Biodegradation of 2,3,7,8 TCDD by anaerobic and aerobic microcosms collected from bioremediation treatments for cleaning up dioxin contaminated soils

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Introduction

There are many microbes that can degrade polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurants (PCDFs) and polychlorinated biphenyls (PCBs) have been isolated including purified bacteria, actinomycetes, white rods, filamentous fungi, anaerobes and also anaerobic and aerobic consortia [1,2,3,4,8,10,11,12,15,16,17,18,19,20,21,22,23].

Bioremediation one of biological remediation has been studied as hopeful alternative to physical and chemical treatments that using for cleaning up PCDDs, PCDFs [5,6,7,9,13,14]. In Vietnam for cleaning up "hot spot" of some former military air bases, bioremediation has been studying in different scales of Danang site. After 18 to 24 month treatments, the reduction of toxicity was significally detected [7].

In order to study biodegradability by different groups and one of dominated strain that are existing microorganisms in our treatments, the investigation of 2,3,7,8 TCDD anaerobic and aerobic degradations was carried out in the laboratory condition. Anaerobic microbial consortium containing three different bacteria such as two Gram- negative vibrio and rod and one gram positive cocoides bacteria. This consortium could degrade 118 pg TEQ/ml 2,3,7,8 TCDD after 133 days under sulfate reduction. Concentration of 2,3,7,8 TCDD in the soil extract that adding to medium at starting point of cultivation was 144.6 pg TEQ/ml. About 81% toxicity was removed. Aerobic consortium containing all three Gram-negative bacteria and one fungal strain. After 9 day shaking at 180 rpm/min and 30^{0}C, 85.6 % of 164.45 pg TEQ/ml 2,3,7,8 TCDD was removed. Other preliminary results of study of 2,3,7,8 TCDD biodegradation as sole carbon and energy by show that this strain FDN30 could remove 43,45 pg TEQ/ml (59%) of 73,1 pgTEQ/ml adding dioxin after two weeks. These findings explain why high concentration of contaminants in treated soil was decreased after two year treatment. Indigenous microorganisms play leading role in the detoxification of 2,3,7,8 TCDD in contaminated soils.

Material and Method

Anaerobic enrichment

Treated sample of 100 DNT treatments was collected after 19 months and enriched in 125 ml bottle. Medium containing acetate, glucose, organic acids and some addities with soil extracts, 2,3,7,8 TCDD, pentachlorophenol and 2,4 D (144.6 pg TEQ/ml: 200 pg TEQ/ml: 200 µm/ml: 200 µg/ml respectively) were used for each treatment. We have been focused in using soil extract, because this extract may contain not only 2,3,7,8 TCDD, but also other chlorinated congeners such as 2,4,5T, 2,4D and other PCDDs, PCDFs included too. Four enrichments with different substrates were transferred three times before taking examined samples for chemical analysis. GC/MS analysis was carried out for anaerobic consortium that added soil extract in 133 day cultivation at room temperature.

***Aerobic enrichment*:**

Sample for aerobic enrichment was collected after two year from several treatments, and shacked at 180 cpm/min at 37^0C for 9 days. Salt medium containing only 2,3,7,8 TCDD was used for enrichment. The same medium with the same concentration of 2,3,7,8 TCDD without treated soil sample had been used for a control. The third transferred enrichment used for chemical analysis and microbial screening in the agar plate containing salt media with the same concentration 2,3,7,8 TCDD.

Study of biodegradation by fungal strain FDN30:

It was carried out by shaking at 180 cpm/min, 30^0C in medium containing 2,3,7,8 TCDD as sole carbon and energy source, after two weeks these inoculums was taken for GC/MS analysis.

Residual concentration of PCDDs/PCDFs was detected by EPA method 8280.

Electron scanning micrographs of bacterial cells was prepared by fixation of the cells in 100mM sodium phosphate buffer with 2.5% glutaraldehyde and taken under JEOL 5410 LV Scanning Microscope.

