BIODEGRADATION OF AN ACTUAL DIOXIN-CONTAMINATED SOIL BY *ACREMONIUM* SP.

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Introduction

Dioxins are some of the most harmful man-made chemicals. In Japan, there are many dioxin-contaminated sites around incineration facilities, with the degree of contamination reaching over 8,000 pg/g of soil. Contaminated soil has been collected and stocked in special holding strage where it will be held until the toxicity has decreased to below the environmental guide line or it can be treated by remediation methods.

In general, dioxins are present in the soil at low concentrations and are widely distributed over the land surface. These low concentrations in the soil, however, translate into high concentrations in the human body as the dioxins pass through the food chain.

Therefore, effective methods for the treatment of soils contaminated by dioxins are necessary. Physico-chemical treatment methods using large amounts of energy and requiring the construction of special facilities have been tested, but as of yet there is no quick, effective method that can be applied to the remediation of dioxin-contaminated soil. Physico-chemical methods are expensive and time-consuming when used for soils with only low to moderate levels of contamination.

Biological methods, for example the use of microorganisms for the degradation of dioxins, can be particularly appropriate for remediation because they do not require special facilities and they can treat large amounts of contaminated soil on site. These methods, however, are comparatively slow when applied on a large scale.

Last year, at this Congress, we reported the isolation of a fungus *Acremonium* sp. from dioxin-contaminated activated sludge that would degrade highly chlorinated dioxins (1). Under aerobic conditions this fungus can over 90% degrade chlorinated dioxins within 1-day. The degradation percentage did not vary with the degree of chlorination of each dibenzo-*p*-dioxin and dibenzofuran. The degradation products from octachlorinated dibenzo-*p*-dioxin (OCDD) were identified as heptachlorinated dibenzo-*p*-dioxin and 1-, or 2-hydroxy dibenzo-*p*-dioxins. It seems that OCDD is degraded by a de-chlorination reaction and then the aromatic rings are oxidized. The temporary accumulation of tetrachlorinated dibenzo-*p*-dioxins, which was reported for dioxin degradation under anaerobic conditions, was not observed during this reaction. The toxicity of the dioxins was rapidly reduced by the action of *Acremonium* sp. This observation indicated that *Acremonium* sp. can possibly be used for bioremediation of dioxin-contaminated soil.

We conducted two experiments; degradation under rotary cultivation (the minimum scale for a bioreactor) and a static culture degradation test (to simulate *in situ* treatment). The dioxin-contaminated soil was added directly to the medium and acted upon by *Acremonium* sp. in the medium. In this report we discuss the use of *Acremonium* sp. for the degradation of dioxins in an actual sample of contaminated soil.

Materials and Methods

Soil The soil used in the study was an actual contaminated soil taken from the area surrounding the incineration plant in Nose City, Osaka, Japan. It was found to contain approximately 10,000 pg-TEQ/g (total dioxin concentration in soil approximately 800,000 pg/g) based on data obtained in our laboratory. The soil was found to contain 20% organic matter based on the loss of weight resulting from heating at 600°C. Before use in the experiment, the soil was dried and passed through a 2-mm mesh sieve. Pre-treated soil was stored in a desiccator until use.

Rotary cultivation system A schematic representation of the reactor system is shown in Fig. 1. Batches of six 50 ml erlenmeyer flasks were placed in a baffle, sited on a callus cultivation apparatus and agitated in an incubator. For the static cultivation the flasks were placed in the incubator without agitation.

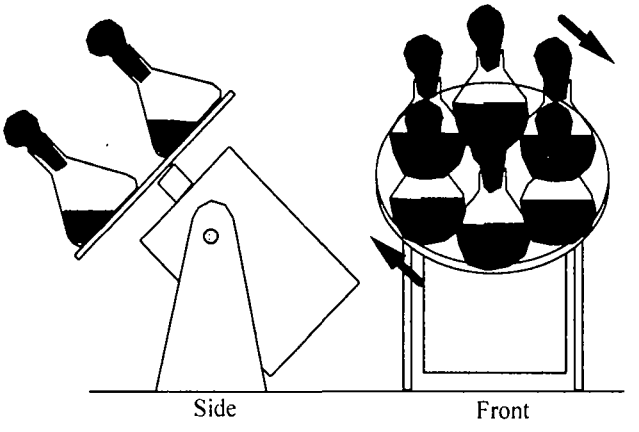

Figure 1. Schematic diagram of the rotary cultivation system used for the degradation of dioxin.

