DEGRADATION CONDITIONS OF POLY CHLORINATED-P-DIOXINS AND FURANS IN DIFFERENT CONTAMINATED SOILS FOR BIOREACTOR SYSTEM

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Introduction

In Japan, soil contamination caused by dioxins in and around sites of incinerators for municipal solid waste (MSW) a concerned. For example, scattering wastewater from a wet gas scrubber at an MSW incinerator facility in Nose, Osaka, caused soil and surface water contamination. The concentration of dioxins in the soil was about 8,000 pg-TEQ/g. Other possible contamination sites can include soils on which fly ash has been improperly stored, or even placed directly, and landfill sites which have been received bottom and fly ash over a long period. Some countermeasures should be carried out immediately at these dioxin-contaminated sites.

We have developed bioreactor systems for dioxin-contaminated water and soil[1-3] because biological methods are inexpensive and have little potential to produce toxic by-products. We showed that a fungus, *Acremonium* sp., isolated from activated sludge treating wastewater that contained dioxins, would degrade highly chlorinated dioxins[2]. A reaction product of octachlorinated dibenzo-p-dioxin (OCDD) was identified as heptachlorinated dibenzo-p-dioxin. Therefore, one of the pathways for degradation of OCDD by this fungus was predicted to be as follows: OCDD would be transformed by dechlorination and then one of the remaining aromatic rings would be oxidized.

Nakamiya et al.[3] showed that dioxins in contaminated soil sampled from Nose could be effectively degraded by this fungus. When water content was 70 % and the temperature was 35 °C, the degradation ratio was nearly 80%. However, the nutrient concentration in the medium was very rich. When one is developing a practical reactor system, the amount of additives, such as carbon and minerals, should be determined by considering the characteristics of the contaminated soils. This is because these factors affect the operating cost and the final quality of the treated soils. Moreover, biodegradation tests should be carried out using lager reactors.

On the other hand, certain constituents of fly ash may inhibit *Acremonium* sp. from degrading dioxins in a soil mixed with fly ash. If the inhibition is actually observed, the inhibitors should be identified and removed before treating the soils.

In this study, some experiments were carried out on two dioxin-contaminated soils. One was the soil sampled from Nose (called soil A), and the other was a soil mixed with fly ash (called soil B), which was prepared in our laboratory. The objectives of this study were to investigate those additives required for degradation of dioxins in soil A, and to identify inhibitors in soil B. An effect on the degradation ratio of dioxins in soil B was observed after removal of inhibitors. In addition, a degradation test using a bioreactor was carried out.

Materials and Methods

Contaminated soils

Soil A was sampled from Nose, Osaka, Japan. The concentration of dioxins was 4,000–8,000 pg-TEQ/g. The ignition loss of soil A (600 °C, two hours) was 20 %. Before the beginning of the experiment, soil A was dried at 100 °C for two hours and passed through a 2-mm mesh sieve. Soil B was prepared in our laboratory by mixing uncontaminated soil and fly ash. The uncontaminated soil was sampled from surface soil within the Hokkaido University grounds, and was dried and sieved as soil A. The fly ash was sampled from an electric precipitator of a mechanical stoker-type incinerator. The concentration of dioxins in the fly ash was nearly 3,000 pg-TEQ/g.

Media

The medium contained glucose, 1.0g; lignin, 0.1 g; $(NH_4)_2SO_4$, 0.2 g; NaCl, 0.2 g; K_2HPO_4, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $CaCO_3$, 0.2 g and 0.1 mL of a trace element solution ($FeSO_4 \cdot 7H_2O$, 0.01 g; $MnCl_2 \cdot 4H_2O$, 0.01 g and $ZnSO_4 \cdot 7H_2O$, 0.01 g per 10 mL of distilled water) per 100 mL of distilled water. In experiments to identify inhibitors of dioxin degradation by *Acremonium* sp., NaCl, $CaCl_2$, KCl, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $PbNO_3$ and $CdNO_3 \cdot 4H_2O$ were added to this medium, respectively.

