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DEGRADATION OF 2,3,7,8-TCDD BY A CONSORTIUM OF BACTERIAL STRAINS ISOLATED FROM HEAVIL HERBICIDE/DIOXIN CONTAMINATED SOIL IN BIENHOA AIRBASE

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SUMMARY

From two different soil sources in Bienhoa airbase (heavy herbicide/dioxin contaminated West-South region and bioremediated cell), five microbial strains were isolated and their 2,3,7,8-TCDD biodegrability in consortium was investigated. Based on the colony and cell morphological characteristics as well as 16S rRNA gene sequences, these strains were classified into 5 genera, including *Methylobacterium* (strain BHBi1), *Hydrocarboniphaga* (strain BHBi4), *Agrobacterium* (strain BHBi5), *Bosea* (strain BHBi7) and *Microbacterium* (strain BH09). Two strains BHBi7 and BHBi4 were the first representatives of the genera *Bosea* and *Hydrocarboniphaga* that were isolated from heavyly herbicide/dioxin contaminated soil. All five strains were able to grow well in mineral salt medium (MSM) supplemented with soil extract (SE) containing 2,3,7,8-TCDD (this congener is the main soil total compound toxicity) and other congeners, including PCDDs, PCDFs, 2,4,5-T, 2,4-D, PAHs and their intermediates. This microbial consortium degraded 2,537.34 ngTEQ/kg of 2,3,7,8-TCDD congener in soil, equivalent to 59.1% lost of total toxicity in comparison to the control without bacterial seeding (4,294.12 ng TEQ/kg). Such a high ratio of dioxin degradation by a bacterial consortium was reported here for the first time, contributing more evidences for convincing the successful dioxin bioremediation of “Active Landfill” technology at large scale in Z1 area at Bienhoa airbase, Dongnai, Vietnam.

Keywords: *Bienhoa airbase, bioremediation, herbicide/dioxin degradation, Bosea, Hydrocarboniphaga*

INTRODUCTION

During the Vietnam war, the US military sprayed more than 100 million liters of herbicides containing 1080 kg of dioxin in the Central and the Southern of Vietnam. After 43 years, a number of “hot spots” with high level of herbicide/dioxin still remains in Bienhoa, Danang, and Phucat military airbases.

There exist different dioxin and POP detoxifying technologies, of those thermal desorption and biological-based landfill and “active landfill” technologies were applied in Vietnam. The thermal desorption was carried out by Terra Thermal company (USA) in Danang airbase with the first step has just finished and is moving on the second. Obviously, this technology required high cost whereas its environmental impact has not yet been evaluated completely. Since 1999, active landfill bioremediation has been performed in Z1 region of Bienhoa, succeeded in detoxification of 3,384 m^3 herbicide/dioxin contaminated soil with total toxicity of 10,000 ngTEQ/kg. After 3 years of treatment, the total toxicity decreased to 14.12 ngTEQ/kg dried soil, met the requirement for agricultural soil according to Vietnam regulation QCVN 45:2012 (40 ngTEQ/kg) (Dang Thi Cam Ha *et al*., 2012). This technology based on stimulation of indigenous microbes and then combination with other technology, showing cost-effective and eco-friendly advantages. Another study using two dioxygenase-containing bacterial strains (US6-1 and IC10) for bioremediation of, a low dose of 100-200 ppt in Asho airbase resulted at 35% detoxification (Nguyen Ngoc Sinh *et al.,* 2017). Furthermore, Nguyen Duy Binh *et al.,* (2015) demonstrated a detoxification efficiency of 10 µg/ml 2,3,7,8-TCDD (reached up to 60%) after 17 week anaerobic and 6 week aerobic

treatment using a microbial consortium enriched from herbicide/dioxin contaminated soil in Bienhoa airbase.

