Detoxification of dioxins and other contaminants in soil by bioremediation treatment

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Introduction

During ten year from 1961 to 1971, 20 herbicides had been sprayed by US to South Vietnam. Pink, purple and orange agents containing more 2,3,7,8 -TCDD than other agents [20]. In some former military bases soil are still heavily contaminated by mainly 2,3,7,8 -TCDD and other PCDDs and PCDFs. In this kind of soils other pollutants such as 2,4-D; 2,4,5-T; DCP, TCP, PAHs were also detected. Recent years, the appearance of many biodegradation researches on dioxin as well as application of bioremediation technology in pilot and field trails indicate that biological method for detoxifying dioxin in soil become one of promising resolution for "hot spot" and contaminated site remediation [1,2,3,4,5,6,7,11,12, 13,14,15,16,17,18,19,21,22,23,24,25].

In order to find out the suitable resolution for detoxification of dioxin contaminated sites in Vietnam we conducted our research on bioremediation technology and particularly using several technologies: isolation, absorption together with bioremediation that named "Active landfill". Field trials with different scales for detoxification of contaminated site in South Vietnam were carried out. Our technology based on stimulation of indigenous dioxin and toxic compound-degrading microorganisms of heavy dioxin contaminated site by providing them necessary sources for their activity in oxydation, reductive decholorination and catabolic processes.

In this report we show our data obtained from one of our biotreatments (1.5DN5) in 1.3 m^3 pilot scale of heavy contaminated soils in Danang former military base. We investigated the change in microbial number, diversity, toxicity as well as biodegradation of high concentration of dioxin, 2,4-D; 2,4,5-T after two year treatment. The change of existing compounds detected by GC/MS scanning reveals that native microbial community may play transforming or degrading roles in reduction of different contaminants at different rate in the treated soils. Our findings also indicated that by the use of bioremediation treatment not only dioxin was reduced but other polluted components in soils (for example, 2,4-D; 2,4,5-T) also transformed or degraded. This biotechnology can be applied for detoxification of heavy dioxin contamination .

Material and Method

1.3 m^3 soils of surface layer of contaminated site (from 0 to 50 cm depth) were carefully mixed up with treated products, substrates, additives, water etc and putted in the 2 m^3 tank with crew cap and small gas getting out hole. This tank was landed in 50 cm in depth of surface soil. Microbial enumeration and chemical analysis have been carried out before, during and after two years. Nine different groups of microbes such as heterothrophic bacteria, actinomycetes, yeast, filamentous fungi, nitrate reducing bacteria, sulfate reducing bacteria, anaerobic microbes in treated medium and anaerobic microbes in mixture of sulfate reducing and methanogen medium with soil extract have been detected during two years. Single strand conformation polymorphism (SSCP) technique was carried out for detection of microbial diversity of this treatment at three different times (between 6 month). The soil extract used in this enumeration containing mainly 2,3,7,8 -TCDD [8]. Concentration of 2,3,7,8 -TCDD of treatment 1.5 DN5 reached to several hundreds ppb/g soil. To study 2,4-D biodegradation of purified fungal strain FDN20 that isolated from treated as well as untreated soils, and this fungal strain was inoculated in the salt medium containing 165.07 mg/ml 2,4-Dichlorophenoxyacetic acid (2,4-D) with 0.1 - 1% glucose.

Residual concentration of PCDDs/PCDFs was detected by EPA method 8280. Detection of composition change in 1.5DN5 treatment by the use of GC/MS scanning. The samples were prepared and analyzed following EPA 8270 method by Gas Chromatography/Mass Spectrometry (Hewlett Packard 6890 GC/MSD 5972 A with EPA/NIST 98 Mass spectral database, scanning from 35 to 500 amu). Residual concentration of 2,4-D detected by HPLC following EPA 8321A method (HPLC/Diod Array Detector HPLC/DAD).

Results and Discussion

Microbial number and diversity

Microbial enumeration was carried out 9 times at starting point (before treatment), 2,4,6,10,15,18,22, and 24 months. Before treatment only heterotrophic bacteria could detected with low number $x10^3$ MPN/g soil and filamentous fungi $x10^2$ CFU/g soil. This group of microbes increased 100 – 1 000 000 times during 9 month microbial analysis (Fig.1A).

Actinomycetes and yeasts were not detected in all samples, except low number of actinomycetes was found in 18 month treated sample. Filamentous fungi almost found with increasing number from $x10^3$ to $x10^5$ CFU/g soil in 2, 4, 6, 15, 22, month samples and decreased in 10 and, 18, month samples.