Biodegradation tests with flasks

The soil (6 g) and the media (14 mL) were placed in a 50-mL Erlenmeyer flask equipped with a baffle. The flask was shaken with a rotator (RT-50, Taitec Inc.) at 45 rpm for 48 hours. The temperature was 30 °C.

Biodegradation tests with a reactor

In this study, a cylindrical reactor (5 L) made of stainless steel, as shown in Figure 1, was used. A mixture of soil A and water (70 % of the total mass) was added and agitated with an angled turbine at 200 rpm. The position of the turbine and the number of rotations was determined by preliminary experiments with another reactor made of PVC and using a similar mixture. The reactor was controlled at 30 °C using a water jacket.

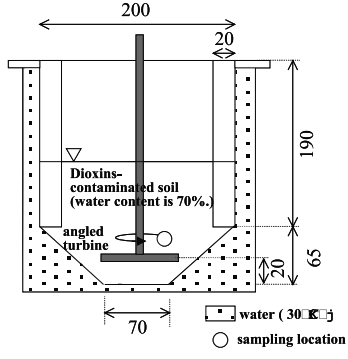

Figure 1. Schematic diagram of a bioreactor (unit: mm).

Dioxins analysis

After biodegradation tests, the culture medium, including soil, was treated with acid to extract dioxins from the cells, and solid and liquid phases were separated with a suction funnel. The solid phase was then freeze-dried. Dioxins in the dried solid phase were extracted with toluene in a Soxleht extractor, and those in the liquid phase were extracted with toluene three times. Both the toluene phases were mixed and applied to a multi-layer silica gel column which was filled from the bottom to top, in order, with 0.5 g of silica gel, 3.0 g of 2 % potassium hydroxide-impregnated silica gel, 0.5 g of silica gel, 4.5 g of 44 % sulfuric acid-impregnated silica gel, 6.0 g of 22 % sulfuric acid-impregnated silica

gel, 0.5 g of silica gel, 3.0 g of 10 % silver nitrate-impregnated silica gel, 0.5 g of silica gel and 3.0 g of sodium sulfate. The dioxins in the column were eluted with 150 mL of hexane, evaporated, and dissolved in 0.1 mL of toluene. Analysis of dioxins was carried out with a GC-MS/MS apparatus (ThermoQuest GCQ plus ion trap mass spectrometer and TRACE GC 2000 gas chromatograph) in accordance with the method of Kemmochi and Arikawa[4]. In this study, the degradation of dioxins was estimated from the decrease in the toxic equivalent quantity (TEQ) based on peaks identified from the GC-MS/MS apparatus.

Chemicals

For calibration and clean-up, a PCDD/PCDF standard mixture, EDF-4931 (CIL, Inc.), and isotopically-labeled chlorodioxin standard ED-900 (Wellington Lab.) were used, respectively. The other chemicals were all laboratory grade.

Results and Discussion

Constituents required in the medium for degradation of dioxins in soil A

This study focused on glucose as the carbon source and carboxymethyl cellulose (CMC) as the dispersant to prevent *Acremonium* sp. from turning into a solid mass. These additives are very expensive. Therefore, biodegradation tests were carried out while changing the amount of glucose and CMC in the medium. Our observations revealed that CMC is not required for biodegradation because *Acremonium* sp. did not turn into a solid mass in the flasks. The soil particles apparently play the role of dispersant.

Figure 2 shows the effect of the amount of glucose on the degradation ratio of dioxins. This result indicated that the amount of glucose in the medium controlled the degradation ratio of dioxins. However, it should be noted that the difference between the degradation ratio with and without glucose decreased with increase in the cultivation time. Therefore, it is possible that *Acremonium* sp. can degrade dioxins in soil A without the addition of glucose to the medium.

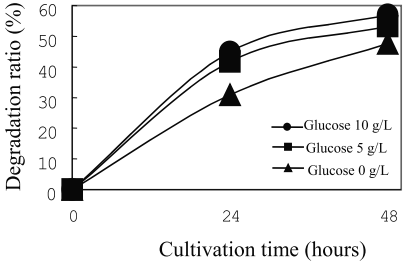

Figure 2. Effect of the amount of glucose on the degradation ratio.

