Environmental Pollution 190 (2014) 43–50

Contents lists available at ScienceDirect

Environmental Pollution

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

Paddy field – A natural sequential anaerobic–aerobic bioreactor for polychlorinated biphenyls transformation

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ARTICLE INFO

Article history:
Received 22 January 2014
Received in revised form
11 March 2014
Accepted 19 March 2014
Available online 11 April 2014

Keywords:
Paddy field
E-waste
Sequential
Bio-dechlorination
Biodegradation

ABSTRACT

The environmental pollution and health risks caused by the improper disposal of electric and electronic waste (e-waste) have become urgent issues for the developing countries. One of the typical pollutants, polychlorinated biphenyls (PCBs), is commonly found in farmland in Taizhou, a major hotspot of e-waste recycling in China. This study investigated the amount of PCB residue in local farmlands. Biotransformation of PCBs was further studied under different water management conditions in paddy field with or without rice cultivation, with a special focus on the alternating flooded and drying processes. It was found that paddy field improved the attenuation of PCBs, especially for highly chlorinated congeners. In the microcosm experiment, 40% or more of the initial total PCBs was removed after sequential flood–drying treatments, compared to less than 10% in the sterilized control and 20% in the constant-drying system. Variation in the quantity of PCBs degrading and dechlorinating bacterial groups were closely related to the alteration of anaerobic–aerobic conditions. These results suggested that alternating anoxic–oxic environment in paddy field led to the sequential aerobic–anaerobic transformation of PCBs, which provided a favorable environment for natural PCB attenuation.

1. Introduction

Crude recycling of electric and electronic waste (e-waste), which releases heavy metals and persistent organic pollutants (POPs) into the environment, continues to be a serious problem in the developing countries (Stone, 2009; Ogunseitan et al., 2009). Taizhou City, one of the largest e-waste disposal centers in China (with an area more than 17.8 km^2), has a nearly 35-year recycling history. There, soil contamination by polychlorinated biphenyls (PCBs), which originates as electrical condensers and transformers are dismantled, is ubiquitous (Shen et al., 2008, 2009). Previous investigation has found severe partial contamination of PCBs near the recycling sites (Shen et al., 2008). Meanwhile, PCB pollution is widespread, though in comparatively low levels, in local farmland (Tang et al., 2009). This is likely to be a consequence of direct input from crude household workshops and indirect input from atmospheric deposition as well as polluted irrigation water, respectively (Harner et al., 1995). The PCBs pose not only a pollution risk to agricultural products but also a great threat to farmland ecosystem (Xing et al., 2010; Zhao et al., 2006). Due to its toxicity, mutagenicity, carcinogenicity, and teratogenicity (Safe, 1993), the behavior and environmental fate of PCBs in farmland are of special significance in terms of food safety.

Rice is the major source of food for as much as 60% of the world's population (Kiritani, 1979), and China is the world's largest rice producer (Kögel-Knabner et al., 2010). Most of the rice growing areas are managed as paddy fields, which are anaerobic during the period of plant growth and aerobic during the period between the harvest of the mature crop and the following planting of rice seedlings (Liesack et al., 2000). In our study area in Taizhou, crop rotation, which is applied by planting vegetables during the first half of the year and single-crop late rice in the second half of the year, also gives rise to a succession of dry and flooded conditions in paddy field. Repeated replacement of reductive and oxidative conditions can be formed as a result of water table fluctuations, O_2 diffusion through the water column and O_2 transport through above ground plant tissues into the rhizosphere (Armstrong, 1971). Such alternation of redox condition in paddy field could induce

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http://dx.doi.org/10.1016/j.envpol.2014.03.018
0269-7491/

staggered appearance of aerobes, facultative and obligate anaerobes, which are expected to exhibit commutative predominance of quantity and activity in different time–space (Liesack et al., 2000; Brune et al., 2000). In addition, increased quantities of readily available carbons derived from root exudation and altered pH and aeration conditions in rice rhizosphere could further result in promotion of microbial activity (Walker et al., 2003).

