A STUDY FOR MICROBIAL TREATMENT OF DIOXINS-POLLUTED SOILS BY THE HALF-ROTATION-TYPE CULTURE APPARATUS

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Introduction

The dioxins-contaminated soils around the solid waste incinerator institutes are one of the serious environmental pollutants in Japan. For example in Nose city, Osaka, Japan, the contaminated soils with high concentration dioxins had been gathered in a building and has not been treated by any treatment procedures, yet. We should study on the technology of reduction and remediation of it.

Recently, we certified the effect of bacteria and fungus on the degradation of dioxins. The bacteria and fungus used in these studies are isolated from ordinary compost, soils and waste water, and these strains are well working for the degradation of every isomer of dioxins, even on the highly chlorinated dioxins include the most toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Therefore, these bacteria and the fungus in the environment has the big potential for the degradation of dioxins.

In this study, we used the mixed culture of bacteria and fungus from the compost and it was applied for any actual dioxins-contaminated soils in the half-rotation-type culture apparatus. And we estimated the effectiveness of degradation on the dioxins by it. Before treatment of the dioxins, isolated strains of bacteria and fungus were temporarily identified.

Materials and methods

(1) Origin of microorganisms

The mixture of microorganisms was accumulatively cultivated from the compost for the treatment of feces from the domestic animals. The strains were named MIX-SF1.

(2) Identification of MIX-SF1

Habitants in the accumulated strains were identified by the homology of their l6S rDNA and the bio-chemical and morphological specificity.

(3) Dioxin-polluted soil

The dioxin-polluted soil is any actual dioxin-contaminated soil. It has been stored as the rest after environmental level analysis in our laboratory.

(4) Dioxin treatment experiment
Using the half-rotation-type culture apparatus in this experiment, we treated the experiment soil with a dioxin-contaminated soil of 960g, 600g water, 400g brewer's grain in mixed bean curd lees out and 240g MIX-*SF1* solutions. The cultivation was carried out with gentle stirring once a day for one time for 120 days at 25 degrees C.

(5) Extraction and analysis of dioxins and Co-PCB
We used the modified standard method of the Japanese Environmental Agency to an analyze dioxins in polluted soils.

After cultivation, from 5 g of cultured soil, the dioxins were extracted to 20 ml of ethyl acetate as shown below. The cultured soil was shaken vigorously for 10 min with a reciprocating shaker. Standing for several minutes separated ethyl acetate phase and solid phase, and Na2SO4 dried the ethyl acetate phase. The extracted dioxins in ethyl acetate phase were diluted and analyzed by gas chromatography-mass spectrometry (selected SIM mode) with JMS-SX102A and a fused silica SP-2331 capillary column (60 m by 0.22 mm i.d.).

The operating parameters for the GC were as follows: injector, 270 ; carrier gas, He; carrier flow, 0.8ml/min; injection method, split less. The oven temperature was initially maintained at 100º for 1 min and then increased to 250 º at 8 º /min and finally to 290 º at 4 º /min and maintained at 290 º for 5 min.

Result
(1) Identification of the bacteria and fungi in MIX-SF1
From MIX-SF1, several bacteria and fungi were isolated and it morphological characters were, for example, rods-shaped aerobic gram-negative, gram-positive rods and a fungus were fungal thread. In these isolated strains, two microbes, named MIX-SF1-2 and MIX-SF1-2 were further identified from their 16S rDNA homology on database as *Achromobacte*r sp. and *Bacillus* sp., respectively. An isolated fungus was not able to identify by 16S rDNA because its genome DNA was hard to extracted from the fungus. But an isolated fungus has the activity of peroxidase, manganese peroxides and laccase. So now we are studying this fungi.

(2) Reduction of dioxins in the polluted soil (pg/g)
The degradation percent of the dioxin-polluted soils by MIX- SF1 was shown in table 1. After 120 days, MIX- SF1 degraded dioxins by 33,200 pg/g. These strains showed an ability to degrade each of the dioxins.

Table 1. Reduction of dioxin in the polluted soil (pg/g)

	Start(A)	After 120days(B)	A-B	Reduction %
Total Dioxins	52,000	38,400	13,600	26.2
Total Dibenzofurans	56,000	40,000	16,000	28.6
Total Co-PCBs	23,200	19,600	3,600	15.6
Total	131,200	98,000	33,200	25.2

(3) Reduction of dioxins (pg-TEQ/g) by MIX-SF1
The reduction (TEQ-pg/g) of MIX-SF1 on dioxin-polluted soil is shown in table 2. After 120 days, MIX SF1 reduction dioxins by 500 TEQ –pg/g.

Table 2. Reductions of dioxins in the polluted soil (TEQ-pg/g)

	Start(A)	After 120days(B)	A-B	Reduction %
Total Dioxins	580	370	210	36.2
Total Dibenzofurans	1,060	780	280	26.4
Total Co-PCBs	150	140	10	6.7
Total	1,790	1,290	500	27.9

Discussion
As shown in the results of an experiment, TEQ decreased 27.9%, and dioxins decreased 25.2% as result of an examination of using half-rotation-type incubator result. The reductions were not high average, but the examination was like onsite. It indicates the data was useful of degraded dioxins by microorganism on site.

Acknowledgements
This study was supported by a grant aid for science and technology by the Ministry of Health and Welfare of Japan.

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