Results and Discussion

Anaerobic enrichments

The growth of anaerobic microcosm of four enrichments were different [Fig.1,2,3,4].

Fig 1 : Growth of anaerobic consortium in medium containing 2,4 D (A) and without microbes (B).

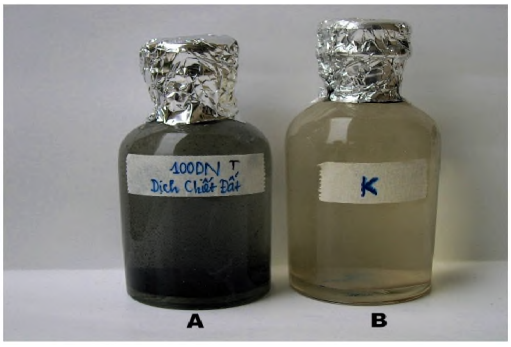

Fig 2 : Growth of anaerobic consortium in medium containing soil extract (A) and without microbes (B).

Figure 3: Growth of anaerobic consortium in medium containing 2,3,7,8 TCDD (A) and without microbes (B).

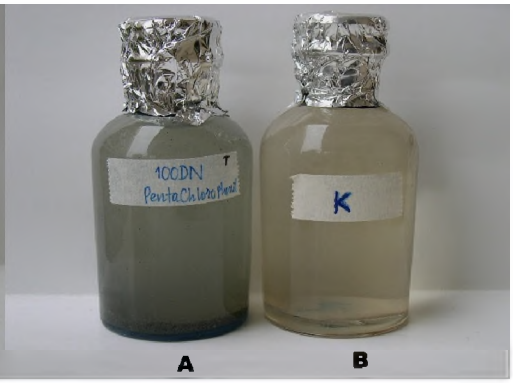

Figure 4: Growth of anaerobic consortium in media containing pentachlorophenol (A) and witthout microbes (B).

The best growth was always observed in the enrichment with 2,4 D [Fig.1] and then soil extract [Fig.2], the 2,3,7,8 TCDD enrichment is in the third order [Fig.4], the last once is enrichment with pentachlorophenol [Fig.4]. As we have learned that 2,4D is easy degrading by microorganisms in comparison to soil extract and 2,3,7,8 TCDD congener, this enrichment had strongest growth. In enrichment with soil extract, main congener is 2,3,7,8 TCDD, but other pollutants such as 2,4D; 2,4,5T and other chemicals are existing there too, therefore the growth of anaerobic bacteria stronger in comparison to medium containing only 2,3,7,8 TCDD. Pentachlorophenol is not found in contaminated soil of "hot spot" therefore the growth of bacteria in this enrichment weaker than other enrichments.

For further investigation we concentrated enrichment with only soil extract. In this enrichment three different bacterial cells was observed under scanning microscope .

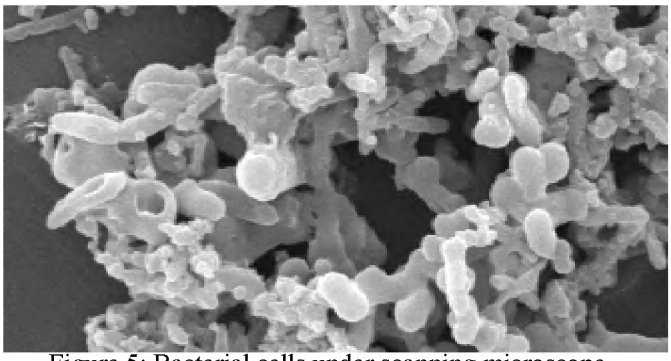

Figure 5: Bacterial cells under scanning microscope

Rod bacterial forms seem dominated cells. This bacterial strain is Gram negative. The Gram negative vibrio cells also present in the medium. The third Gram positive bacteria have coccal shape.

GC/MS analysis of 133 day anaerobic consortium revealed that these bacteria could degrade 118 pg TEQ/ml 2,3,7,8 TCDD (81% toxicity was removed).

