JOINT STUDY OF BIOREMEDIATION AT PILOT SCALE FOR DETOXIFICATION OF HERBICIDE/DIOXIN IN DA NANG HOT SPOT, VIETNAM

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Introduction

Biodegradation of tetrachloro dibenzo-*p*-dioxin (TCDD) has been reported in the scientific literature, in the laboratory, and in pilot studies. From 1999 to 2009, Vietnamese researchers conducted several studies to detoxify heavily-contaminated soil in the former Da Nang military base[1]. Full-scale bioremediation of 3,384 m^3 of dioxin-contaminated soil was demonstrated in Bien Hoa, Vietnam, in 2009. Several international scientific work groups have concluded that bioremediation is the most environmentally responsible and cost-effective remedy for cleaning up Agent Orange residues at the former air bases in Vietnam.

More than 30 years after the US-Vietnam War, spilled Agent Orange defoliant solution containing traces of the dioxins, TCDD and octachloro dibenzo-*p*-dioxin (OCDD), 2,4,5-T, 2,4-D, and chlorophenols (TCP and DCP) remains in the soil and in lake sediment affected by contaminated soil, which had been carried by runoff from the former military airbase in Da Nang[2]. Natural attenuation of the herbicides and dioxins has not been effective in detoxifying the soil or sediment. This first joint study by Vietnamese and American researchers was conducted to demonstrate whether the soil in Da Nang can be bioremediated effectively using aerobic or anaerobic microbial processes. This study also sought to provide engineering design guidance to support the selection of either an aerobic or an anaerobic amendment recipe and an operating strategy to optimize biological treatment.

Materials and Methods

Eleven pilot reactor units were constructed. The reactors included: an M1 anaerobic "reference" cell (unamended, dry, sealed off from air), four anaerobic treatment cells (three M2, M3 and M4 cells for VAST and M5 cell for EPA) (amended, seeded with active soil [bioaugmented], flooded, and sealed), an M6 aerobic reference unit (unamended, watered, and untilled), and five active land treatment units (LTUs) (M7 cell for EPA and four M8, M9, M10 and M11 cells for VAST) (amended, watered, and tilled or aerated with forced air). Soil samples from M5 cell were taken both shallow and deep (designed as M5A and M5B, respectively). Each reactor contained approximately 2 cubic meters (3.6 to 4 tons) of Da Nang site soil, which had been pre-mixed to achieve a nominal dioxin concentration of about 100,000 (pg/g). Although the actual starting concentration for the full-scale remediation is estimated to be between 5,000 and 10,000 ppt, a high concentration was desired for the pilot study to overcome data variability and to enable a more valid estimate of the degradation rate. Bioremediation of soil can be expected to achieve about 90% removal, or between 3 and 4 half-lives for log-normal kinetics.

Representative composite samples were collected monthly for six months and sent to the United States and Germany for dioxin congener analysis. These soils were thoroughly mixed, dried, and sub-sampled (split) for analysis. Many of these samples also were analysed by DR-Calux in the Netherlands and by GC/MS in Vietnam laboratory of MOD. Other laboratory partners included Eurofins GfA in Germany and the Vietnam-Russian Tropical Research Center. All laboratory results met the EPA-approved analytical and quality assurance/quality control (QA/QC) requirements. VAST analyzed the soil composites for pH and soil moisture content (%). The samples which came to the USA were received by the ERT and transshipped to a NELAC certified contract laboratory (SGS Systems, Wilmington, NC). The samples were extracted and analyzed using high resolution gas chromatography/ mass spectrometry (GC/MS) for dioxin congeners (EPA Method SW-8290A) with a 30-day delivery date. After the results and the quality assurance packages were received by the ERT and transmitted to Vietnam, the sample codes were revealed to ERT and VAST, and the results were reduced to descriptive form. The ERT assessed the results using standard methods of analysis, supported by the MS Excel spreadsheet statistical tools. A one-tailed confidence level of 90%

(α = 0.1) was selected for determining the significance of the results. The null hypothesis was that there is no downward trend for any of the treatments.
The bacterial cell morphology was observed by scanning electron microscopy JEOL 5410LV. Laccase activity was measured in soil and also in microbial enrichment solution from soil according to Eggert et al[3]. Primer and probe were designed and used to detect *C23O* gene copies by Real Time-Polymerase Chain Reaction (RT-PCR)-based on the Roche program (https://www.roche-applied-science.com).

