A Biodegradation System Considered Localization of Polychlorinated Dioxins in Contaminated Soil and River Bottom Deposits

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Introduction

Polychlorinated dioxins are generated mainly by (1) the burning of wastes and (2) the manufacture of agricultural chemicals. While the amount of polychlorinated dioxins emitted has sharply decreased due to recent trends towards stricter controls, a large proportion of the dioxins emitted in the past has accumulated in the soil and bottom sediment in rivers, lakes, marshes, and harbors where it remains untreated. The physical/chemical degradation methods for removing polychlorinated dioxins usually require high temperatures and pressures (i.e., high energy input) and are inappropriate for the direct treatment of wet soil and sediment dredged from rivers, etc. This is why attention is being given to bioremediation using enzyme catalysis reactions that work at normal pressures and temperatures.

Among the various bacterial strains reportedly able to degrade polychlorinated dioxins, research is most advanced on *Sphingomonas* sp. RW1[1)]. However, the RW1 strain cannot degrade tri-chlorinated or more chlorinated dioxins[2), 3)]. While the *Dehalococcoides* sp. CBDB1 strain, reportedly able to degrade dioxins in reductive de-chlorination reactions, can grow only under strictly anaerobic conditions. In sum, much of the research on bioremediation cannot yet be put to practical use.

The SH2B-J2 strain was isolated by Dr. Hoshina et al. for use in composting fallen leaves[4)]. The strain, a gram positive bacterium belonging to the genus *Geobacillus*, is an aerobic thermophile whose optimal cultivation temperature is 65℃. The 48-hour co-cultivation of the strain with fly ash contaminated water (5,300 pg -TEQ/L) in a bubble tower reactor with a capacity of 0.01 m ^{3} degraded 70% of the dioxin TEQ. It was verified that tetra -chlorinated or more chlorinated dioxins in the fly ash were degraded and that no tetra -chlorinated dioxins were re-synthesized.

Method and Results

1. Dioxin degradation Potency of the SH2B-J2 strain and Breakdown Mechanism

An assay was developed using specially synthesized substrate which fluoresces only when the ether bond of dioxin is cleaved[5)]. When the SH2B-J2 strain was grown on this substrate for 18hours at 65℃, strong 450-nm fluorescence was observed indicating the initial phase of the dioxin cleavage reaction [6)]. This degradation activity shown by the strain was only observed in the cell membrane fraction [6)]. A large amount of the intermediary metabolite that showec fluorescence on the thin layer chromatography was collected and analyzed using GC/MS. As results, the degrading enzyme from the strain was discovered to be glutathion -s-transferase, able to detoxify any substance harmful to microorganisms by adding hydrophilic sulfur to it [6)]. The co-cultivation of 2,3,7,8-TCDD substituted with ^{14}C and the crude cellular membrane enzyme also led to the discovery of an intermediary dioxin metabolite in the water layer fraction, which attempts are now being made to identify [7)]. These results indicate that the strain can detoxify polychlorinated dioxins by producing a degradation enzyme that cleaves the ether bond of the dioxins.

On the other hand, the dioxins from contaminated water and soil differ in their uniformity. So we studied the localization of dioxins using dredged soil from a river in Osaka City. The granularity of the sediment was analyzed according to the test method established in JIS A 1204 -1999. This produced the granularity composition shown in Table 1.

Table 1 Granularity composition and dioxin TEQ in the dredged soil

Fraction	Range of mesh	Composition	Polychlorinated dioxins
Gravel	d ≧4.75 mm	5.4 %	9.9 pg-TEQ/g
Sand	75 mm < d ≦4.75 mm	47.6 %	46 pg-TEQ/g
Silt/Clay	d < 75 mm	47.0 %	710 pg-TEQ/g

This sediment of 1 kg was suspended in water and, using sieves with a 75 mm and ≦5 mm mesh, was separated into silt/clay fraction, sand fraction and gravel fraction. The sand/ gravel fractions were rinsed with running water to wash away the adhering silt. Through Soxhlet extraction, polychlorinated dioxins were extracted from each of the fractions and their concentrations determined. Assuming that the silt/clay and sand/gravel have the same concentration, the sum total of the dioxin TEQ for each fraction multiplied by its relative proportion was equal to 356 pg-TEQ/g. It was verified that this value is approximately equal to the dioxin of the original sediment, 410 pg-TEQ/g[7].

Only the silt/clay fraction, collected from the polychlorinated dioxin contaminated sediment and sterilized, was used for this degradation test. An Erlenmeyer flask of 1 L was filled with 200 mL of TSB+YE liquid medium and the sediment sample of 50 g was added. A freshly cultivated SH2B-J2 strain was inoculated and then co-cultivated for 72-hours at 65℃ while stirring at 150 rpm. Additional SH2B-J2 strain was inoculated every 24-hours. A control was prepared by adding a sediment sample to the liquid medium but without inoculation with the strain. After centrifugal separation for 15 minutes, the sediment sample of 4,000 g was collected for quantitative analysis of dioxins content. Table 2 shows the dioxin TEQ obtained as a result of this quantitative analysis[7].

