Bioresource Technology 102 (2011) 5905–5911

Contents lists available at ScienceDirect

Bioresource Technology

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

Response of compost maturity and microbial community composition to pentachlorophenol (PCP)-contaminated soil during composting

Guangming Zeng [a,b,*], Zhen Yu [a,b], Yaoning Chen [a,b,*], Jiachao Zhang [a,b], Hui Li [a,b], Man Yu [c], Mingjie Zhao [a,b]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China
[b] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, PR China
[c] Environmental Resources and Soil Fertilizer Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, PR China

ARTICLE INFO

Article history:
Received 16 December 2010
Received in revised form 10 February 2011
Accepted 22 February 2011
Available online 24 February 2011

Keywords:
Composting
Compost maturity
Pentachlorophenol
DGGE
Microbial community composition

ABSTRACT

Two composting piles were prepared by adding to a mixture of rice straw, vegetables and bran: (i) raw soil free from pentachlorophenol (PCP) contamination (pile A) and (ii) PCP-contaminated soil (pile B). It was shown by the results that compost maturity characterized by water soluble carbon (WSC), TOC/TN ratio, germination index (GI) and dehydrogenase activity (DA) was significantly affected by PCP exposure, which resulted in an inferior degree of maturity for pile B. DGGE analysis revealed an inhibited effect of PCP on compost microbial abundance. The bacteria community shifts were mainly consistent with composting factors such as temperature, pH, moisture content and substrates. By contrast, the fungal communities were more sensitive to PCP contamination due to the significant correlation between fungal community shifts and PCP removal. Therefore, the different microbial community compositions for properly evaluating the degree of maturity and PCP contamination were suggested.

1. Introduction

Pentachlorophenol (PCP) is a highly chlorinated organic compound which has been extensively used as wood preservative, pesticide and general biocide in agriculture and industry. Although most of PCP in the environment is utilized and destroyed, a high percentage is released into the water and soil, representing a potential environmental hazard (Alexander, 1995; Scelza et al., 2008). As with many organic compounds, PCP can be irreversibly bound to soil. As a result, a legacy of contaminated soils requiring attention exists, and a variety of remediation techniques, including the physical and chemical remediation strategies and biological techniques, have been developed for the clean-up of PCP in soils (Semple et al., 2001).

As a promising bioremediation technology, composting has been successfully applied to the bioremediation of PCP-contaminated substances with the evidence that mineralization of the xenobiotic (Laine and Jorgensen, 1997) or the maturity and stability of composts (Jiang et al., 2006) is/are achieved. A composting bioremediation strategy relies on mixing the contaminated substances with other necessary materials, wherein the pollutants are degraded by the active microflora as the compost matures (Semple et al., 2001). Currently, compost maturity has been widely recognized as one of the most important factors concerning the compost process and the application of these by-products (Chikae et al., 2006). It could be a criterion for the success of bioremediation of PCP-contaminated soil and mineralization of the xenobiotic compound PCP by composting.

Microorganisms are the essential factors for the successful operation of composting, in which biodegradable organic wastes are stabilized and converted by the activities of all kinds of microorganisms under controlled conditions (Khalil et al., 2001; Tang et al., 2004). Usually, microorganisms that tend to dominate within the contaminated ecosystems are those capable of utilizing and/or surviving toxic contamination. The structure and diversity of the dominant microbial communities changed substantially (MacNaughton et al., 1999). Hence, in addition to studies of the bioavailability of hazardous chemicals, investigations of the stress of these chemicals, PCP included, are needed to properly understand the shifts in microbial communities.

Up to date, several studies have been carried out to explore the effect of PCP on the microbial community composition in soils. Relative abundance changes of microbial communities have been detected in the PCP-amended soils (Mahmood et al., 2005). While, microbial community succession in the composting system might be different from that in the soil. Microorganisms present in compost are metabolically active and able to degrade contaminants under optimized composting conditions (Antizar-Ladislao

* Corresponding authors at: College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China. Tel.: +86 731 88822754, fax: +86 731 88823701.
E-mail addresses: zgming@hnu.cn (G. Zeng), imscyn@263.net (Y. Chen).

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.02.088

et al., 2007; Miller et al., 2004; Tang et al., 2009). Thus, the implementation of composting technology as a remediation strategy requires an understanding of the succession of compost microbial communities. Advanced molecular biological techniques such as polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) have proved useful in detecting this succession (Cahyani et al., 2003; Nakasaki et al., 2009). The effects of different treatment regimes on the microbial community composition can be evaluated using such technique at molecular level (Vivas et al., 2009).

For complete elucidation of the biota responsible for the bioremediation of PCP-contaminated soil, the present study aimed at revealing the advances in composting process. This was accomplished by evaluating the compost maturity with such parameters as the ordinary physico-chemical properties, dehydrogenase activity (DA), germination test and PCP removal, and the microbial community composition using PCR–DGGE analysis.

2. Methods

2.1. Composting materials

PCP was purchased from American ADL Co. with a purity >98%. The raw soil was obtained from Yuelu Mountain (Changsha, China). The soil was air-dried and ground to pass through a 2 mm sieve, and then stored at 4 °C in amber-colored jars. The common physico-chemical characteristics were as follows: water holding capacity 26.1%, organic matter (OM) 9.5%, total organic C (TOC) 5.5%, total N (TN) 0.24%, and pH 4.73.

