Journal of Hazardous Materials 261 (2013) 351–361

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Bioremediation potential of soil contaminated with highly substituted polychlorinated dibenzo-*p*-dioxins and dibenzofurans: Microcosm study and microbial community analysis

Wei-Yu Chen, Jer-Horng Wu *, Yong-Yu Lin, Hung-Jun Huang, Juu-En Chang

Department of Environmental Engineering, National Cheng Kung University, Taiwan, ROC

HIGHLIGHTS

- We demonstrate that near-fully and fully chlorinated dioxins could be biodegraded.
- We uncover the microbial composition in the dioxin-degrading microcosm.
- Microbial populations are subjected to great dynamics when dioxins are degraded.
- We obtain four bacterial cultures that can degrade octachlorodibenzofuran.

ARTICLE INFO

Article history:
Received 5 April 2013
Received in revised form 10 July 2013
Accepted 16 July 2013
Available online 24 July 2013

Keywords:
Polychlorinated dioxins
Octachlorodibenzofuran
Bioremediation
Microbial community structure

ABSTRACT

Highly chlorinated dibenzo-*p*-dioxins/dibenzofurans (DD/Fs) are main hazardous dioxins, and ubiquitously distributed in the environment. To study the feasibility of bioremediation for remedying contamination of highly chlorinated dioxins, closed microcosms were constructed with soil from a chronological site under oxygen-stimulated conditions. The results showed that high levels of near-fully and fully chlorinated DD/Fs, particularly octachlorodibenzofuran were effectually reduced without accumulation of less substituted congeners. The clone library analysis of PCR-amplified 16S rRNA gene from the octachlorodibenzofuran-degrading consortia showed that 98.3% of the detected sequences were affiliated with *Proteobacteria*. The obtained strains with putative aromatic dioxygenase genes and abilities to repetitively grow in octachlorodibenzofuran-containing agars were closely related to members within *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. Among them, certain *Rhodococcus*, *Micrococcus*, *Mesorhizobium* and *Bacillus* isolates could degrade octachlorodibenzofuran with efficiencies of 26–43% within 21 days. Hierarchical oligonucleotide primer extension analysis further showed that *Micrococcus*, *Rhizobium*, *Pseudoxanthomonas*, and *Brevudimonas* populations increased largely when high concentrations of octachlorodibenzofuran were reduced. Overall, our results suggest that a distinctive microbial composition and population dynamic could be required for the enhanced degradation of highly chlorinated DD/Fs in the batch microcosm and highlight a potential of bioremediation technologies in remedying polychlorinated dioxins in the polluted sites.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs), which encompass a group of 210 congeners, are among the hazardous pollutants of most concern. These compounds, including 17 laterally substituted (2,3,7,8) congeners that are legislatively regulated, display potent endocrine-disrupting activity and are associated with numerous health disorders and carcinogenicity [1]. PCDD/Fs could be formed through various natural and anthropogenic combustion processes, rendering them ubiquitous in the environment [2]. Because of their hydrophobicity, recalcitrance, and strong partition to particles, the concentrations, although usually low in the soil (~ppb level), build up in soil, sediment, and biota. High concentrations of PCDD/Fs could be inadvertently generated with the manufacture of commercial chlorinated chemicals, such as pentachlorophenol (PCP) and chlorophenoxy pesticides [3]. This could lead to heavy pollution in an area, resulting from mass usage of chemical formulations, improper disposal, and storage and waste, or during accidents [4,5]. Therefore, remediation measures for such contaminated sites are required.

The microbiological approach represents an environmental friendly and cost-effective remediation technology for

* Corresponding author at: Department of Environmental Engineering, National Cheng Kung University, No. 1, University Road, Tainan City 70101, Taiwan, ROC. Tel.: +886 910385159; fax: +886 6 2752790.
E-mail address: enewujh@mail.ncku.edu.tw (J.-H. Wu).

0304-3894/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2013.07.039

PCDD/Fs-contaminated soil [6]. Several studies have demonstrated that chlorinated DD/Fs can be degraded by defined bacterial isolates or microbial consortia [7–11]. Under aerobic conditions, the bacterial catabolism of DD/F and lightly chlorinated counterparts is initiated by various aromatic ring-hydroxylating dioxygenases involved in the upper pathway, from which the catechol related monoaromatic metabolites can be yielded, and then transformed by the catechol dioxygenase in the lower pathway [12,13]. The degradation of highly chlorinated congeners is restricted to a few specialist strains, such as *Sphingomonas wittichii* RW1 and *Pseudomonas veronii* PH-03 [14]; however, chlorinated dioxins with 4–6 chlorine substitutions could only be co-metabolized under aerobic conditions or undergo the reductive dechlorination under anaerobic conditions [8]. The underlying knowledge was based on studies that focused mainly on low-chlorinated or the congeners that did not include the 17 toxic counterparts. The field investigations indicated that the highly chlorinated dioxin congeners, specifically hepta- and octa-chloro ones accounted for the majority of toxic dioxins in soil environments regarding total toxicity equivalency quantity (TEQ, the sum of the products of individual concentrations multiplied by their respective toxicity equivalency factors) and molar concentrations [15–18]. Despite a lower toxicity (toxicity equivalency factor is 10^3–10^4 times lower than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)) [19], the biotransformation of highly chlorinated dioxins (>6 chlorines) may lead to more toxic dechlorinated congeners [20]. Recently, simultaneous dechlorination and oxidative degradation of PCDD/Fs could be observed in the less anoxic microcosms [21,22]. However, the relevant microbial community structures were not sufficiently addressed.