Cultivation conditions The medium for use in the degradation tests contained glucose, 1.0 g; 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; $ZnSO_4 \cdot 7H_2O$, 0.01 g per 10 ml of distilled water) per 100 ml of distilled water. The pH was adjusted to 7.0 with hydrochloric acid. To make the solid medium, purified agar 1.5% was added to the above medium. The cultivation temperature was kept at 35°C and conditions were kept aerobic by use of a cotton plug. A portion of culture (20 g) including soil was placed in each flask.

Preparation and analysis procedure After cultivation, the culture medium, including the soil, was freeze dried, and then extracted with toluene in a soxhlet extractor. The toluene (50 ml) was then dried over anhydrous sodium sulfate, washed with sulfuric acid and subjected to silica gel (3 g) column chromatography. The dioxins were eluted with 150 ml of hexane, and the volume was decreased to 100 µl.

One microliter out of 100 µl was analysed by a GC-MS apparatus (ThermoQuest GCQ plus equipped with a TRACE GC 2000) set to GC-MS/MS mode (2). As a result of these procedures, approximately 100% of spiked dioxins were recovered from the culture medium. Degradation of the dioxins was estimated from the decrease in the GC-MS/MS peak areas for the tetra- to octachlorinated dibenzo-*p*-dioxins and dibenzofurans.

Chemicals PCDD/PCDF STANDARD MIXTURE EDF-4931 (Cambridge Isotope Laboratories

Inc.) was used for the quantification of the dioxins. The other chemicals used in this experiment were all laboratory grade.

Results and Discussion

Rotary cultivation Under sterilized conditions, the effect of soil on the degradation of dioxins was estimated. The ratio of soil to medium was varied between 40 and 90 % (w/v). The weight of inoculum was fixed at 8 mg (dry weight). The mixture was agitated at 54 rpm for 36 hr. When the soil content in the medium was increased, the extent of dioxin degradation was found to decrease. We were not able to select a soil-to-medium ratio, based on degradation efficiency, but with soil at 70 % of the medium content (resulting in 45% degradation) the mixture was found to agitate well (low viscosity) and we selected these conditions for the next test.

The optimum agitation speed and size of inoculation were estimated. The degradation efficiency remained constant up to approximately 30 rpm and then decreased. A degradation of 65% was achieved in relation to the total dioxins. The agitation speed was fixed at 30 rpm, and the initial inoculation amount was varied betweem 5 mg and 40 mg. Finally, 85% of total dioxins were degraded when 35 mg of inoculum was used (Fig 2). However, cultivation under non-sterilized conditions resulted in about 20% less degradation than under sterilized conditions. Wild strains of fungus were not observed by light microscopy. It seems that this decrease was not caused by contamination with wild strains of fungus. Possibly it was caused by the solubilization of dioxins in soil particles by the effect of autoclaving.