The investigation of dioxin detoxification has been conducted in laboratory scale, focusing on less harmful and/or degradable dioxin in contaminated soil at the present time. Twenty three microbial strains isolated from soil containing polychlorinated dioxin (6.8-4,600 pgTEQ/g dried soil), dedicated dibenzofuran (DF) degrading capacity (Futamata *et al.,* 2014). Two strains *Sphingomonas* sp. HL7 and *Kliebsiella* sp. HL1 were shown to use DF as sole carbon source (Fukuda *et al.,* 2002). Some other bacterial strains were shown to involve indioxin degradation such as *Terrabacter* sp. strain DBF63, *Pseudomonas* sp. strain CA10 (Habe, 2001); *Pseudomonas* sp. HH69, *Sphingomonas wittichii* RW1, *Terrabacter* sp. DPO360 and *Burkholderia* sp. JB1 (Akira Hiraishi, 2003). Especially, the strain *Sphingomonas wittichii* RW1 proved its degrading ability of many dioxin congeners, including 2,3,7,8-TCDD (Fukuda, 2002) etc. Some *Rhodococcus* and *Pseudomonas* strains isolated from polluted soil were reported for the ability to degrade polychlorinated biphenyls (PCBs) (Garrido-Sanz, 2018). However, there is very little knowledge about bacterial that degrade of dioxin congener 2,3,7,8-TCDD. From the point of view that local bacterial community in dioxin contaminated soil play leading role in degradation, transformation and mineralization of the toxic compounds, studies on microorganisms originated from the contaminated sites and their degradation capability toward toxic compounds could provide very important scientific basics for scaling up dioxin detoxification *in situ* condition.

This study aims to look for bacterial strains from heavy herbicide/dioxin soil in Bienhoa airbase that can grow and degrade well in mineral medium containing herbicide/dioxin. Combination five bacterial strains detoxify of 2,3,7,8-TCDD will prove it.

METHODS AND MATERIALS

Materials

Two soil samples were used for bacterial isolation. The first sample was heavily herbicides/dioxin contaminated soil collected from Bienhoa airport (a mixture of soil from 5 hole in the West-Southern area with an average total toxicity was 21,605 ngTEQ/kg dry weight). The second sample was bioremediated soil collected from a successfully working bioremediation cell with after 40 month operation, the toxicity was as low as 14.12 ngTEQ/kg (the sample was a mixture of soil from 7 points in bioremediation cell).

The soil sample used for evaluating dioxin degradation was taken in contaminated sites with a total toxicity > 4,000 ngTEQ/kg.

Methods

Soil extraction (SE)

The soil samples were homogenized and dried absolutely with Na_2SO_4 and divided into vials. Subsequently, a mixture of methanol:toluene (1:4) (v/v) was added into vials with ratio 1:1 (w/v) and mixed for solvent permeation. Sonication was carried out and repeated at 60^oC, 90 kHz in 30 minutes, shaker at 110 rpm for 6 to 8 hours. The vials were let to stand for soil settle down for 5 to 7 hours before transferring the upper solution into new vials containing concentrated H_2SO_4. The mixture was shaken and the upper phase (soil extract, SE solution) was transferred into new flask for evaporation in the air. The SE solution was in yellowish brown color when the volume decreased 6 times after evaporation, ready for use in the further experiments (everage of total toxicity 3.500 pgTEQ/ml).

Bacterial enrichment and isolation

Contaminated soil samples were used as inoculate for the bacterial enrichment in mineral salt medium (MSM) supplemented with SE at a high concentration (ratio of SE in medium about of 500µl SE/1000ml medium). The enrichment was achieved via three times of subsequent transfer in the same culturing condition and the sample at the last transfer was used for isolation of bacteria. One hundred µl of the enrichment culture was spread out on MSM-SE agar and incubated at 30^oC in 3 days. Colonies of different shapes and colors appeared on the plates were picked up, purified and transferred into new MSM-SE tube. The bacterial growth in MSM was determined based on OD detection at wavelength of 610nm.

Bacterial classification based on 16S rDNA sequences

Genomic DNAs of the selected strains were extracted following the method of Sambrook and

Russell (2001). The 16S rRNA genes were amplified using Thermocycler Eppendorf Mastercycler personal/PTC 100. The PCR was carried out in 25 μL reaction volumes containing 1 μL DNA template (300 ng/μL), 15 μL Master mix 2x (Promega), and 1 μL (10 pmol/μL) of each primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Wilson *et al*., 1990). The thermocycle for the PCR has initial DNA denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 1 min, which was followed by a final extension at 72°C for 5 min. 5 μL PCR product was analyzed by electrophoresis in 1% agarose gel and purified by QIAGen kit

The sequencing of 16S rRNA gene was conducted using genetic analyzer (ABI Prism 3730 Genetic Analyzer). The sequences were edited to exclude the PCR primer binding sites and manually corrected using ClustalX software. The full gene sequences of five strains were compared with 16S rDNA sequences available in GenBank using the BLAST tool. The phylogenetic tree was constructed by using MEGA 5 version software.