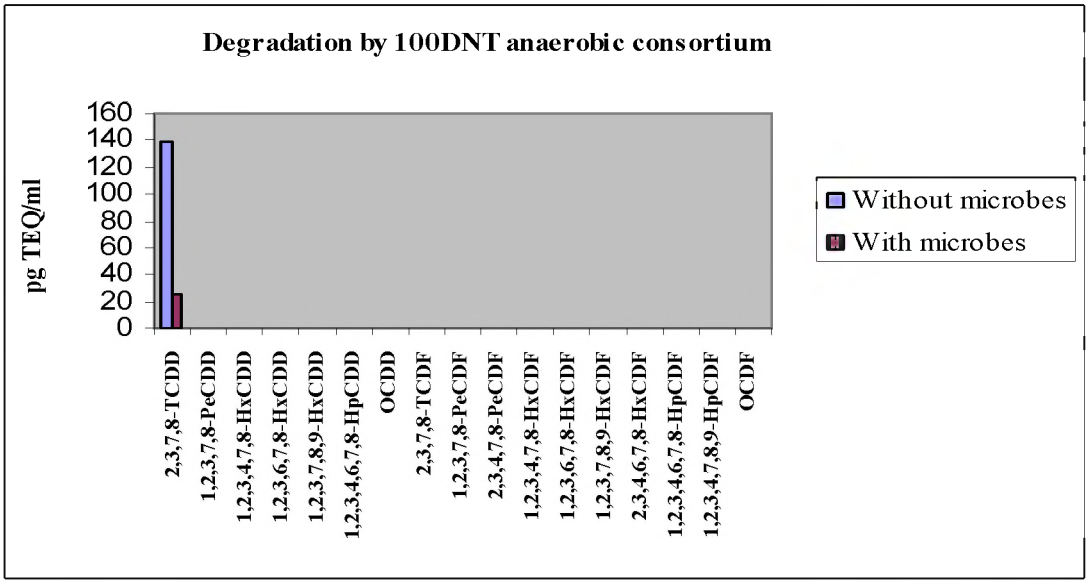

Figure 6: Dioxin degradation by 100DNT anaerobic consortium.

Aerobic enrichment

The growth of aerobic enrichment appeared after only 5 day inoculation. Three different bacterial strains and one actinomycete strain was isolated from this enrichment. Three of them belong to Gram negative bacteria with different colonies and cell shapes, actinomycete strain [Figure 7: A, B, C, D]. These microbes were purified and they are now under further investigation.

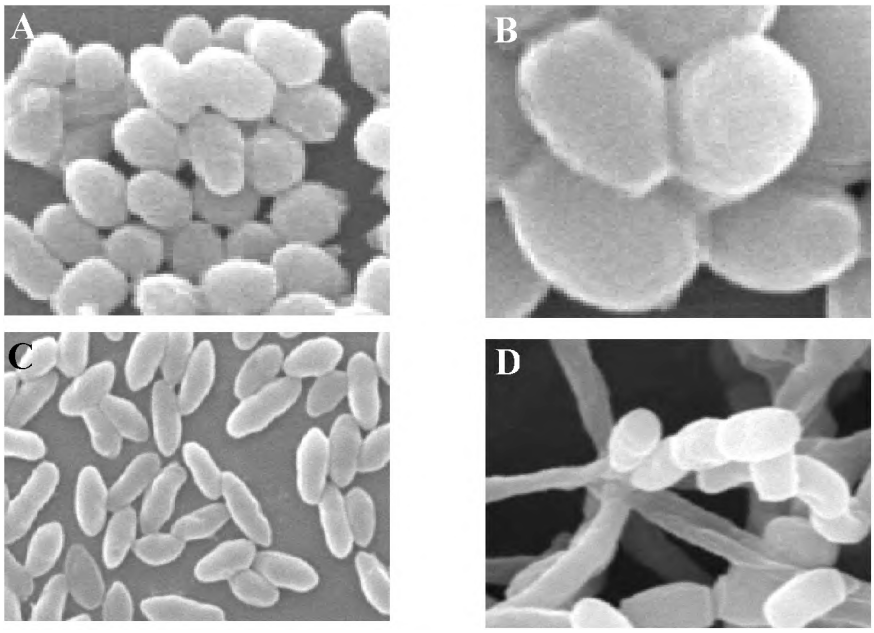

Figure 7: Three bacterial strains (A) DDiBDN11, (B) DDiBDN12, and (C) DDiBDN13 and actinomycete strain (D) XKDN18 isolated from aerobic consortium.

