APPLICATION OF BIOREMEDIATION TREATMENT FOR CLEANING UP ORANGE/DIOXIN CONTAMINATED SOIL

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Introduction

Polychlorinated dibenzo-p-dioxin (PCDDs) and polychlorinated dibenzofurans (PCDFs) are recognized as toxic pollutants and persists in an environment. These compounds are unintentionally formed in the process of producing chlorine-containing herbicides, and in other industrial processes such as bleaching of paper pulp, combustion of domestic and industrial waste etc. These kinds of contaminants have been found in many environmental matrices such as air, soil and plant [2, 5]. There are more and more reports on capacity of microorganisms that are capable of degrading PCDDs, PCDFs and PCBs. Particularly, native microorganisms existing long time in heavy contaminated sites by toxic pollutants are considered promising candidates for detoxination of these contaminants. Studies on degradation and *in situ* bioremediation to clean up PCDDs and PCDFs by the use of microbial communities and purified cultures as *Phanerochaete sordida* YK-624, *Phanerochaete chrysosporium* and *Sphingomonas sp.* RW1. etc. show that examined microorganisms could degrade these toxic chemicals in different rate [1,2, 6,7].

In some areas of South Vietnam orange/ dioxin contamination with different levels have been detected. In order to find out the suitable method for cleaning up dioxin and other pollutants in such kind of soil, bioremediation treatments were carried out in laboratory conditions. In this report we demonstrate our premelinary results that obtained after 6-9 month treatment.

Materials and methods

5-kg orange/dioxin contaminated soils collected from polluted sites were used for each treatment. pH in starting point ranging from 3-5. Microbial enumeration of different groups of microorganisms before and during treatment had been evaluated. For in situ bioremediation treatment we used products that provide nutrients, substrates, microelements, and some additives for microbial community that involves in the process of detoxination.

Residual concentration of dioxin and its congeners detected and determined by GC/MS following the three step procedure:

- *Soxhlet extraction*:

20 µl of 100pg/µl 2378- ^{13}C - TCDD, 100pg/µl 2378-^{13}C -TCDF 100pg/µl 12378-^{13}C - PCDD, 200µl/ml 123789-^{13}C - HCDD, 200pg/µl 1234678-^{13}C-HpCDD, 300pg/µl ^{13}C-OCDD were

added to each sample as a surrogate standard. 20 gram of soil sample in the soxhlet thimble was placed in the soxhlet apparatus. Add an aliquot of the surrogate standard solutions to the sample in the soxhlet thimble. Heat the sample under reflux for 24 hours using 80:20 toluene: acetone (300ml) as the solvent. Extracts were reduced in volume on a rotary evaporator to approximately 5 ml. Extracts were transferred into a series of clean-up columns.

- *Clean up:*

Method of preparation of chromatography columns as well as clean up procedures was performed in accordance with [3,4]. The volume of last clean up procedure was reduced to 15 µl with a stream of purified nitrogen. The extracts were stored at 4°C until analysis.

- *Quantitative analysis:*

All quantitative analyses were performed on a Hewlett Packard GC/MSD system using a 60 m x 0.25 mm x 0.10 um cross-linked BD-5 capillary column with an injection port temperature of 270°C and the transfer line into the 5972 Mass Selective set at 280°C. The column temperature was maintained at 90°C for 1 min followed by a 15°C/min ramp to 150°C and a further 5°C to 280°C for 25 min. The MSD was used in the selected ion monitoring SIM mode with the m/z values determined by the molecular ions of Dioxins and Furans and internal standard used in the particular run.

Result and Discussion

Several microbial enumerations were detected. Number of microorganisms 10-10000 times increased during the treatment [Tab.1]. Microbes were not diverse.

Tab. 1: Number of Representative Microbial Groups

Bioremediation treatment	Heterotroph bacteria (MPN/g)	Filamentous fungi (CFU/g)	Sulfate-reducing bacteria (MPN/g)	Nitrate-reducing bacteria (MPN/g)
Before treatment	1.9×10^3	10^3	4.3×10^2	4.3×10^1
Treatment -DN3	1.1×10^7	10^3	7.5×10^3	4.6×10^6
Treatment -DN5	4.6×10^6	10^3	1.1×10^6	2.1×10^4

After 6-9 months treated, 3 from 10 experiments were harvested for chemical analysis. The preliminary results obtained by GC/MS demonstrate that more than 40 % dioxin and its congeners were removed from the soil which indicate that the native microbes in the contaminated sites have potential in situ bioremediation for cleaning up dioxin and other toxic congeners. Microorganisms play an important role in biodegradation of such toxic chemicals that are persistent for a long time in the soil of Vietnam. These findings also show that the products produced in NCST have been successfully used especially for this bioremediation treatment to stimulate in situ biodegradation process. It may give us a real possibility in detoxination dioxin of contaminated soil in different levels.

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