Comparing the sample where the SH2B-J2 strain was inoculated with the sample where it was not, it was discovered that the dioxin contents were decreased by over 30%.

Table 2 Dioxins contents by each test sample

Test sample	Polychlorinated dioxins
Original silt/clay fraction in river bottom	730 pg-TEQ/g
Cultivation without SH2B-J2 strain	680 pg-TEQ/g
Co-cultivation with SH2B-J2 strain	450 pg-TEQ/g

2. Establishing technologies for planning and implementing a dioxin degradation System

This degradation system is designed for the on-site treatment of chlorinated dioxins as shown in Fig. 2. After water and soil contaminated with dioxins is pretreated while wet, the silt/clay that has been made into slurry will then be directly decontaminated. The granularity of the sand/gravel fractions, if any, will decrease the stirring force inside the bubble tower reactor. Since the reactions of the crude cellular membrane enzymes with the dioxins are dependent on the concentration of dioxins, they are first-order reaction[6]. A decrease in the stirring force inside the reactor results in a decrease on the reaction speed. This is why, before biodegradation, a pretreatment unit is used to reduce the volume of contaminated water and soil by separating it into sand/gravel and silt/clay components and, at the same time, concentrating the dioxins. This pretreatment unit, consisting of two parts, adds an appropriate amount of water to the contaminated soil and then washes it by milling and rubbing. The first part thus physically separates the silt/clay fraction, including organic matter deposited on the surface and in the gaps between sand grains, while the second part classifies the soil into sand/gravel and silt/clay components. The advantages of this pretreatment unit are that it (1) can wash the sand/gravel using only water without any added chemicals, (2) improves the efficiency of degradation by the enzymes by concentrating the dioxins and reducing the amount of subsequent biodegradation required, and (3) allows the silt/clay slurry to go directly into the reactor by adjusting the amount of water added and then recycling it.

The reactor is a batch-operation system equipped for two functions: heating the contaminated water or silt/clay slurry mixed with SH2B-J2 strain at the 65 ℃ treatment target for co-cultivation, and then cooling vapor from the culture medium. This system, comprising multiple reactors connected in series and using the batch culture process, constitutes a semi-automatic treatment unit. This enzymatic dioxin degradation process also provides a means for preventing secondary contamination by dioxins entering the atmosphere because almost all the treatment process can be done in a closed system and under water. The effluent from the pretreatment unit will be reused in the reactor and the final liquid waste will be properly disposed of after being checked for dioxin contents.

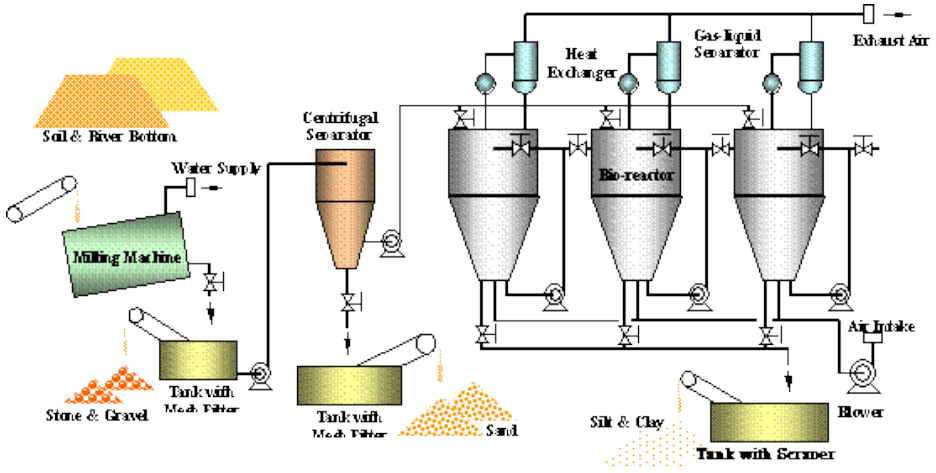

Fig. 2 Schematic Diagram of Biodegradation system using SH2B-J2 strain

Results and Discussion

The SH2B -J2 strain is a microorganism that directly breaks down polychlorinated dioxins by cleaving the ether bond, the backbone of the dioxin structure. The enzyme produced by the strain is glutathion-s-transferase. If the intermediary metabolite can be identified, then the description given for the metabolic pathway of dioxin degradation by the strain will be complete. We propose that the reactor be used as part of a biodegradation system based on the unique enzymatic activity of the strain and able to be applied both to water and soil contaminated with polychlorinated dioxins. For contaminated soil, in particular, we have demonstrated the necessity of a pretreatment unit to improve reaction efficiency, reducing the amount of treatment required by targeting the soil fractions where most dioxins are localized. In the future, we will verify the effectiveness of the crude cellular membrane enzymes of the strain in the biodegradation of soil contaminated by polychlorinated dioxins and determine the technological limits of this treatment system.

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