This group of microorganisms was not found in 24 month sample when water was completely filled in the treated tank. Nitrate and sulfate reducing bacteria were detected from $x10^2$ to $x10^7$ MPN/g soil in almost examined samples. The enumeration of sulfate reducing bacteria describes in figure 1C. Two other kinds of anaerobes cultivating in the media with soil extracts appeared only in few samples with low number, in the 24 month sample, bacteria were found at high number $x10^7$ MPN/g soil.

Figure 2B**:** Neighbour-joining tree showing the phylogenetic positions of cloned PCR amplified a part of the 16S rRNA genes obtained from bio-treatment 1.5DN5 of long-term herbicide contaminated soil, and closed representative 16S rDNA sequences from GenBank. Genbank assession are given and boostrap values are shown at banch points when higher than 60%. Bar, 2 sustitution per 100 nucleotide.

The PCR-SSCP result of microbial community profile in treated samples at three deferent collections is shown in figure 2A. Data presenting in figure 2B indicates phylogenetic positions of cloned PCR amplified a part of the 16S rRNA genes obtained from bio-treatment 1.5DN5 of long-term herbicide contaminated soil, and closed representative 16S rDNA sequences from GenBank. Several bacteria such as *Pseudomonas* sp SETDN1*, Sphingomonas* sp. BDN19 and some representatives of genus *Actinomyces* were isolated and characterized. Other isolated microbes from 1.5DN5 bioteatment will be identified. The examined microorganisms are capable to degrade 2,3,7,8-TCDD; 2,4,5-T; 2,4-D or PAHs.

We also analyzed soil sample before and after two year treatment. Result of GC/MS analysis indicates that 51% of toxicity of several hundreds mg TEQ/g was removed after two year .

Figure 3 shows composition change in soil sample before treatment in comparison to sample after two year treatment. Not only 2,3,7,8 -TCDD congener was reduced, but also other herbicide contents were decreased too. Comparing chemicals that analyzed in 1.5DN5 sample before treatment and two year treated sample, the change of biodegrading products was detected in treated sample. Some diesel oil compositions were also degraded. This finding shows that bioremediation treatment can be applied for soil with high concentration of 2,3,7,8 -TCDD, 2,4,5-T and 2,4-D. Obtained data from GC/MS scanning analysis of the main existing chemicals in soil before and after two year treatment, also shows that bioremediation treatment by 'Active landfill" technology providing promising tool for detoxification of heavily contaminated soils by dioxin and other toxic compounds.

For example, strain FDN20 was found in almost untearted and treated soils and it was able to degrade 2,4-D. This fungal strain could remove 72.92 mg/ml (44.17%) after 7 day inoculation.

Among many microbes isolated from treatments, fungi and actinomycetes (data not shown) seem play an important role in 2,4-D detoxification.

These examined results also drive us go to study in detail the mechanisms of 2,3,7,8 -TCDD, 2,4-D. 2,4,5-T and other toxic chemical transformation, biodegradation as well as mineralization by different microbes in contaminant mixture in soil. The relationship of each microbial group or role of each microorganism in the process of degradation has to be also studied in order to enhance the reduction of all such kind of toxic contaminants in the "hot spot". In 1.5DN5 treatment, elevation of biodegradation effect was detected in comparison to the same other pilot treatments (data not shown). This biotechnology is one of remediations for detoxification of heavy dioxin contamination. This bioremediation can be used for cleaning up complex of pollutants in soil.

Acknowledgments

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Reference

1. Armengaud Jean and Kenneth N. Timmis .1997. J. Microbiology 35: 241-252.

2. Bunz, v. p and Schmidt, S. Biotech. Advances. 1997. 15: 621-632.

3. Bunge, M., Hendrik Ballerstedt and Ute Lechner.2001. Chemosphere 43:675-681.

4. Bunge, M.A., Kraus, A., Opel, M., Lorenz, W. G., Andreesen, J. R., Gorisch,H., Lechner, U. 2003. Nature. 421:357-360.

5. Dang, T. C.H., Nguyen, B.H., Nguyen, V. M., Do, Q.H., Pham, H. L., Dang, V. M. 21 th International Symposium on Halogenated Environmental Organic Pollutants and POPs, 2001. Korea. Vol 54: 259-261

6. Dang, T. C. H., Nguyen, B.H., Pham, T.Q.V., Nguyen, T.D., Nguyen, Q. V., Nguyen, D. N., La, T. P., Tran, N. H., Mai, A. T., Pham, H.L., Nguyen, V. M., Le, V. H., Do, Q.H., Dang, V. M. 22 th International Symposium on Halogenated Environmental Organic Pollutants and POPs,2002. Spain. Vol 56: 433-436.