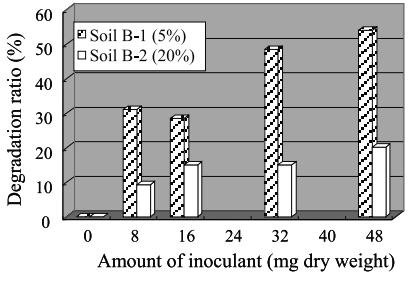

Figure 3. Degradation ratio of dioxins in soil B.

Effects of inhibitors in fly ash on the degradation of dioxins in soil B

Preliminary experiments were carried out to ascertain whether the dioxin-degrading ability of *Acremonium* sp. is inhibited by constituents of fly ash and, if so, what causes inhibition. The growth rate and lignin decolorization rate of *Acremonium* sp. were observed in media that included extracts from fly ash. We had previously used the lignin decolorization rate as an indicator of the degradation of dioxins[2]. As a result, we observed an inhibition in the growth rate, which caused a decrease in the lignin decolorization rate. Since fly ash generally includes a large amount of heavy metals (Pb, Zn, Cu and Cd) and salts (NaCl, KCl and $CaCl_2$), the effects of added Pb, Zn, Cu, Cd and salts were investigated. Observations showed that salinity is one of inhibitors. When the concentration of chloride ion in the medium was over 20 g/L, notable inhibition was observed.

Biodegradation tests with soil B were carried out to confirm whether the inhibition occurs in actual soils. The proportions of fly ash to soil were 5 % (soil B-1) and 20 % (soil B-2). It was predicted that soil B-1 would not inhibit the dioxin-degrading ability of *Acremonium* sp., but that soil B-2 would. In practice, biodegradation tests for soils B-1 and B-2 were carried out by changing the amount of inoculant (0 to 48 mg dry weight). Figure 3 shows that soil B-2 inhibited the dioxin-degrading ability of *Acremonium* sp., and the degradation ratio was reduced to 20 % because of the larger amount of inhibitors. The result suggests that the inhibitors in fly ash should be removed from actual contaminated soils in which the proportion of fly ash to soil is large.

Removal of the inhibitors in fly ash may be accomplished by washing. Both soils B-1 and B-2 were washed with distilled water as a pretreatment. A 50-mL Erlenmeyer flask containing 6 g of either soil B-1 or soil B-2, and 14 mL of distilled water was shaken for one hour. After the water phase was replaced with the new medium, biodegradation tests were carried out, similar to those described above. Figure 4 shows that the pretreatment caused an increase of the degradation ratio, compared to results shown in Figure 3. This result suggests that dioxins in soils mixed with fly ash can be degraded by *Acremonium* sp., but that pretreatment, such as washing, is necessary when the proportion of fly ash to soil is large.

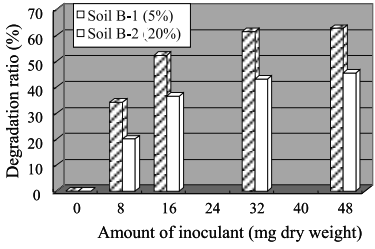

Figure 4. Effect of pretreatment for soil B on degradation ratio of dioxins.

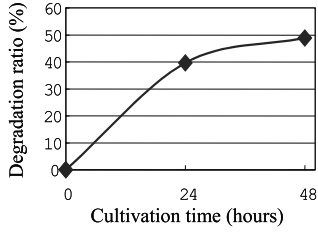

Figure 5. Result of bioreactor tests.

Biodegradation tests with the reactor

Next, 1 kg of soil A and 2.33 L of the medium that included glucose 1.0 g/L and no CMC were mixed and were agitated in the reactor shown in Figure 1 at 30 °C for 48 hours. Two samples were taken from the reactor after the agitation was temporarily stopped at 24 and 48 hours. The sampling location is shown in Figure 1. Figure 5 shows that the degradation ratio was about 50% when the reactor was used. This result suggests that we can obtain the same degradation ratio as when 50-mL flasks are used. We are now carrying out additional experiments to confirm the effects that the amount of glucose addition has on the degradation ratio.

Acknowledgments

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