First step toward biodegradation of dioxins by means of a reactor

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Introduction

Dioxins are some of the most toxic human-made organic chemicals and their density increases in the natural world by incineration of chlorinated carbons and other molecules (1). Some methods for degrading dioxins have been developed by fundamental works. Physical and chemical treatments, for example, which involve burning waste at high temperatures and incorporation by a metal catalyst (2), are very effective for high density and low volume dioxin-containing waste from industries. However, once spread dioxins cannot be burned effectively since they exist on the surface of soil or sediment under the water; processing, under such conditions, is not cost effective and burns fossil fuel. Further, these treatments are needed special incinerator or equipment. The microbial degradation method is appropriate for the treatment of low level environmental dioxins. It does not need special incinerator or a substantive amount of energy, because its reactions occur by means of enzymes in microorganisms.

Recently, some reports have appeared regarding the microbial degradation of dioxins. The research presented therein indicates that low-chlorinated dioxins are degraded by aerobic microorganisms, which take a period of a few days to accomplish degradation (3,4). In contrast, high-chlorinated dioxins are dechlorinated by anaerobic microorganisms, which take a period of a few months or more to complete the reaction (5, 6). For purposes of application of these microorganisms to the degradation of dioxins, the anaerobic reaction is too slow to use in the reactor system. Consequently, we did not use the high-chlorinated dioxin-degrading microorganisms. On the other hand, ultraviolet degradation offers well studied methods by which to degrade high-chlorinated dioxins to low-chlorinated dioxins (7). We decided to link ultraviolet degradation of high-chlorinated dioxins to microbial degradation of low-chlorinated dioxins.

We have been studying microbial degradation of dioxins throughout the past year. Our primary aim is to establish application methods for the degradation of dioxins in eluted water from fly ash in landfill sites. We are currently engaged in a search for low-chlorinated dioxin-degrading microorganisms for use with the reactor system. In these trials, we investigated two bacteria for degradation of dioxins. One strain is *Pseudomonas* sp., which degrades coplanar PCB (8), and the other is *Bacillus midousuji* SH2BJ2, which degrades polyethylene and can grow at temperatures over 62°C (9). Herein, we demonstrate the dioxin-degrading ability of these two bacteria and estimate the possibility of using these strains for application of the reactor system.

Materials and Methods

Chemicals

2,3-dichlorodibenzo-p-dioxin, 2,8- dichlorodibenzo-p-dioxin, 2,3,7-trichlorodibenzo-p-dioxin and 2,3,7,8- tetrachlorodibenzo-p-dioxin were purchased from AccuStandard Inc. Dibenzofuran was purchased from Wako Pure. Chem. Co. Ltd. Other chemicals used in this study were all reagent grade.

Cultivation and maintenance of strains.

Pseudomonas sp. SN4995: cultivation was done on PAS medium containing biphenyl 1 g, $(NH_4)_3SO_4$ 1 g, KH_2PO_4 0.2 g, K_2HPO_4 1.6 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, NaCl 0.1 g, $FeSO_4$ 0.01 g and $CaCl_2$ 0.015 g in 1 liter of deionized water, and pH was adjusted to 7.5 by using hydroxyl chloride or sodium hydroxide. Cultivation temperature was set at 30°C (8). For solid medium, 1.5% of purified agarose was added. Pre-cultivation of the reactor experiment was done on LB medium.

B. midousuji SH2BJ2: all cultivation was done on Trypticasesoy Broth and temperature was maintained at 65°C (9).

Laboratory scale reactor system

The laboratory scale reactor system, which has 15 L of working volume and contains the mobilizing strains in the reactor by means of a ceramic membrane, was used for the reactor experiment. The composition of the reactor system is shown in Fig 1. When *B. midousuji* SH2BJ2 was cultivated in the reactor, the pooling of effluent and drop-wise addition of fresh medium was stopped because Trypticasesoy Broth would otherwise not be cost effective for the application. In the case of *Pseudomonas* sp. SN4995, fresh culture was at a continuous flow and with pooling effluent, so we were able to estimate the continuous handling of this reactor.

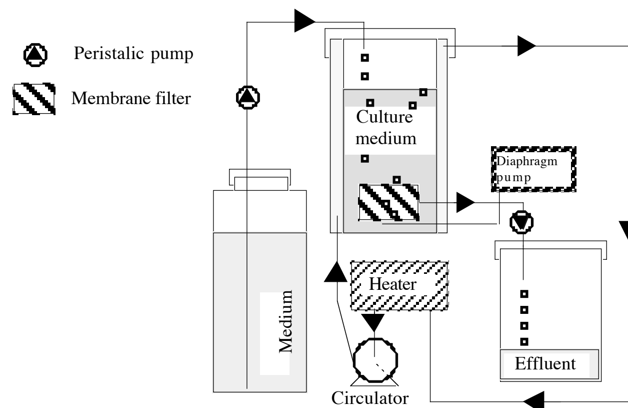

Fig.1. Schematic diagram of a laboratory scale reactor system used for degradation of dioxins.