Morphological characteristics

Cell morphology was observed under Scanning Electron Microscope – SEM. Gram stain was carried out with Gram's method (Colco, 2005).

Experiment on bacterial detoxification of 2,3,7,8TCDD

The five selected bacterial strains were co-inoculated in MSM with SE at 30°C, shaking at 150 rpm in 15 days and transferred 3 times. This complex was used as starter culture in this study. In order to minimize data input errors, the contaminated soil was homogenized and autoclaved at 121°C for 60 min (to kill microbial cells in the soil). A 500 ml flask containing 100 ml of MSM medium, 50 ml of tap-water, 1 ml of compost extract (dissolve 100 g matured compost produced from mixture of agricultural residual and chicken manure by using thermophilic *bacillus, streptomyces* and filamentous fungi in 200 ml of water), 50 µl of natural surfactant (preparation from 5 gram dry fruit containing biosurfactant dissolve in 20 ml water) and 1 ml extract (from fresh fruits and vegetables which pressed by ErgoMixx (Bosch). The obtained extracts was filtrated through a 0,5 µm milipore membrane. This mixture was supplemented with 200 g of sterile heavy herbicide/dioxin contaminated soil. A ratio of 15% of starter culture was added before shaking at 200 rpm, 30°C in 30 days. This experiment was conducted in triple. In the control sample, the bacterial consortium was replaced by distilled water. The conversion of toxic compounds was analyzed by HRGC/HRMS, Model DFS, Thermo Model SOP02/DXL, US EPA.

RESULTS AND DISCUSSION

Selection of bacterial strains for consortium

The bacterial strains isolated from two sources, the bioremediated soil and the enrichment culture of heavy dioxin contaminated soil were evaluated for their growth capacity on MSM-SE medium. The best five strains showing high cell density after on MSM-SE were selected, i.e. four strains BHBi1, BHBi4, BHBi5, BHBi7 originated from the bioremediated soil and the strain BHBO9 from heavy herbicide/dioxin contaminated soil.

Colony and cell morphology of the five selected strains were analyzed, showing that they were different to each other in color, size and shape (Figure 1, 2 and Table 1).

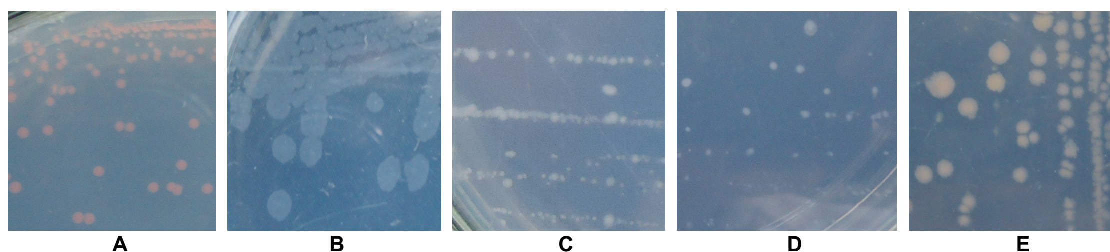


Figure 1. *Morphological colonies of five bacterial strains on MSM-SE medium.* A: BHBi1; B: BHBi4; C: BHBi5; D: BHBi7; E: BHO9.