Results and Discussion

Five anaerobic treatment units (cells), including one reference cell, and six aerobic land treatment units (LTUs), including one reference LTU yielded 140 paired data points for TCDD and OCDD. These data were analyzed using least-squares analysis for downward trend over the course of the study. Aerobic treatment by stimulating the activity of indigenous microbes emerged as the most effective treatment, but anaerobic treatment using contaminated lake sediment as a bioaugmenting agent was also promising. Changes in the microbial numbers and community structure were also monitored over the course of treatment. Microbial numbers and diversity (Figure 1A, 1B) varied depending upon differences in treatment type (aerobic vs. anaerobic) and management methods (mixed vs. vented), and amendment and watering practices.

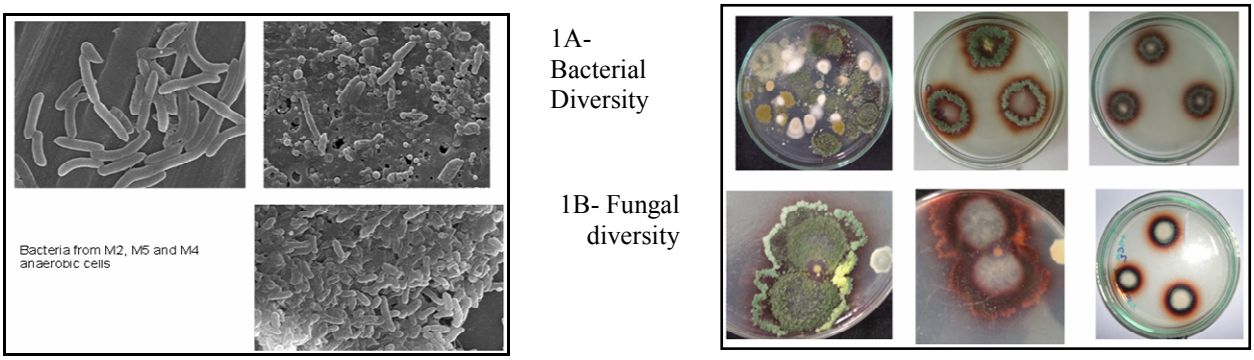


Figure 1. Bacterial diversity of soil samples from anaerobic bioremediated cells (A) and the fungal diversity and extracellular enzyme activity (color zones) from filamentous fungi (B) in bioremediation cells

The environmental condition created within each treatment design tested permitted different microbial groups to dominate the microbial community. Dominant filamentous fungi, actinomycetes, and some bacteria (pseudomonads-data not shown) tolerate the combination of toxic pollutants in the soil matrix and produce extracellular enzymes (Figure 1B), such as laccase (Table 1), which are capable of detoxifying dioxin.

Table 1. Laccase activity of bioremediation cells and their enrichments

Cells	Laccase activity (Units/l)				
	Four month soil		Five month soil		Six month soil
	Direct detection	Enrichment	Direct detection	Enrichment	Direct detection
M1	0.9	ND	0.3	2.6	ND
M2	0.8	3213.2	0.2	0.1	1.1
M3	2.8	2869.7	1.7	1112.5	6.8
M4	1.0	3771.9	0.5	1250	2.6
M5A	1.2	312.3	ND	88.2	ND
M5B	0.2	3463.1	1.3	41.9	ND
M6	ND	38.9	0.6	ND	ND

M7	23.6	105.5	0.5	1427.7	1.2
M8	0.7	3560.2	0.3	713.9	0.7
M9	0.4	77.03	0.2	ND	ND
M10	1.7	2786.4	ND	26.7	ND
M11	2.2	1842.6	ND	1051.4	4.4

ND- not detected

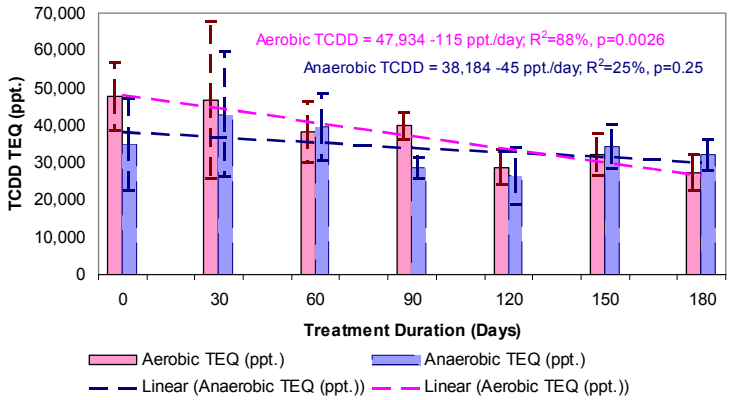
Catechol 2,3-dioxygenase (C23O) is a key enzyme in the metabolism of aromatic compounds. C23O is encoded by several genes[4]. These genes have been studied widely and also are used in monitoring bioremediation of aromatic compounds. As shown in Table 2, the *C23O* gene copies in soil samples in aerobic bioremediation treatments in this study initially ranged from 2.1 x 10^5 to 2.6 x 10^6 per dry soil gram by the use of the RT-PCR technique. The *C23O* gene copies increased 10-fold after 3 months of bioremediation in all samples except the M11-3 soil samples.