A stock solution of PCP (10 g L^{-1}) was prepared in acetone and stored under refrigeration at 4 °C. Approximately 100 g of the sieved soil was spiked with 40 ml of acetone and 9.8 ml of the PCP-stock solution. Soil was gradually added to a 2 L specimen container in 100 g aliquots and extensively mixed with the spiked soil. This procedure was repeated until the entire amount of soil (1 kg) was added and mixed. The specimen containers were hermetically closed and left to shake for inversion. Then the acetone was left to evaporate overnight under a flow hood. The final concentration of PCP-spiked soil was 133 mg kg^{-1} (dry weight).

The other composting materials were collected from suburb of Changsha, China. Rice straw, which was air-dried and cut into 10–20 mm lengths, was used as recalcitrant organic composting materials. Several kinds of vegetables chopped into 10–20 mm pieces were used as easy metabolizing materials. Bran was used to adjust the initial TOC/TN ratio of composting.

2.2. Composting set-up and sampling

Two experimental composting piles were set up indoors in this study. Soil, rice straw, vegetables, and bran were homogenized at a ratio of 38:43:12:7 (moist weight). Soil contained in the control (pile A) was free from PCP contamination. In the treatment (pile B), the raw soil was replaced by PCP-contaminated soil. The initial concentration of PCP in compost mixture was 50 mg kg^{-1} (dry weight). These compost materials (40 kg moist weight) were packed in 120 dm^3 polyethylene bins (65 cm long, 45 cm wide, 42 cm height). The dimension of the bins ensured adequate homogenization of the initial mixture of composting materials and self heating. The initial OM content and TOC/TN ratio of this mixture were 58% and 30:1, respectively. The initial water content was adjusted to approximately 60% and composts were monitored every 3 days throughout the process, with sterile deionized water being added when needed to maintain appropriate moisture. To provide some aeration, the piles were turned twice a week during the first 2 weeks and then once a week afterwards.

The experiment was conducted for 60 days. Samples were collected on day 1, 3, 6, 9, 15, 21, 36 and 60, respectively. Combination samples of 5–10 different subsamples in each pile were mixed by shaking and sieved through an 8 mm sieve. Samples for total DNA extraction were stored immediately at −20 °C until use.

2.3. Composting parameters and PCP analyses

Average temperature was monitored daily by inserting a thermometer into five different locations of the piles. The pH was determined after mechanically shaking the fresh sample in water suspension at a ratio of 1:10 (w/v) at 200 rpm for 40 min. Moisture content was measured after drying the samples overnight at 105 °C. The dried sample was analyzed for TOC by dry combustion at 550 °C, and TN content by the Kjeldahl method (K435, Buchi, Switzerland).

Water soluble carbon (WSC) and germination index (GI) were determined according to the method described by Cayuela et al. (2009) and Jiang et al. (2006), respectively. DA was measured with the substrate of 3% of 2,3,5-triphenyltetrazolium chloride following the modified Thalmann method (Benito et al., 2003).

PCP was extracted with acetone and *n*-hexane as described by Scelza et al. (2008). The PCP was analyzed and quantitated by HPLC (Agilent Technologies, USA) using an UVD detector and an Eclipse XDB-C18 (4.6 × 150 mm) column with 5 μm particle size. Analysis was conducted using 68% of acetonitrile and 32% of buffered water (1% acetic acid) as mobile phase with a column temperature at 25 °C and 1.0 ml min^{-1} flow rate. Detection was carried out at 220 nm.

2.4. DNA extraction and PCR–DGGE

Total genomic DNA was extracted according to the method described by Yang et al. (2007). Fragments of 16S rDNA and 18S rDNA genes were amplified with bacterial universal primers 338F/518R (Muyzer et al., 1998) and fungal universal primers NS1/Fung (Hoshino and Morimoto, 2008), respectively. GC clamp was attached to the forward primers to prevent complete separation of the strands during DGGE.

The PCR reaction mixture was prepared with 1 μl of template DNA, 5 μl of 10×PCR buffer, 1 μl of dNTPs (10 mM each), 10 pmol of each primer, 2 μg of bovine serum albumin, 2 U *Taq* polymerase, and adjusted to a final volume of 50 μl with sterile deionized water. The PCR thermal cycling scheme of 16S rDNA consisted of 5 min at 94 °C, then 45 s at 94 °C, 40 s at 56 °C, 40 s at 72 °C (35 cycles) followed by a final chase at 72 °C (10 min), and end at 4 °C. The 18S rDNA PCR program consisted of 5 min at 94 °C, then 45 s at 94 °C, 50 s at 55 °C, 1 min at 72 °C (35 cycles) followed by a final chase at 72 °C (7 min), and end at 4 °C.

DGGE was carried out using a Dcode Universal Mutation Detection System (Bio-Rad, USA). Approximately equal amounts of PCR samples (30 μl) were loaded onto the 0.75 mm thick 8% (w/v) polyacrylamide gels in 1×TAE buffer using a denaturing gradient ranging from 30% to 65% and 15% to 50% for bacterial and fungal PCR samples, respectively. Electrophoresis was performed at 60 °C and 120 V for 12 h. After stained with SYBR Green I nucleic acid gel stain for 30 min, the gels were scanned and analyzed for understanding the DGGE profiles.

2.5. Data analysis

All data were expressed on dry basis except for moisture, which was expressed on a moist basis.

Three replicates were used for each analysis. Data were presented as the mean values of triplicates. Student–Newman–Keuls test (S–N–K test) was performed to compare the mean values of