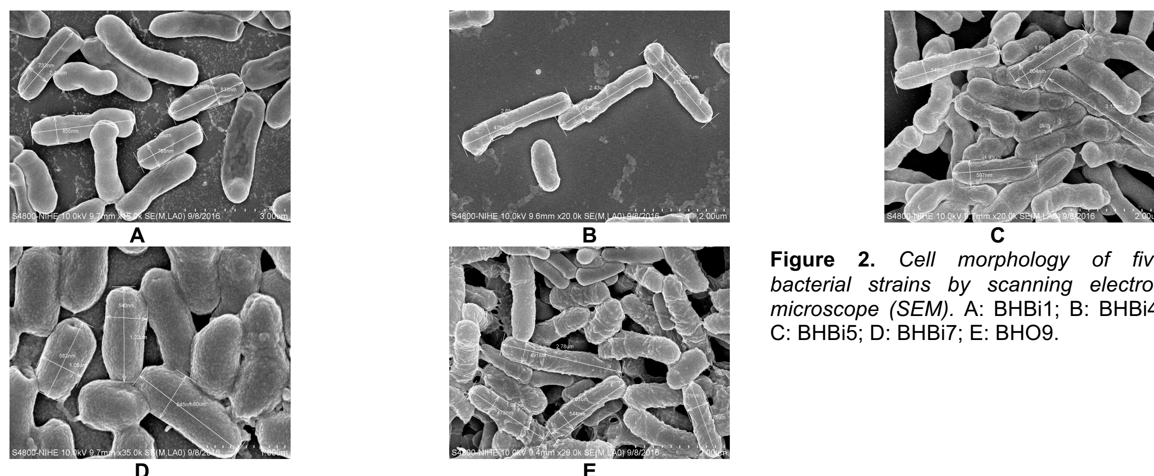


Figure 2. *Cell morphology of five bacterial strains by scanning electron microscope (SEM).* A: BHBi1; B: BHBi4; C: BHBi5; D: BHBi7; E: BHO9.

Table 1. Morphology characteristics of five selected bacterial strains

Strain	Colony color	Gram	Cell shape	Cell size (μm x μm)
BHBi1	Dark pink	Negative	Rod linear	2.15–3.15 x 0.73–0.86
BHBi4	Fuzzy, spangle	Positive	Rod, slightly curved at one end	1.31–3.01 x 0.45–0.49
BHBi5	White opaque	Negative	Long rod	1.93–3.13 x 0.55–0.6
BHBi7	White	Negative	Rod, short, rough surface	1.05–1.60 x 0.54–0.65
BHBO9	Orange	Negative	Single rod	1.75–2.78 x 0.47–0.54

Comparative analyses of 16S rRNA gene sequences of these strains show that five strains belonging to five different genera. Strain BHBi4 belonges to the genus *Hydrocarboniphaga* with 90% similarity with *Hydrocarboniphaga* sp. FSBRY8, strain BHBi5 belonges to the genus *Agrobacterium* with 98% similarity to *Agrobacterium* sp. Van101*,* strain BHO9 belonges to the genus *Microbacterium* with 100% similarity to the *Microbacterium* sp. Atl-19*,* strain BHBi7 belonges to genus *Bosea* with 85% sequence homology to the *Bosea* sp. CRIB-10, whereas strain BHBi1 is most closely related to species *Methylobacterium organophylum* with only 97% similarity to the *Methylorubrum rhodesianum strain* S3-128 (Figure 3). Representatives of these five genera are abundant in soil, however, so far *Bosea* (*Bosea* sp. BHBi7) and *Hydrocarboniphaga* (*Hydrocarboniphaga* sp. BHBi4) genera have never been reported to grow with herbicide/dioxin as the only carbon and energy sources.

Methylobacterium is an important genus frequently found in soil, on leaves and other plant parts. They often use methylamine, methanol, C2, C3, C4 compounds for growth (Lidstrom and Christoserdova, 2002). However, little is known for the *Methylobacterium* species isolated from herbicide/dioxin contaminated soil. Few strains of this genus have been reported to degrade some aromatic compounds, such as *Methylobacterium populi* VP2 isolated from the heavily PAH contaminated soil showing capacities of xenobiotic compound degradation and stimulating plant growth (Ventorino, 2014); *Methylobacterium mesophilicum* RD1 isolated from hydrocarbon contaminated tropical soil was capable of degrading engine oil (1,274.85 mg/L) at 65 mg/L day after 12 first days and 40 mg/L day after 9 following days (Salam, 2014). Beside soil and plant parts, hospital wastewater in Japan was proven to be a source for isolation *Methylobacterium* strains such as *M. aquaticum* and *M. fujisawaense* that contained antibiotic resistant genes (Furuhata, 2006).

Similarly, *Hydrocarboniphaga* genus has not been published for involving directly into herbicides/dioxin degradation till now. Palleroni *et al.,* (2004) found *H. effusa* sp. nov with a broad substrate spectrum, including phenol, toluene and other organic compounds. *H. effusa* AP103 originated from oil contamined soil in New Jersey, on the other hand, is capable to use n-alkanes as

carbon and energy sources. In the present study, strain *Hydrocarboniphaga* sp BHBi4 showed ability to grow on toxic substrate (herbicide/dioxin extracted soil) is reported for the first time. Ramos Monroy et al (2013) indicated a microbial consortium composed of *Hydrocarboniphaga* and *Methylobacterium* strains with efficiency in treatment of water containing three herbicides frequently found in agricultural runoffs (Ramos Monroy *et al.,* 2013).