Table 2. Number of catechol 2,3-dioxygenase (*C23O*) gene copies initially and after three months of treatment in Aerobic LTUs

Cells	*C23O* gene copy/g dry soil
M7-0	$2.5x10^5$
M7-3	$2.7 x10^6$
M8-0	$3.7 x10^5$
M8-3	$6.7 x10^6$
M9-0	$2.1 x10^5$
M9-3	$2.2 x10^6$
M10-0	$4.6 x10^5$
M10-3	$2.4 x10^6$
M11-0	$2.6 x10^6$
M11-3	$1.3 x10^6$

For the TCDD chemical results, the simplest picture of successful bioremediation applies a 90% one-tailed confidence interval (α=0.1) to the average concentrations in each sampling period. For the entire data set, the average dioxin concentration decreased significantly at a linear rate of over 100 ppt per day aerobically (43% in 6 months) and about 40 ppt per day anaerobically (21%) (Figure 2).

Figure 2. TCDD degradation in Da Nang pilot study all aerobic and anaerobic treatments.



The highest rates measured were 283 ppt TCDD/day for aerobic (LTU) biostimulation treatment (74% in 6 months) where a buffered amendment recipe was used. These amendments may be cost prohibitive in Vietnam. The highest

anaerobic treatment achieved was 114 ppt TCDD/day (46% in 6 months) where buffered conditions were maintained. In some other aerobic cells, degradation shows high rate too (highest rate is 122 ppt per day). This treatment is feasible in both amendment recipes and technology in the result the cost for biotreatment significant reduced (1/4 in comparison to buffered) and this treatment is friendly.
The aerobic results also exhibited a concurrent 6-month downward trend in TCDD/OCDD ratio (81%), indicating that TCDD was indeed being degraded, while the OCDD (the biomarker) was not. The anaerobic TCDD/OCDD ratio did not show a statistically significant downward trend. The herbicides, 2,4-D and 2,4,5-T, were also degraded (Figure 3) during treatment (approximately 78% and 68%, respectively, in 6 months).

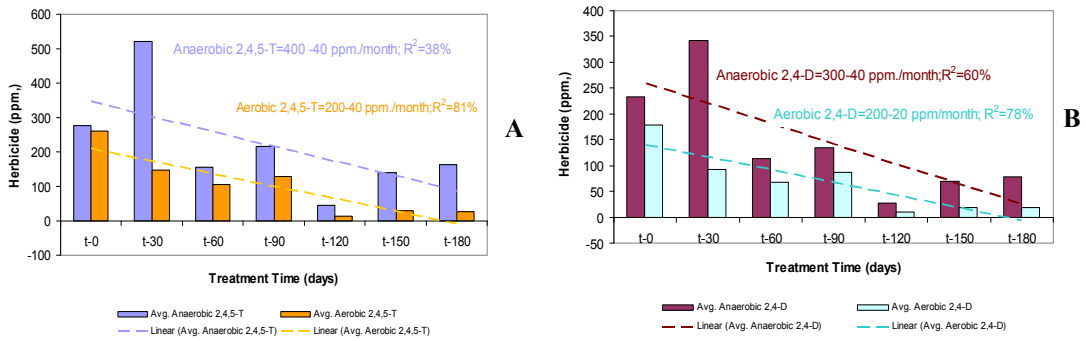


Figure 3. Biodegradation of 2,4,5-T (A) and 2,4-D (B) in all aerobic and anaerobic treatments

Conclusions

Aerobic bioremediation is capable of significantly reducing TCDD toxicity (p=0.0026). Bioaugmentation with small amounts of treated soil or contaminated sediment may be effective for anaerobic treatment. However, if suitable growth conditions are provided, the indigenous microbes in the mixed soil and sediment at Da Nang appear capable of degrading TCDD without adding another source of microbes. Anaerobic bioremediation rate is about half the rate of aerobic treatment, but the results are not as significant (p=0.25). From our of point active landfill containing both aerobic and anaerobic degradation become feasible resolution for detoxification of heavy herbicide/dioxin in full scale in Vietnam.
Bioremediation is recognized as a "Green Technology," which has a very low energy requirement and produces few emissions. Bioremediation is a permanent solution which produces a soil which can be returned to beneficial use.
Knowledge gained from this project by both Vietnamese and US scientists will allow for design of customized recipes suitable for addressing dioxin and other persistent organic pollution problems throughout Vietnam and elsewhere

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