The An-Shun site located close to the seacoast in the northwest of Tainan, Taiwan, was heavily contaminated with PCDD/Fs due to large production of PCP from 1965 to 1982 [5]. A site investigation uncovered that TEQ ranged from several to hundreds of milligram TEQ per kilogram of soil (mg TEQ/kg) in the hot-spot area, and the dominant forms of congeners were highly chlorinated, including heptachlorodibenzo-*p*-dioxin (H_pCDDs), heptachlorodibenzofuran (H_pCDFs), octachlorodibenzo-*p*-dioxin (OCDD), and octachlorodibenzofuran (OCDF) [23]. These levels of PCDD/Fs are at least 1000 times higher than the regulation standard (1 μg TEQ/kg). Because the degradation of fully or nearly fully chlorinated DD/Fs is poorly understood, the application of bioremediation technology to sites such as An-Shun remains challenging.

The main purpose of this study was to explore microbial potential for the bioremediation of PCDD/Fs at the An-shun site. This study demonstrates a rapid and effectual degradation of H_pCDD/Fs and OCDD/F in soil microcosms. Culture-dependent and culture-independent biological tools were used to characterize the community structure of microbial consortia that were capable of reducing high concentrations of OCDF. The bacterial populations over the timeframe of degradation were studied by using a quantitative hierarchical oligonucleotide primer extension method [24].

2. Experimental

2.1. Soil samples

In this study, soil samples (10–30 cm depth) were collected from the An-Shun site (120°07.467′E, 23°02.173′N) northwest of Tainan, Taiwan, and stored at the ambient temperature. The site was close to the seacoast and was contaminated with high concentrations of PCDD/Fs for decades [2]. The moist soils sieved with a 20-mesh stainless steel sieve (particle size <0.85 mm) were used in the experiments and were characterized as a sandy loam texture with a pH of 8.0, water content of 9.9%, total organic carbon of 1.17%, total nitrogen of 0.10%, phosphorus of 2.23 mg/kg dry soil, and chloride of 0.19–1.42 g/kg dry soil.

2.2. Batch microcosm assays

To inspire the activity of soil microorganisms, the first-batch soil microcosms were prepared by resuspending 10 g (wet weight) of soil with 20 ml of a minimal medium [25] containing (per liter) 2.2 g of Na_2HPO_4, 0.8 g of NH_2PO_4, 1.0 g of NH_4Cl, 0.2 g of $MgSO_4{\cdot}7H_2O$, 10 mg of $FeSO_4{\cdot}7H_2O$, 10 mg of $CaCl_2{\cdot}2H_2O$, and 20 mg of yeast extract (pH 7.2) in 125 ml serum bottles in duplicates. The headspaces of the serum bottles were flushed with a pure oxygen stream for 3 min, capped with Teflon-coated rubber septa, and subsequently sealed with aluminum caps to maintain oxygen-rich conditions (approximately 1.5 atm of oxygen) at the onset of the incubation. A total of 12 bottles were prepared and 6 (control bottles) were heat-sterilized twice at 1.21 atm and 121 °C for 30 min to inhibit microbial activity. The bottles were incubated at 28 °C in the dark in an orbital shaker that provided excellent mixing action (300 rpm). During the tested period, every two bottles with and without sterilization were opened at time 0, 6, and 12 weeks for the analysis of dioxin congeners, respectively. Because the bottles were tightly closed, the water content of samples remained relatively constant (about 70%) during incubation. After about 3 months (the bottles capped were kept static at 28 °C in the dark), microbial consortia (50%, v/v) were transferred using sterilized glass pipettes to serum bottles containing a fresh minimal medium and 50 mg of OCDF powder (purity >98%, purchased from AccuStandard Inc., New Haven, USA) for further enrichment. In the second-batch microcosm, the decapped bottles were replenished with oxygen and resealed with Teflon-coated rubber septa and aluminum caps, after the enrichment cultures of 2 ml were sampled using glass pipettes on a weekly basis. The slurry samples for microbial analysis were stored at −20 °C, whereas those for the PCDD/Fs measurement, whole slurry samples were air-dried, homogenized and preserved in the brown glass vials at 4 °C before analysis.

2.3. PCDD/Fs analysis

The concentrations of 17 toxic PCDD/Fs congeners (2,3,7,8-substituted) were analyzed using the isotope dilution high resolution gas chromatography–high resolution mass spectrometry (HRGC-HRMS) method described in [10,26]. Prior to analysis, the samples were subjected to the treatment procedures including internal standardization, Soxhlet extraction, Synder column concentration, and nitrogen gas vaporization. For the batch microcosm assay, soil samples of approximately 0.1–1 g (dry weight) were extracted for 24 h into a Soxhlet apparatus with HPLC-grade toluene. For the OCDF degradation by the isolated strains (see section 2.6), the whole culture samples were extracted three times (30 min each time) with hexane/acetone (1:1, v/v, HPLC grade), and concentrated on a rotary evaporator. Each extract was re-dissolved in hexane and cleaned on a column with layers of silica impregnated by sulfuric acid. The sample was subsequently split on a Carbopack C/Celite column, and the collected PCDD/Fs were dissolved in toluene. Seventeen stable isotope $^{13}C_{12}$- or $^{37}Cl_4$-labeled PCDD/Fs (Cambridge Isotope Laboratories and the Wellington Company) were spiked as the internal standards to ensure recovery efficiencies in the pretreatments. Quantification of PCDD/Fs was performed on a gas chromatograph (GC: Agilent 6890 series) equipped with a capillary column, CP-5MS (60 m × 0.250 mm × 0.25 μm) and a high-resolution mass spectrometer (HRMS: JMS 700D, JEOL, Japan). The temperature was programmed as follows: the injector was set at 280 °C, the initial oven temperature was 170 °C (maintained for 5 min), heated to 220 °C at 20 °C/min (maintained for 5 min), heated