Some *Bosea* species such as *B. vestrisii* 34,635^T, *B. eneae* 34,614^T and *B. massiliensis* 63,287^T were isolated from hospital water supplier showed their potentials not only in treatment of new infections but also in oxidation of thiosulphate, like in the case of *B. thiooxidans* (La Scola, 2003). *Bosea* species are mainly aerobic bacteria able to oxidize sulfur compounds and isolated from various sources such as soil (*B. thiooxidans* Bl-42), anaerobic digestion sludge (*B. minatitlanensis* sp) etc. (Das Subizata, 1996; Ouattara, 2003). Interestingly, strain *Bosea* sp BHBi7 of this study grew fast on MSM medium supplemented with SE and it might be a novel point in study of herbicide/dioxin degradability of *Bosea* sp BHBi7 in future.

In contrast, *Microbacterium* genus has been reported for inhabiting dioxin contaminated soil in Japan (Hiraishi A, 2003) as well as in Da Nang military airport (previous results). In addition, some strains such as *Microbacterium* ZD-M2 strains isolated from sludge could degrade 4,6-dimethyl-DBT, thiophene, benzothiophene and diphenylsulfide (Li, 2005). *Microbacterium* sp. BR1 was capable of breaking down sulphonamide antibiotics for use in industrial wastewater treatment (Benjamin, 2015). On the other hand, *M. esteraromaticum* sp. SL6 isolated from tropical hydrocarbon polluted soil was capable of oxygenation and mineralization of carbazole (Lateef, 2015).

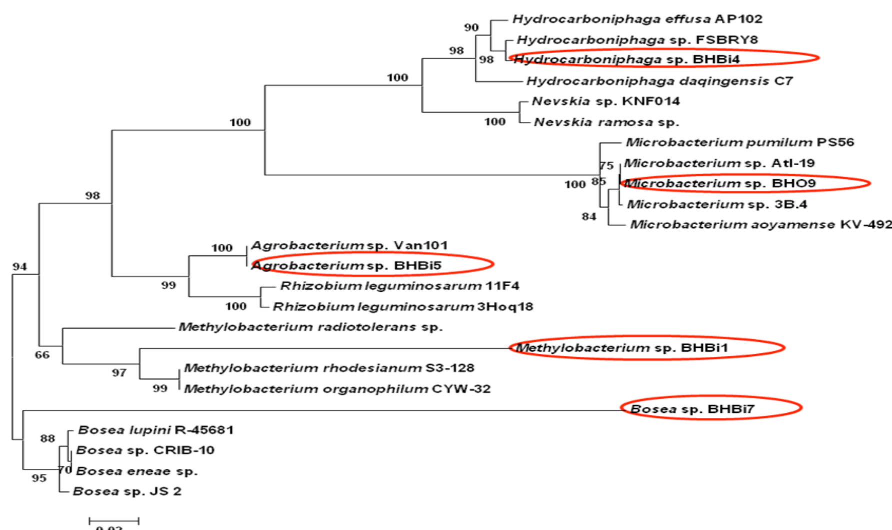


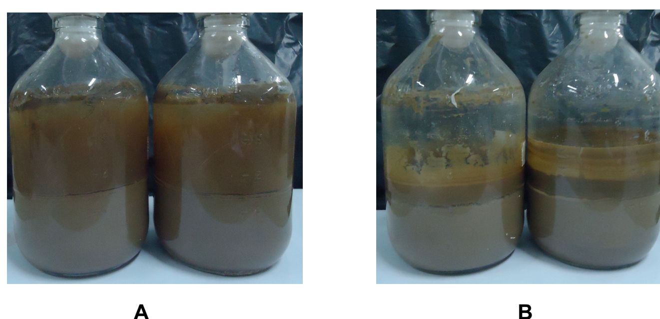
Figure 3. Phylogenetic tree showing taxonomic positions of the five bacterial strains

Degradability of herbicides/dioxin by consortium of the five selected bacterial strains

All five selected strains grew well on MSM containing 2,3,7,8-TCDD from herbicide/dioxin soil extracts, therefore, dioxin degrading efficiency of the consortium of these strains was carried out in laboratory scale. Significant differences between the culture with bacterial consortiun and control (without bacteria) bottles after 30 day incubation under shaking condition was appearance of dispersion in cultivated internal bottle wall (Figure 4). For quantitative assessment, total toxicity in whole mixture in flasks was analyzed by GC/MS (Table 2).