7. Dang,T. C. H., Nguyen, B.H., Nguyen, T.D., Mai, A. T., Nguyen, D. N., La, T. P., Nghiem, N. M., Nguyen, Q.V., Nguyen T.T., Hoang, T. M. H., Pham, H.L., Nguyen, V. M., Le, V. H., Do, Q.H., Dang, V. M. Nov- 2003. Hanoi, Vietnam. Vietnam-US Scientific Workshop: Dioxin screening remediation methodologies, and site characterization: 104-112.

8.Dang,T. C. H., Mai A. T, Nguyen Q.V, Trinh K. S, PapkeO, Nguyen T. S. 24 th International Symposium on Halogenated Environmental Organic Pollutants and POPs. 2004, Germany. Vol 66: 3695- 3701.

9. Habe Hiroshi**,** Kazuki Ide, Mizuyo Yotsumoto, Hirokazu Tsuji, Takako Yoshida, Hideaki Nojiri. Toshio Omori.2002. Chemosphere 48:201-207.

10. Haraishi A, H. Miyakoda, B-R.Lim, H.Y. Hu, K.Fujie, J. Suzuki.2001. Appl. Microbiol Biotechnol. 57:248-256.

11. Halden Rolf U.**,** Barbaka G. Halden and Daryl Dwyer.1999. Appl. Environ. Microbiol. 65:2246-2249.

12. Hong Hyo-Bong**,** Seok-Hwan and Yoon-Seok Chang.2000. Water. Res. 34: 349-353.

13. Hong Hyo-Bong, Yoon-Seok Chang, In-Hyun Nam, Peter Fortnagel, and Stefan Schmidt. 2002. Appl. Environ. Microbiol. 68:2584-2588.

14. Kao C. M., Chen S.C., Liu J.K., Wu M.J . 2001. Chemosphere 44:1447-1454.

15. Kao C.M. and M.J. Wu. 2000. J Hazardous Material, B74: 197-211.

16. Kimura Nobutada and Yoshikuni Urushigawa.2001. J. of Bioscience and Bioengineering 92:138-143.

17. Fukuda Kazumasa, Sumio Natsumi Nagata, Hatsumi Taniguchi. 2002. FEMS Microbiol. Lettres. 100337:1-7.

18. Lida Toshiya**,** Yuki Mukouzaka. Kaoru Nakamura and Toshiaki Kudo. 2001. REKEN review. 42:27-30.

19. Mori Toshio, Ryuichiro Kondo.2002. FEMS Microbiol. Let.. 213:127-131.

20. Sato Akira**,** Tsuneo Watanabe, Yoshio Watanabe, Koichi Harazono, Takema Fukatsu.2002. FEMS Microbiol. Letters 213: 213-217.

21. Stellman, J.M., Stellman, S.D., Christian, R., Weber, T. and Tomasallo C. 2003. Nature 442:681-687.

22.Tomotada Iwamoto and Masao Nasu. 2001. J. Bioscience ans Bioengineering. 92: 1-8.

23.Toshiynki Sakaki, Raku Shinkyo, Teisuke Takita, Miho Ohta, and Kuniyon Inouye. 2002. Arch. Biochemis. Biophs. 40: 401:91-98,

24. Vargas C., Fennell D.E., M.M. Haggblon. 2001. Appl Microbiol. Biotechnol 57:786-790.

25. Wilkes Heiz**,** Rolf-Michael Wittich, Kenneth N. Timmis, Peter Fortnagel, and Wittko Francke.1996. Appl. Environ. Microbiol. 62:367-371.