Estimation of dioxin degradation by batch or reactor cultivation

On the batch reaction, each dioxin was toluene-diluted and added to screw-capped vial, then the toluene was evaporated via the cold-dry system. After capping, it was autoclaved for 10 min at 121°C, then 1 ml of each culture medium was applied under sterilized conditions. These strains were incubated for 4 days under aerobic conditions at the temperature most conducive to growth. Degradation rate was estimated from the ratio of dioxin contents in the inoculated medium per non-inoculated medium.

In the case of the reactor system, 1 ppm of solid dibenzofuran was added in the reactor and elution was stopped for 2 min to adsorb it well on the microbial cells, then incubation was conducted for 4 days. Degradation was estimated based on the ration of added amount of dibenzofuran to collected amount of it after 4 days incubation.

Preparation and analysis procedure

Samples from the batch culture: each dioxin was extracted by toluene in triplicate, then dried by using anhydrous sodium sulfate, after which the toluene solution was washed by hydroxyl sulfite until the yellow pigment in hydroxyl sulfite was eliminated. The cleaned-up toluene solution was rinsed against distilled water twice, then dried by using anhydrous sodium sulfate. Volume of the toluene solution was decreased to 100 μl under nitrogen gas flow.

Samples from the reactor: culture medium was centrifuged (10 min at 10,000 rpm) at 3 °C. The precipitate and the supernatant were separately extracted by toluene. The precipitate was washed by toluene then centrifuged (10 min, 10,000 rpm) at 3 °C. This procedure was repeated 10 times. Further treatment of the supernatant of the centrifuged culture medium and the toluene solution from which the precipitate was performed as described for the sample from batch culture. By these procedures, approximately 100% of spiked dioxins were recovered from the culture medium.

One μl out of 100 μl was measured by GC-MS apparatus.

Results and Discussion

Batch cultivation

We investigated for a low-chlorinated dioxin-degrading bacteria appropriate for use with the reactor system. *B. midousuji* SH2BJ2 was degraded 1 ppm of low-chlorinated dioxins (2,3-dichlorodibenzo-p-dioxin and 2,8-dichlorodibenzo-p-dioxin) and 0.01 ppm of 2,3,7-trichlorodibenzo-p-dioxin (Table 1). The degradation rates for these bacteria reached 60% to 90%. 0.01 ppm of the most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin, was degraded and its degradation rate reached to 12.5%. This degradation rate was increased when its concentration was decreased. Usually, degradation of dioxins by aerobic bacteria only occurred with non-, mono-, or di-chlorinated dioxins. To our knowledge, this is the first report of degradation of tri- or tetra-chlorinated dioxins by an aerobic bacterium.

Further, *Pseudomonas* sp. SN4995, which degraded 86% of coplanar PCB (4 ppm), degraded 60% of dibenzofuran (10 ppm).

Table 1. Degradation rate of dioxins by *B. midousuji* SH2BJ2

Dioxins	Degradation rate (%)

	1 ppm	0.01 ppm		
2,3-dichlorodibenzo-p-dioxin		95.5	-	
2,8-dichlorodibenzo-p-dioxin		87.5	-	
2,3,7-trichlorodibenzo-p-dioxin			31	60.4
2,3,7,8-tetrachlorodibenzo-p-dioxin		5.4	12.3	

-, not determined

Reactor experiment

To minimize the chemical hazards of dioxins, dibenzofuran was applied for the reactor experiment. As shown in table 2, after incubation for 4 days, *Pseudomonas* sp. SN4995 degraded about 96% of dibenzofuran. The elimination rate, on top of the degradation rate, reached 99%,.

B. midousuji SH2BJ2 was similar to *Pseudomonas* sp. SN4995 in that it could degrade almost 100% of dibenzofuran from the reactor. If this activity were to be maintained by using the minimum culture of this strain, it could be applied under conditions for exclusive elimination of the contaminate of bacteria, including high temperatures over 62°C.

These results are from a single run of fermentation; more rapid and continuous treatment will be expected for both strains.

Table 2. Mass balance of the degradation of dibenzofuran by two strains.

Pseudomonas sp. SN4995

Remaining rate of dibenzofuran in culture medium (%)	Remaining rate of dibenzofuran in eluted water (%)	Total degradation rate (%)
4.1	0.04	95.9

B. midousuji SH2BJ2

Remaining rate of dibenzofuran in culture medium (%)	Total degradation rate (%)
0	100

We have been studying another biodegradation method, which uses activated sludge. This method degrades high-chlorinated dioxins by application of bacteria alone. In this experiment, 15 ng / 15 *l* of a high-chlorinated dioxin mixture was degraded to about 96% when incubated under anaerobic conditions for 4 weeks. We are in the process of confirming this data. We will show some of the results on Dioxin 99.

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