BIOREMEDIATION TREATMENT FOR CLEANING UP TOXIC CHEMICAL CONTAMINATED SOIL IN FIELD TRIALS

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Introduction

At present, in South and Midle of Vietnam there are some US old military bases were contaminated by toxic chemicals (Orange/Dioxin). These soils were heavily contaminated by exposure of toxic chemicals for a long time (30-40 years). Recently, several groups of researches working on detoxination by one or other ways and they obtained promissing results. However, up to now there are no single and promising solutions that help government to select effective projects to cleanup these contaminated areas. In order to find down complex of cleaning methods for remediation of these heavy dioxin contaminated sites based on the results of distribution of native microbial populations in toxic chemical contaminated sites and laboratoty detoxination experiments that were performed [3] we carried out field trial in different scales directly in the site of Central Vietnam.

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 [9, 16]. In recent years, there are more and more reports on capacity of microorganisms that are capable of degrading PCDDs, PCDFs and PCBs [6,7,8,9,10,11,12,16,18,19]. Particularly, research of German scientists showed that there are many genes that encoded for enzymes involved in PCDDs, PCDFs and PCBs degrading pathways were found in bacteria and in several fungal genera etc [6, 20]. Enzymes were involved in oxidation, dechlorination, catalysis or direct ring cleavage. PCDDs, PCDFs and PCBs degrading pathways in microorganisms are providing knowledge and experiments for us to study of cleaningup these contaminants in Vietnam [4,8,9,10,11,12,16,17,18,20]. Several representative microbial generas are capable degrade dioxin such as: *Phanerochaete sordida* YK-624, *Phanerochaete chrysosporium; Sphingomonas* sp. RW1, *Sphingomonas wittichii*; *Pseudomonas* st. CA10, *Terrabacter* sp. DBF63, yeast etc and recently in International Symposium of Dioxin 2001 there are several new 2,3,7,8 TCDD or 3Cl-DD and chlorinated compound degrader such as: *Bacillus midousuji* (mesophilic bacterium), *Proteus* sp., *Bacillus* sp., *P. putida*, fungus *Acremonium* sp. and other fungal dioxin-degarder [9,14]. The most recent, Hiraishi et. al. had informed capacities of native microbial population could removed 22% dioxin after three month treatment [7]. Some new microbes such as *Klebsiella* sp. HL1 and *Sphingomonas* sp. HL7 and *Rhodococcus opacus* SAO101 were isolated very recently in

Japan showed capability to degrade TCDD and TCDF [5, 13].

In this report, we demonstrated our results in field trails on detoxination of very heavy contaminated toxic chemical sites by the use of bioremediation in scale of 1.3 to 100 m^3 in combination with landfill treatment that called "active landfill" (Fig.1). We also show some preliminary results of 2,3,7, 8 TCDD degradative capacity by native bacterial strain BDNE1 and fungal train FDNE1 that were dominated during treatment .

Materials and methods

- 1,5 m^3 tanks contain 1,3 m^3 contaminated soil with different level of dioxin were bioremediationly treated that named 1.5 DN1, 1.5DN2, 1.5DN3, 1.5DN4 and 1.5DN5 (Fig. 2). In one treament, we used natural surfactant.
- 100 m^3 contaminated soil has been used for active landfill trail, these soil also undergoing bioremediation 100DN9 and untreatment 100DN8 (Fig.3).
- pH in starting point ranging from 3-5. Microbial enumeration of different groups of microorganisms before and during treatment had been evaluated. Media for microbial enumeration prepared with modification accoding Atlas [1]. For these bioremediation treatments, we used products that provide nutrients, substrates, microelements, and some additives for microbial community that involves in the process of aerobic as well as anaerocic detoxination.
- Two strains, one was dominated bactrial strain BDNE1 and other dominated within fungi, strain FDNE1 have been used for study of dioxin degradation capacity.

Residual concentration of dioxin and its congeners determined by both immunoassay and GC/MS detection followed protocol have been reported in Procceding "Dioxin 2001" [2]

Immunoassay was performed according the EnviroGard™ dioxin test kit [17]

Result and Discussion

Microbial enumeration of the main four groups was detected. Number of hetetrophs in 10-1000 times increased after 47 day treatment and slightly decreased after 123 day treatment. In trail 100 m^3 hetetrophs were increased in the number 10 000 and 100 000 time after 47 and 123 day treatment respectively (fig. 1). Bacteria are not diverse in all treatments (fig. 2,3). Number of fungi was not significant changed, one or two strains of fungi have been shown domination in an initial step and during treatment steps (fig. 4, 5). Number of bacteria that could grow in sulfate and nitrate reducing bacterial media significantly changed (fig.2, fig.6). Some actinomyces strains were isolated during the treatment too. pH reached to 7 in the treated soil.

In media containing sole carbon and energy sources is 2,3,7,8 TCDD bacterial BDNE1 and fungal FDNE1 strains were growing very nice. About 30-35% 2,3,7,8 TCDD added in to the cultivating media was removed. Forming products after dechlorination was illustrated in GC/MS – graph 1. The main products of degradation were congeners which less chlo atom than four and cleavaged aromatic ring products with oxygen linkage. In the case of fungal degradation chlo of 2,3,7,8 TCDD substituted by groups $-OCH_3$ and -OH.

After 123 day treatment soil from 1,5DN5 bioremediation treatment and 100DN9 were harvested for chemical analysis. The preliminary results obtained by GC/MS and immunoassay demonstrate that about 30 % dioxin and its congeners were removed from the soil. This finding indicate that the native microbes in the contaminated sites have a potential in situ bioremediation trails for cleaning up dioxin and other toxic congeners. Microorganisms may play leading role in biodegradation of such toxic chemicals that are persistent for a long time in the soil of heavy dioxin contaminated site of Central Vietnam. Obtained data also show that the products were used in these field trails successfully applied

Figure 1. Before " active landfill " of toxic chemical contaminated soils in combination with bioremediation treatment at 100 m^3 tria

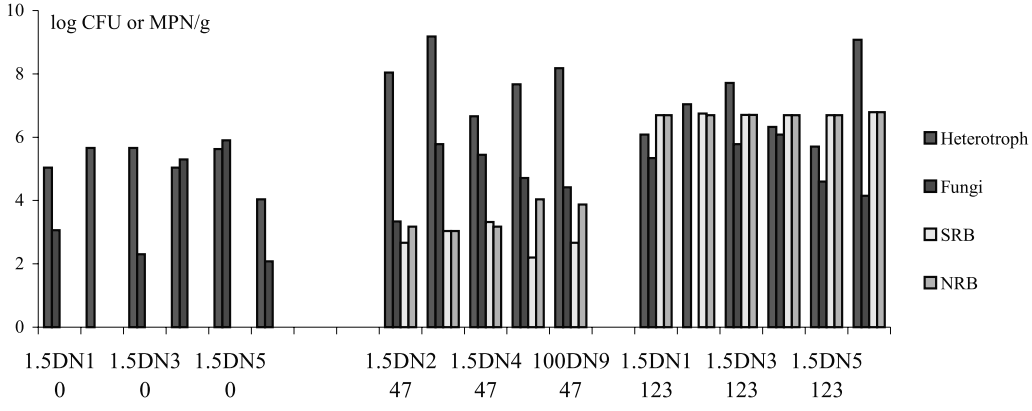

Figure 2. Number of representative microbial groups in treatment in field trials

to stimulate *in situ* biodegradation process. It may give us a real possibility in detoxination dioxin of contaminated soil in Vietnam.

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