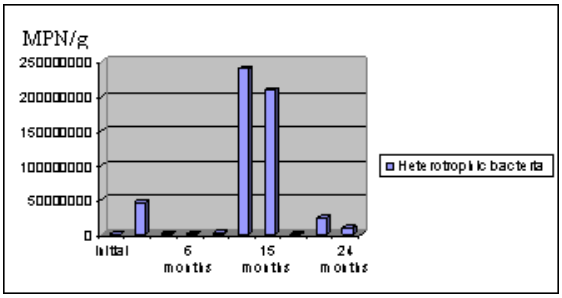

Figure 1A: Number of heterotrophic bacteria in 1.5DNT

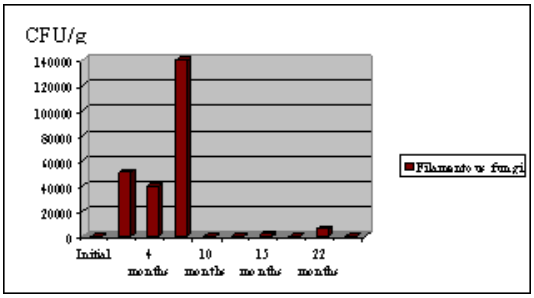

Figure 1B: Number of filamentous fungi (CFU) in 1.5DN

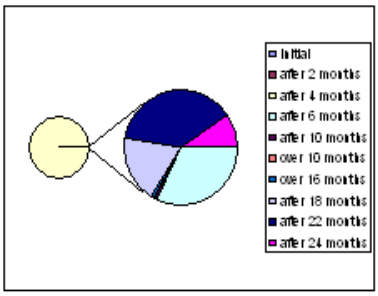

Figure 1C: Number of sulfate bacteria (MPN) in 1.5 DNT

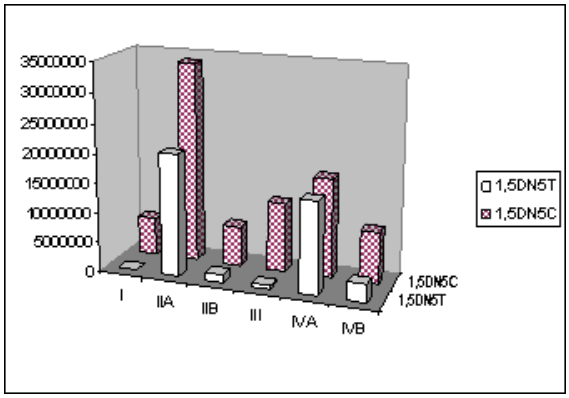

Figure 3: Composition change in soil sample before and after two year treatment

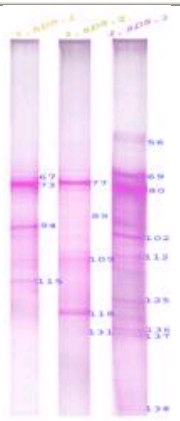

Figure 2A: PCR – SSCP community profile of 1.5

Figure 4: (A) Colony morphology of strain FDN20 and (B) Sporophore and spore

DN5 biotreatment	

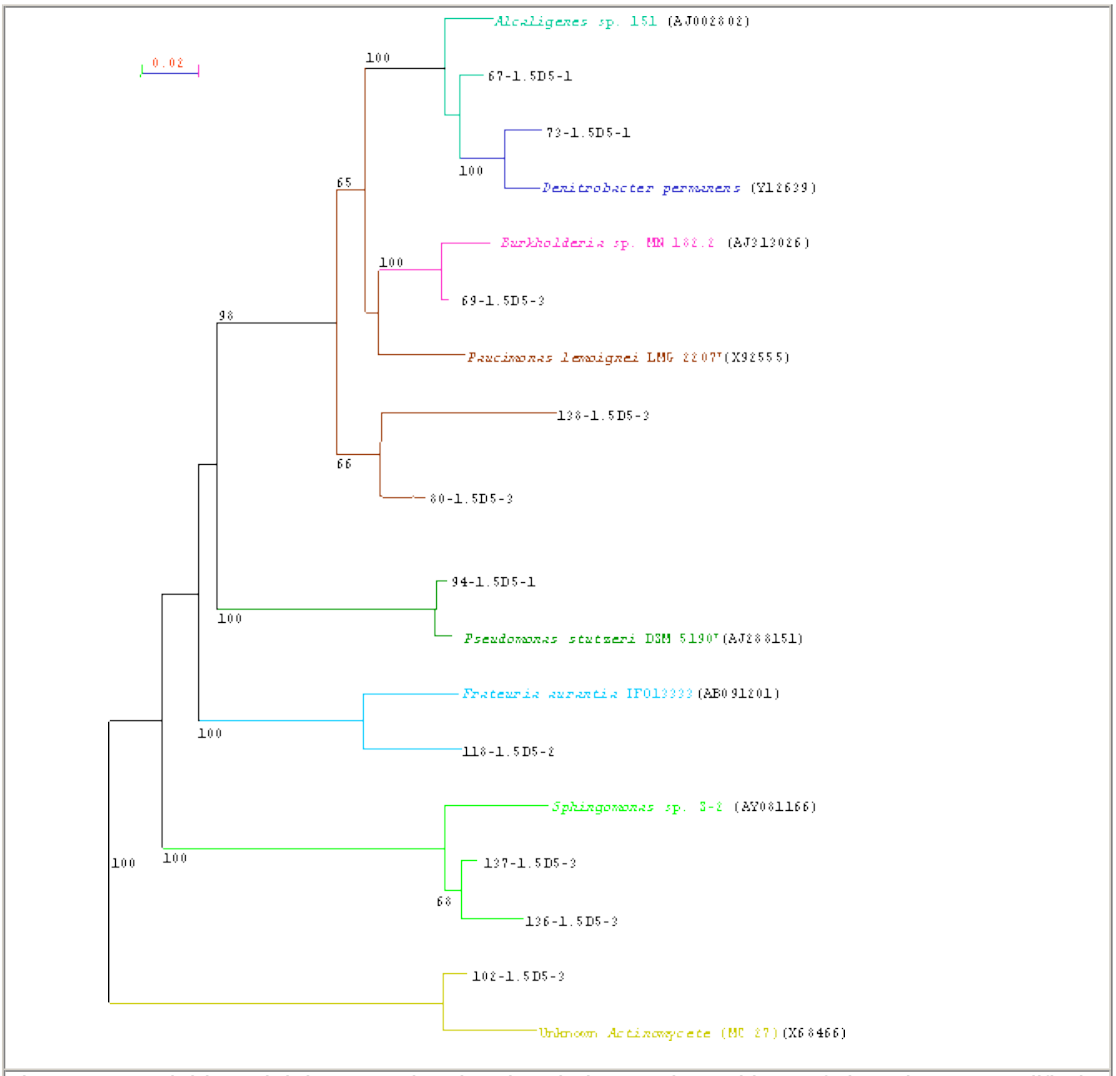

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Table 2: Soil composition change before and after treatment

	1,5DN5C (Before treatment)	1,5DN5T (After treatment)
I	2,4,5-T Methyl ester; 2,4-D methyl ester; Acetic acid, (2,4-dichlorophenoxy)	2,4,5-T Methyl ester
IIA	Phenol, 2,4-dichloro-; Phenol, 2,4,5-trichloro-	Phenol, 2,4-dichloro-; Phenol, 2,4,5-trichloro-