The degradation of 2,3,7,8-TCDD by microbes at high concentration in laboratory condition is not well known till now. Researchers have been mainly focusing on less toxic and more easily biodegradable compounds such as 2,3-dihydrogen isomers (TCDDs). According to previous results, *Rhodococcus* sp HDN3 isolated from herbicides/dioxin contaminated soil in Da Nang

could degrade completely DBF after 24 hours. In addition, *Terrabacter* sp. was known to degraded DMA and DBF (4 mM) up to 71.54% and 100% after 24 hours and 48 hours, respectively. Iida *et al.,* (2006) showed that *Paenibacillus* sp. YK5 can use 2 mM DBF as sole carbon source after 34 hours at 37°C. The *Nocardioides aerate* strain isolated from soil and dioxin-contaminated river sludge nearby an incinerator in Japan broke down 0.18 mM DBF after 96 hours at 30°C. Hong *et al.,* (2004) reported that *Pseudomonas veronii* PH-03 degraded 90.7%, 79.7%, 88.3% and 78.6% of DD; DBF; 1-MCDD and 2-CDD, respectively, after 60 hours cultivated with these compounds at initial concentration of 1 mM of each.



A **B**

Figure 4. Biodegradation of herbicide/dioxin by the consortium of five bacterial strains. A: Control sample without cultures; B: Biotreated samples with consortium of five bacterial strains

Several studies have also proven capacity of different microorganisms, including bacteria, actinomycete and fungi, in herbicide degradation, such as PAHs or/and dioxin congeners. *Pseudomonas* sp. BDN15 was known to degrade 39.37% of 2,4,5-T at an initial concentration of 1000 ppm after 90 day inoculation. Moreover, *Aspergillus* sp. FDN41 degraded 393.5 ppm of 2,4,5-T (1,500 ppm at initial cultivation) after 20 days. *Streptomyces* sp. XKDN19 degraded 78.7% of anthracene, 22.36% of fluoranthene at 100 ppm of each after 21 days, whereas *Streptomyces* sp. XKDN12 was able to convert 32.72% of phenanthrene, 39.01% of anthracene; and 39.01% of fluranthene after 7 days cultivated. (Nguyen Thanh Thuy, 2006; Nguyen Duong Nha, 2005; Dang Thi Cam Ha, 2008).

Recently, Briefly carrying out the 1ml reaction mixture contained 7.5 μM 2,3,7,8-TCDD dissolved in dimethyl sulfoxide (final concentration 5%) and the cell-free extract of *Geobacillus* sp. UZO3 (about 2 mg of protein). The enzymatic reaction was performed at 65°C 18 hours (Suzuki, 2016). Matsumura's group reported 2,3,7,8-TCDD degradability of *Bacillus megaterium* and *Nocardiopsis sp.* was as high as 49.7 and 29.4%, respectively, after 12 and 25 months incubation (Matsumura, 1983).

In this paper, consortium of the 5 selected bacterial strains showed 59.1% degradation of 2,3,7,8-TCDD (equivalent to 2,537.34 ngTEQ/kg dried soil) after 30 day inoculation under shaking condition (Table 2). With a high initial toxicity (> 4,000 ng TEQ/kg dried soil), the microbial consortium presented its special ability to grow on medium containing one of the most persistent dioxin congener 2,3,7,8-TCDD as sole carbon and energy sources. The average detoxification rate in laboratory scale reached 84.58 ng TEQ/kg/day, higher than that in previuos reports. Huynh Thi Mai Trang *et al.,* (2012) analyzed the detoxification of 13,400 pg TEQ/kg in 2.4 kg soil, and reported that the total toxicity still remained at 9,615 pg TEQ/kg, i.e. 28.2% was degraded with a rate of 39.4 pg TEQ/kg/day after 96 day cultivation.

Applying bioremediation technology in Danang contaminated soil Dang Thi Cam Ha et al (2005) showed that a remove rate of 40 - 100 pgTEQ/day was achieved in field trials of scales from 0.5 - 100 m^3 after 16 weeks of treatment, 44.1% of total toxicity was removed. Thus, 2,3,7,8-TCDD degradation efficiency performed by the microbial consortium in this study was higher than that in the field trial scale when extract of herbicide/dioxin contaminated soil was used as sole carbon and energy sources.

