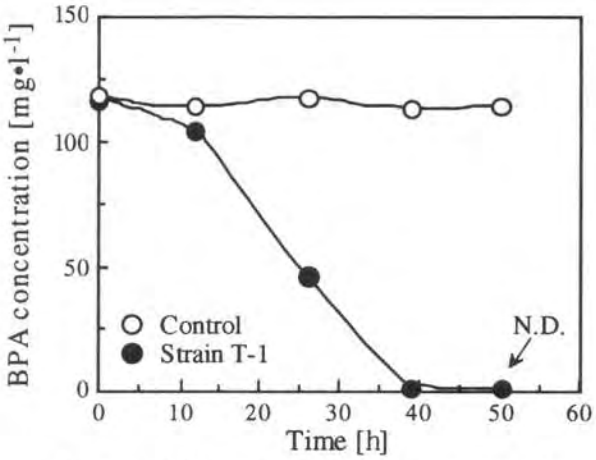

Fig. 1 BPA-biodegradation by strain T-1.

Figure 2(a) shows the resultant HPLC chromatogram of the MBPA in which strain T-1 was cultivated for 36 h. Comparing retention time of the occurred peaks with that of authentic standards, a peak at 10.0 min is considered to indicate 4HBA. Since the result of the spike test showed no change in the retention time of 4HBA (Fig. 2(a) and (b)), its presence was confirmed, thereby indicating that BPA-biodegradation by strain T-1 produced 4HBA.

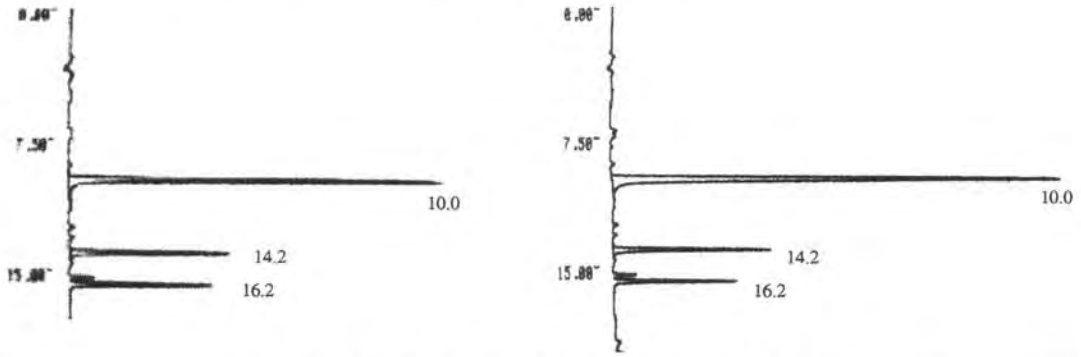

Fig. 2 Chromatograms of the MBPA; (a) not spiked, (b) spiked with a standard sample (4HBA).

Due to this result, we decided to analyze 4HBA together with DOC in the MBPA during BPA-biodegradation. As indicated in Fig. 3, the analysis showed that approximately 20 $mg \cdot l^{-1}$ of DOC remained in the MBPA and that 4HBA in the MPBA was equivalent to 60% of the remaining DOC through BPA biodegradation, thereby confirming that 4HBA was the main product of the remaining DOC in BPA-biodegradation by strain T-1. Note that strains MV-1 [3] and FJ-4 [4] could not completely mineralize BPA and that the reason why these strains could not mineralize BPA was not investigated.

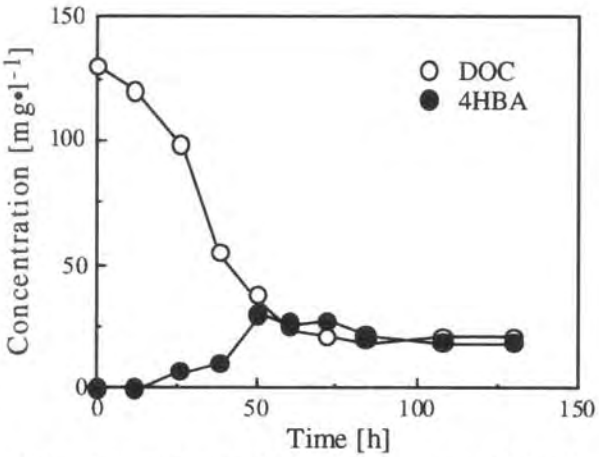

Fig. 3 Variation in the concentrations of DOC and 4HBA of the MBPA in which strain T-1 is cultivated.

4HBA biodegradation inhibition test After only 24 h of inoculating strain T-1 into the M4HBA, it was confirmed that 50 mg·l^{-1} of 4HBA completely vanished (data not shown). Although strain T-1 could biodegrade 4HBA, 4HBA was the main product of the remaining DOC in BPA-biodegradation by strain T-1 (Fig. 3). Accordingly, it was hypothesized that BPA biodegradation by strain T-1 produced inhibitory compound(s) to strain T-1 itself. In order to verify this hypothesis, we decided to investigate effects of the BPA-biodegraded products on 4HBA-biodegradation behavior of strain T-1.

Figure 4 shows variation in the 4HBA concentrations of the MMIX. As the concentration of the MBPA increases, the MBPA demonstrates some degree of inhibitory effects on 4HBA-biodegradation by strain T-1, which confirms that BPA-biodegradation by strain T-1 produces inhibitory compound(s) to strain T-1 itself. These results suggest that mineralization of BPA could be accomplished by using strain T-1 and other bacteria capable of biodegrading such inhibitory compound(s).

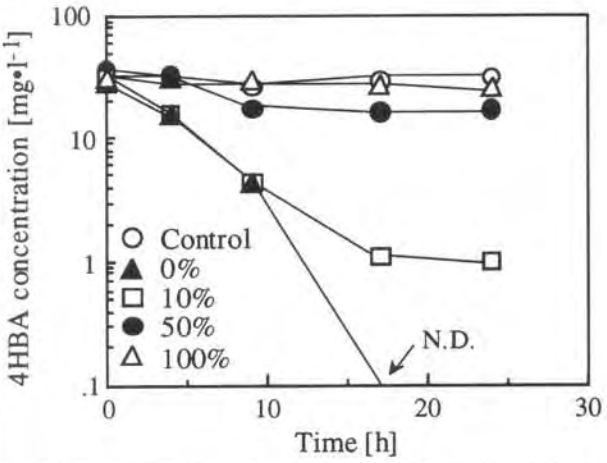

Fig. 4 4HBA-biodegradation by strain T-1 as affected by the MBPA in which strain T–1 was cultivated for 50 h.

Conclusions

The obtained BPA-biodegrading bacterium strain T-1 could completely remove BPA (120 mg·l^{-1}) from the MBPA at the first-order rate constant of 0.2 h^{-1}, while BPA-biodegradation by strain T-1 produced 4HBA. Although strain T-1 could easily biodegrade 4HBA, it was the main product of the remaining DOC in BPA-biodegradation by strain T-1.

These results provide the evidence showing that strain T-1 is capable of complete removal of BPA from wastewater discharged from factories producing plastics and resins, however the remaining DOC of BPA biodegradation still exists. Because BPA biodegradation by strain T-1 produces inhibitory compound(s) to strain T-1 itself, identification of such inhibitory compound(s) and isolation of bacteria strain capable of biodegrading them should be focused as future research needs.

References

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