IIB	Phenol, 4,5-dichloro-2-methoxy-; Phenol, 2,4,6-trichloro-; Phenol, trichloro-; Phenol, 2,6-bis(1-methylpropyl); Phenol, 2,3,4,6-tetrachloro-; Phenol, 2,3,5,6-tetrachloro-; Phenol, 2,3,5-trichloro-; Phenol, 2,3,6-trichloro-	Phenol, 2,4,6-trichloro-; Phenol, trichloro-; Phenol, 2,6-bis (1,1-dimethylethyl); Phenol, dichloro-; Phenol, 2,6-dichloro-

III	Benzene, 1,2,4-trichloro-3-methoxy; 1,2-Dimethoxy-4,5-dichloro-benzene; Benzene, dichlorodimethoxy- ; Naphthalene, 1,3,7-trichloro-; Benzene, 1,2,4-trichloro-5-ethoxy-	Benzene, dichlorodimethoxy-; Benzene, 1,2,3-trichloro-4-methoxy
IVA	1-Nonadecene; 9-Tricosene, (Z)-; Nonadecane, 2-methyl-; Heptadecane; Tetradecane; Nonadecane; Eicosane; Pentadecane; Octadecane; Hexadecane	1-Octadecene; 1-Nonadecene; Heptadecane;Tetradecane; Nonadecane; Eicosane; Octadecane; Docosane
IVB	9-Octadecenamide, (Z)-; 9-Octadecenoic acid, (E)-; Dodecanoic acid; Dodecanoic acid, 1-methylethyl ester; Octadecanoic acid; n-Hexadecanoic acid; Tetradecanoic acid	9-Octadecenoic acid, (E)-; Dodecanoic acid; Dodecanoic acid, 1-methylethyl ester; Octadecanoic acid; n-Hexadecanoic acid; Tetradecanoic acid; Nonanoic acid; Hexadecanoic acid, methyl ester; Z-7-Hexadecenoic acid