Aerobic consortium in shaking culture at 180 rpm/min and 37^{0}C after 9 days shows that 85.6 % of 164.45 pg TEQ/ml 2,3,7,8 TCDD was removed. Aerobic consortium could degrade 2,3,7,8 TCDD much fast than anaerobic consortium.

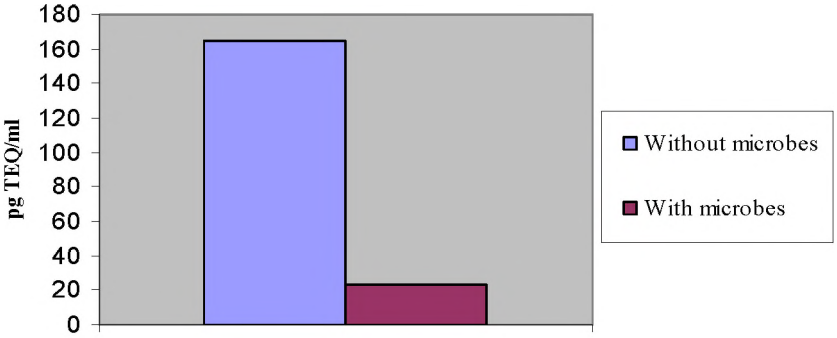

Figure 8: 2,3,7,8 TCDD degradation by aerobic consortium

2,3,7,8 TCDD degradation by filamentous fungal train FDN30

This actinomycete strain was observed in almost 9 microbial enumerations of 12 treatments and in soil of contaminated site. After two week shaking at 30° C, FDN30 degraded 43,45 pg TEQ/ml 2,3,7,8 TCDD. The strain could remove 59% adding dioxin to the medium. Other congeners that are less toxic were detected with elevation [Fig. 9].

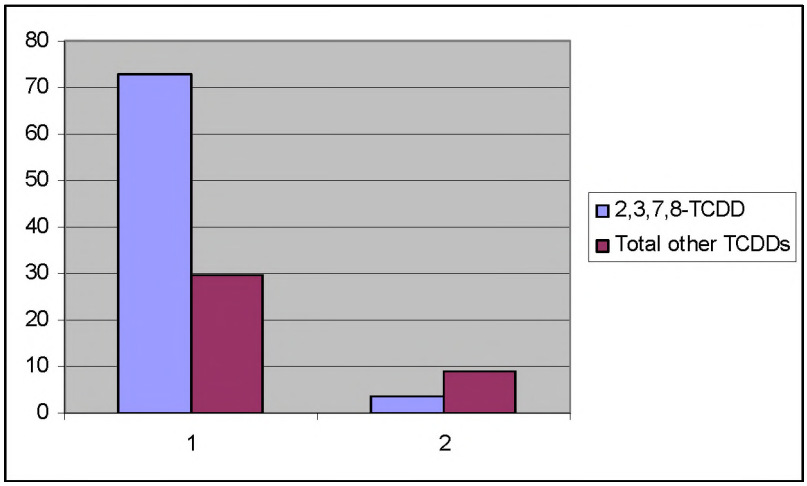

Figure 9: Dioxin biodegradation by FDN30

These findings may explain why high concentration of contaminants in treated soil was decreased after two year treatment. Indigenous microorganisms of long time exposure by dioxins and other contaminants play leading role in detoxification of 2,3,7,8 TCDD in contaminated soils. Obtained results driving us to develop further research in the enhancement of bioremediation for cleaning up dioxins and other pollutants in former military bases in Vietnam.

Acknowledgments

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