Though the more chlorinated biphenyls that pose high environmental risk are extremely resistant to degradation, they may be potentially transformed through halorespiration, which involves naturally occurring microorganisms that use PCBs as an electron acceptor and thus obtain energy (Holliger et al., 1998). It is generally accepted that highly chlorinated congeners can only be fully biodegradable in a sequence of anaerobic reductive dechlorination followed by the aerobic mineralization of less chlorinated products (Abramowicz, 1990; Field and Sierra-Alvarez, 2008). Researchers have proposed and implemented the combination of these two processes to effectively destroy PCBs (Evans et al., 1996; Master et al., 2001). However, thus far, studies involving sequential PCB transformation have relied on the preliminary enrichment of functional guilds or bioaugmentation, which is not suitable for a large-scale application in situ (Payne et al., 2013).

Based on results of our field investigation, it is hypothesized that in paddy field, anoxic environment induced by flooding is necessary to produce the redox condition required for reductive dechlorination, without compromising the cultivation of rice. Oxic conditions formed during fallow period provide a favorable environment for subsequent degradation of dechlorination products. Furthermore, PCB transformation is enhanced and accelerated in rice planted soil with ameliorated micro-environment. The results from this study will improve our knowledge regarding the fate of PCBs in paddy field and confirm the function of paddy field as a natural sequential anaerobic–aerobic bioreactor for PCBs transformation.

2. Materials and methods

2.1. Field investigation

In our study area in Taizhou, drylands and paddy fields had a random geographical distribution and tend to co-exist. Surface soils (0–20 cm) were collected from 34 locations in the town of Fengjiang (approximately 10 km^2), Taizhou city. Sampling avoided area with direct impact of PCBs and was designed to characterize the widespread contamination in local agricultural soil from e-waste recycling. A global positioning system was employed to identify the precise location of each sample. At each sampling site, five sub-samples were collected from a 100×100 m^2 plot and homogenized in pre-cleaned aluminum containers to obtain an approximately 1000 g representative sample. Pebbles and twigs were removed and samples were stored at −20 °C.

2.2. Microcosm experiment under simulated flooded and dry conditions in paddy field

Surface soils (0–20 cm) for the microcosm experiment was collected from a paddy field in Taizhou e-waste recycling area. The soil was mainly contaminated by a mixture of commercial PCBs. According to the grain size analysis, soil was silty sandy loam. Soil pH (1:2.5 water) was 6.95.

The microcosm experiment was performed in pots. The pots were placed at random on the same bench in a greenhouse with temperature (25 °C) and relative humidity (60%) control.

The experiment consisted of four treatments: rice cultivation under sequential flooded–dry condition (R), no rice cultivation under sequential flooded–dry condition (U), no rice cultivation under constant dry condition (D) and a sterilized soil control without plants (S). Sequential flooded–dry conditions referred to the treatments with a prior waterlog incubation, following by a drying cultivation. For the cultivated treatment, the Xiuyou 5 variety of rice seed (*Oryza sativa*) was used. Two rice seedlings were transferred to each pot that contained 2.0 kg soil. A water layer of 5 cm above the soil surface was maintained during the flooding period. The plants were harvested after 180 days. The shoots were removed by cutting above the soil surface, and soil moisture content was then adjusted to 60% for another 60 days. Therefore, the paddy soil underwent alternating submerged and drained conditions. The same water management was also set for the soils in the U and S treatments. However, soil moisture content was consistently kept at 60% throughout the incubation for the constant drying treatment (D). No mineral nutrients were added to the soil during the whole experimental period. Three replicates were performed for each treatment.

Five sub-samples were collected in deep areas of pots at different intervals (50, 100, 180 days after seedling transplant and flooding 60 days after rice harvested and drying) using a sterile cork borer and 5 g of soils were taken from each pot. The three sampling times during the rice cultivation period corresponded, respectively, to three rice growth stages: the tillering, booting and maturation stages. For ease of making comparison among each treatment, unified nomenclature of sampling intervals which accorded with the rice growing stages was also applied in unplanted treatment. Soil turned dry after rice harvest, and the drying periods were referred to as drying stage. Soil samples were freeze-dried overnight and stored at −20 °C until to residual PCB concentration analysis and microbial molecular analysis.