The results of this study provided more evidences explaining the success of the *in situ* biodegradation process via stimulating the indigenous microbes by feeding them with suitable nutrients and environmental condition for the most effective detoxification. Therefore, investigation of enhancing and accelerating the native microorganisms as well as suitable environmental condition for them is neccessary for targeting an effective biodegradation when scaling up of herbicide/dioxin as well as POP detoxification.

Table 2. Efficiency of 2,3,7,8-TCDD detoxification by bacterial consortium after 30 day cultivation

Bacteria	Total toxicity (ngTEQ/kg dried-soil)	Efficiency (%)
Control (without cultures)	4,294.12	0
Bacterial consortiun of five strains	1,756.78	59.1

CONCLUSION

Five bacterial strains were isolated from heavy contaminated herbicide/dioxin soil and bioremediated treatment cells in Bienhoa airport. These bacteria were classified as *Methylobacterium* sp. BHBi1, *Hydrocarboniphaga* sp. BHBi4, *Agrobacterium* sp. BHBi5, *Bosea* sp. BHBi7, *Microbacterium* sp. BH09 strains. The consortium of these five bacteria showed high efficiency of dioxin degradation. After 30 day cultivation in shaking culture, the consortium detoxified 2,537 ngTEQ/kg of 2,3,7,8-TCDD with 59,1% efficiency.

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REFERENCE

Binh ND, Imsapsangworn C, Oanh NT, Parkpian P, Karstensen KH, Giao PH, Delaune RD (2016) Sequential anaerobic-aerobic biodegradation of 2,3,7,8-TCDD contaminated soil in the presence of CMC-coated nZVI and surfactant. *Envir Technol* 37(3): 388-98.

Benjamin R, Oliver F, Hans-Peter EK, Andreas S, Philippe François-Xavier C, Boris AK (2015) Degradation of sulfonamide antibiotics by *Microbacterium* sp. strain BR1 – elucidating the downstream pathway. *New Biotechnology* 32(6): 710-715.

Daubaras, Danganan DL, Hubner CE, Ye A, Hendrickson RW, Chakrabarty W (1996) Biodegradation of 2,4,5-trichlorophenoxyacetic acid by *Burkholderia cepacia* strain AC1100: evolutionary insight. *Gene*. 179: 1-8.

Das S, Ajit K, Mishra, Brian J, Tindall, Fred, Rainey A, Erko (1996) Oxidation of Thiosulfate by a New Bacterium, *Bosea thiooxidans* (strain BI-42): Analysis of phylogeny based on chemotaxonomy and 16s ribosomal DNA sequencing. *Int JSystem Bacteriol* 46(4): 981-987.

Dang Thi Cam Ha, Nguyen Ba Huu, Mai Anh Tuan, Nguyen Duong Nha, Nguyen Quoc Viet, Nguyen Nguyen Quang (2008) Survey of microbial community in herbicide/dioxin contaminated site at Danang airport area and detoxification of contaminated soil under laboratory conditions by bioremediation technology, *J Biotechnol* 6(4A): 837-846(in Vietnamese).

Furuhata K, Kato Y, Goto K, Hara M, Yoshida S, Fukuyama M (2006) Isolation and identification of methylobacterium species from the tap water in hospitals in Japan and their antibiotic susceptibility. *Microbiol Immunol* 50(1):11-7.

Haugland RA, Schelenm DJ, Lyons RPIII, Sferra PR, Chakrabarty AM (1990) Degradation of the chlorinated phenoxyacetate herbicides 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid by pure and mixed bacterial cultures. *App Environ Microbiol* 56: 1357-1362.

Hiraishi A (2003) Biodiversity of dioxin-degrading microorganisms and potential utilization in bioremediation, *Microb Environ*, 18: 105-125.

La Thi Thanh Phuong, Nghiem Ngoc Minh, Dang Thi Cam Ha (2005) Degradation of 2,4,5-Trichlorophenoxyacetic acid by bacterial strain BDN15 isolated from toxic chemical contaminated soil. *J Biotechnol* 3(3): 389-396 (in Vietnamese).