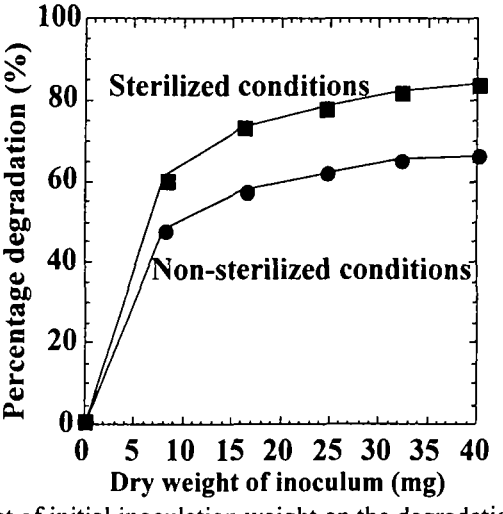

Figure 2. Effect of initial inoculation weight on the degradation of dioxins

Static cultivation Under sterilized conditions, the effect of the amount of soil on the degradation of the dioxins was estimated. The ratio of soil to medium was varied between 10 and 80 % (w/v). The weight of inoculum was fixed 8 mg (dry weight). The mixtures of soil and medium were incubated for 14 days at 35°C. When the soil content was increased the extent of degradation of the dioxins was found to decrease. However, with a water content of over 40 %, the degradation percentage was 30 %. We selected a soil to medium ratio based on a degradation efficiency of 40 %

The weight of inoculum was estimated. The initial inoculation weight was varied between 5 mg and 40 mg (dry weight). Finally, under sterilized conditions, when 35 mg of *Acremonium* sp. was

inoculated in the medium, 45% of the total dioxins were degraded (Fig 3). However, under non-sterilized conditions, with less than 30 mg of inoculum, the percentage degradation was decreased. Under the light microscope many wild strains of fungus other than the culture medium were observed. When the weight inoculated was inadequate *Acremonium* sp. may be overrun by wild strains.

We thought 45 % degradation was not enough. We then tried fed-batch cultivation. Thirty five mg (dry weight) of *Acremonium* sp. was added every 6-days to the culture medium. After the first inoculation, the percentage of dioxin degradation was increased and reached 75%. A second inoculation was not effectivein dioxin degradation in this experiment. However, using Fed-batch cultivation, dioxin degradation was promoted as is shown in Figure 3.

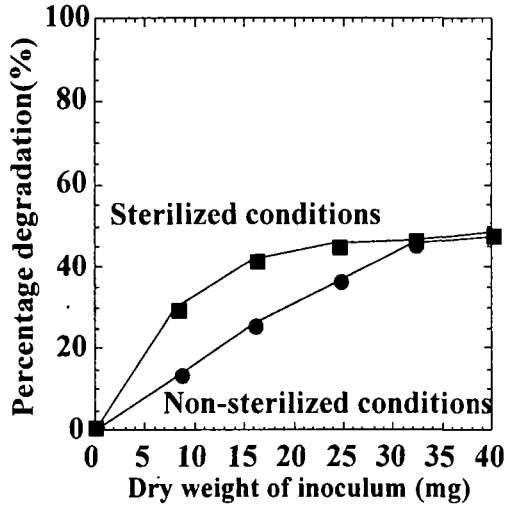

Figure 3. Effect of initial inoculation weight on the degradation of dioxins.

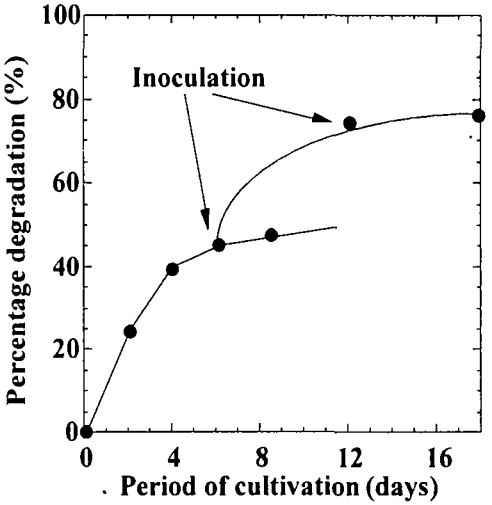

Figure 4. Effect of fed-batch cultivation on the degradation of dioxins.

These data indicated that *Acremonium* sp. can remove about 80 % of dioxins from actual dioxin-contaminated soil both with and without agitation. After treatment with *Acremonium,* about 20 % of the dioxins were not removed from the contaminated soil. It is possible that this resulted from adsorption of the dioxins on soil particles which hindered the approach *Acremonium* sp. and its enzyme systems,

Acknowledgements

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