2.3. Chemical analyses

For PCBs analysis, 5.0 g soil was extracted in a Soxhlet apparatus for 24 h with 250 mL of a hexane-acetone (1:1, v/v) mixture. The extracted solutions were subsequently concentrated to 4–5 mL by a rotary evaporator. The extracts were passed through a column packed with layers of florisil and anhydrous sodium sulfate. Each eluate was evaporated to 1 mL prior to further analysis. Congener-specific PCB analysis was performed on an Agilent 7890 gas chromatograph (Agilent, USA) equipped with a ^{63}Ni electron-capture detector (Shen et al., 2009). PCBs were quantified by using customized calibration standards prepared from the AccuStandard Aroclor 1242 plus Aroclor 1254 as described (Frame et al., 1996). All procedures were rigorously monitored for quality control in order to meet the USEPA requirements.

The variation in soil redox potential with depth in rhizosphere and bulk soil was measured with a microelectrode (Unisense, Aarhaus, Denmark).

2.4. DNA extraction and qPCR

Total genomic DNA was extracted from 0.25 g of each soil sample and the control according to the manufacturer's instructions (Power SoilTM DNA Isolation Kit, MoBio Laboratories Inc., Carlsbad, CA). The concentration of the nucleic acid was determined by a Nanodrop-1000 instrument (NanoDrop Technologies, Wilmington, DE, U.S.).

As is well known, the oxidative degradation of PCBs or biphenyl in aerobic bacteria consists of four steps. Four enzymes catalyze these steps, among which *bphA* is a large subunit of the biphenyl dioxygenase catalyzing the initial step in the oxidative pathway for PCBs degradation by inserting a molecule of oxygen into the aromatic ring to form a chlorinated cis-dihydrodiol. And *bphC* is the component of 2,3-dihydroxybiphenyl dioxygenase which involved in extradiol meta cleavage of biphenyls and PCBs (Abramowicz, 1990). Usually, it is an efficacious strategy for determining the potential for in situ bioremediation of PCBs by the assessment of *bphA* or/and *bphC* gene diversity on field samples (Erb and Wagner-Dobler, 1993; Hoostal et al., 2002). Therefore, based on existing information, we aligned all complete sequences of gene *bphA* and *bphC* retrieved from NCBI database, and then obtained sequences with high similarity via MEGA and CLUAX constructed evolutionary tree. Finally, qPCR primers targeted for microbes carrying *bph* degradation gene were designed on the base of aforementioned grouped sequences via Primer Premier. They could cover almost all microbes carrying *bphA* or/and *bphC* gene. qPCR targeting a broad range of species within the "dechlorinating Chloroflexi" including *Dehalococcoides* spp. and o-17/DF-1 group 16S rRNA genes was performed with primers and conditions described previously by Sower et al. (2006) and with minor modifications. Wherein the forward primer C-DehalF (CGCTTTAAGTGTCCCGCC) is specific for putative dechlorinating species and reverse primer C-1100R (GGGTTGCGCTCGTTG) is used to detect bacterial in general. A calibration curve was obtained by using 10-fold serial dilutions of known plasmid (pEASY-T1) DNA concentrations. The standard curves spanned a range of 10^2–10^8 gene copies per μL of template DNA. Nuclease-free water or plasmid without an insert was used as the negative control.

By testing, it was found that a variety of targeted PCBs degrading organisms were under the detection limit or in a small number (Tables S1 and S2). Therefore, according to the quantitative results, primer C3 was chosen to represent the abundance of aerobic PCBs degraders in paddy field.

2.5. Statistical analyses

The means were compared using Student–Newman–Keul's multiple comparison test. ANOVA was used to investigate the interactions between PCBs and PCB-degrading bacteria, as well as their effects on the dissipation of PCBs in soil. The statistical analyses were carried out using SPSS version 18.0.0 (SPSS Inc., Chicago, USA).

3. Results

3.1. In situ PCB dissipation in polluted farmlands

Fourteen paddy field samples and twenty dryland samples were used for analysis. The amount of PCBs in each sample site is shown