La SB, Mallet MN, Grimont PA, Raoult D (2003) "*Bosea eneae* sp. nov., *Bosea massiliensis* sp. nov. and *Bosea vestrisii* sp. nov., isolated from hospital water supplies, and emendation of the genus *Bosea*". *Int J Syst Evol Microbiol* 53 (Pt 1): 15–20.

Lateef BS, Matthew OI, Olukayode OA (2015) Carbazole degradation in the soil microcosm by tropical bacterial strains. *Braz J Microbiol* 46(4): 1037-44.

Li W, Zhang Y, Wang MD, Shi Y (2005) Biodesulfurization of dibenzothiophene and other organic sulfur compounds by a newly isolated *Microbacterium strain* ZD-M2. *FEMS Microbiol Lett.* 247(1): 45-50.

Lidstrom, Mary E, Ludmila Ca (2002) Plants in the pink: Cytokinin production by *Methylobacterium*. *J Bacteriol* 184(7).

Mai P, Stig Jacobsen O, Aamand J (2001) Mineralization and co-metabolic phenoxyalkanoic acid herbicide by a pure bacterial culture isolated from an aquifer. *Appl. Microbiol. Biotechnol*. 56: 486-490.

Matsumura F, Quensen J, Tsushimoto G (1983) Microbial Degradation of TCDD in A Model Ecosystem. *Environ Sci Res* 26:191-219.

Nguyen Thanh Thuy, Hoang Thi My Hanh, Nghiem Ngoc Minh, Dang Thi Cam Ha (2006) Study on identification and degradng capability of the fungal strain FDN22 isolated from bioremediating soil toxic chemically contaminated, *Journal of Biotechnology,* 4(1), page.125-132. (in Vietnamese)

Nguyen Duong Nha, Nghiem Ngoc Minh, Nguyen Ngoc Bao, Dang Thi Cam Ha (2005) Degradation of polycyclic aromatic hydrocarbons (PAHs) and dibenzofuran by actinomyces XKDN12, *Journal of Biotechnology*, 3(1), page.123-132. (in Vietnamese)

Ouattara AS, Assih EA, Thierry S, Cayol JL, Labat M, Monroy O, and Macarie H (2003) *Bosea minatitlanensis* sp. nov., a strictly aerobic bacterium isolated from an anaerobic digester. *Int J Syst Evol Microbiol* 53: 1247-1251.

Palleroni NJ, Port AM, Chang HK, Zylstra GJ (2004) *Hydrocarboniphaga effusa* gen. nov., sp. nov., a novel member of the *γ-Proteobacteria* active in alkane and aromatic hydrocarbon degradation. *Int J Syst Evol Microbiol* 54: 1203–1207.

Ramos-Monroy O, Ruiz-Ordaz N, Galíndez-Mayer J, Juárez-Ramirez C, Nava-Arenas I, Ordaz-Guillén Y (2013) Operational Stability to Changes in Composition of Herbicide Mixtures Fed to a Laboratory-Scale Biobarrier. *Biotechnol Appl Biochem* 169(4): 1418-1430.

Sambrook J and Ruslee DW (2001) *Molecular Cloning. A Laboratory Manual*. 3rd ed. Cold Spring Harbor Laboratory Pree, Cold Spring Harbor, NY.

Salam LB, Obayori OS, Raji SA (2015) Biodegradation of Used Engine Oil by a *Methylotrophic Bacterium, Methylobacterium Mesophilicum* Isolated from Tropical Hydrocarbon-contaminated Soil, *Petrol Sci Technol* 33(2): 186-195.

Stellman JM, Stellman SD, Christian R, Weber TA, Tomassalla (2003) The extent and patterns of usage of agentorange and the herbicides in Vietnam. *Nature* 422: 681-687.

Suzuki Y, Nakamura M, Otsuka Y, Suzuki N, Ohyama K, Kawakami T, Umeka Y, Maninang JS, Izawa-Sato K, Hishiyama S, Inoue K, Kameyama T, Takahashi A, Katayama Y (2016) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) degradation by the thermophilic *Geobacillus* sp. UZO 3. *J Envir Biotechnol* 15(2): 105–108.

Ventorino V, Sannino F, Piccolo A, Cafaro V, Pepe O (2014) *Methylobacterium populi* VP2: plant growth-promoting bacterium isolated from a highly polluted environment for polycyclic aromatic hydrocarbon (PAH) biodegradation. *Sci World J* 2014.