Bioresource Technology 255 (2018) 16–21

Contents lists available at ScienceDirect

Bioresource Technology

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

Degradation of organophosphate esters in sewage sludge: Effects of aerobic/anaerobic treatments and bacterial community compositions

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ARTICLE INFO

Keywords:
Organophosphate esters
Biodegradation
Treatment
Microbial communities
Sewage sludge

ABSTRACT

In this study, the degradation of organophosphate esters (OPEs) in sewage sludge with aerobic composting and anaerobic digestion was investigated. The total concentrations of six OPEs (ΣOPEs) in the whole treatment process reduced in the order of anaerobic digestion combined with pig manure (T3) > aerobic composting combined with pig manure (T1) > aerobic composting (T2) > anaerobic digestion (T4). The addition of pig manure significantly enhanced the removal rate of OPEs in both aerobic and anaerobic treatments. The abundance and diversity of bacterial community reduced after the treatment process. Shannon index, principal component analysis, network analysis, and heat map further confirmed the variation of bacterial community compositions among different treatments. Five genera (i.e., *Flavobacterium*, *Bacillus*, *Alcaligene*, *Pseudomonas*, and *Bacillus megaterium*) might be responsible for the degradation of OPE compounds in sewage sludge.

1. Introduction

Organophosphate esters (OPEs) are widely used as flame retardants and plasticizers in recent years. Because some polybrominated diphenyl ethers (PBDEs) have been totally forbidden by European Union since 2008, the production and usage of OPEs as an important substitution of flame retardants are surging from 204,000 tons in 2005 to 370,000 tons in 2013 (http://www.cefic-efra.com). Generally, OPEs are physically mixed into the appliances instead of chemically bonding and thus they are prone to be released into the environment via abrasion, dissolution, and volatilization (Cristale et al., 2013). Hitherto, different pollution levels of OPEs have been found in aquatic, sewage sludge, terrestrial, and atmospheric environments (Brommer and Harrad, 2015; Matsukami et al., 2015; Pang et al., 2016). Furthermore, previous studies have also found the presence of OPEs and their metabolites in human hair, nail, and urine (Butt et al., 2016; Hoffman et al., 2015). Since the potential risks of OPEs pollution such as carcinogenicity, neuro-toxicity, endocrine disruption and reprotoxicity (Dishaw et al., 2011; Meeker and Stapleton, 2010), OPEs are regarded as a class of emerging pollutants.

Generally, the effluent from wastewater treatment plants (WWTPs) is considered as one of the main contributors for OPEs pollutions to aquatic environment (Fries and Puttmann, 2003). The adsorption on the waste activated sludge is one of the important factors on the removal of OPEs during the wastewater treatment process and thus relative high levels of OPEs enriched in the sludge (Liang and Liu, 2016). Vast amount of sewage sludge generated from WWTPs without treatment would cause serious environmental problems. Composting is an effective way to realize the sludge recycling and harmless disposal (Awasthi et al., 2017; Soobhany et al., 2017). Generally, sewage sludge product is suitable to be used as a great substitute for chemical fertilizer because of containing abundant organic carbon, phosphorus, and nitrogen (Biederman and Harpole, 2013). However, heavy metal, organic pollutants, and pathogen contained in the sludge may cause potential risks on agricultural ecological security (Awasthi et al., 2016). Organic pollutants have become a major concern nowadays because of their high resistance of removal from sewage sludge. Therefore, it is still tightly regulated on the use of sewage sludge for agriculture in many countries including China.

The degradation of organophosphorus compounds by microorganisms was mainly carried out by hydrolysis of phosphate ester group with enzymes such as organophosphate hydrolase or phosphotriesterase (Waaijers and Parsons, 2016). The biodegradation of organophosphorus compounds was mainly focused on organophosphorus pesticides and has been reviewed extensively (Singh and Walker, 2006). However, there are still limited researches on the OPE compounds. Kawagoshi et al. (2002) reported that OPEs exhibited high biodegradability in leachate under both aerobic condition and anaerobic condition within 20 days, although some of OPEs such as tris (2-chloroisopropyl) phosphate (TCPP) was recalcitrant even over a long period of time. When

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https://doi.org/10.1016/j.biortech.2018.01.104
Received 22 November 2017; Received in revised form 16 January 2018; Accepted 22 January 2018

tris (2-chloroethyl) phosphate (TCEP) and TCPP were used as the sole phosphate sources, *Sphingomonas sp*. and *Sphingobium sp*. were capable of degrading them into 1,3-dichloro-2-propanol and 2-chloroethanol (Takahashi et al., 2010). Besides, it was demonstrated that microorganisms could degrade and mineralize OPE compounds, such as triphenyl phosphate (TPhP), resorcinol bis(diphenylphosphate) (RDP), and bisphenol A bis(diphenylphosphate) (BDP), through aerobic composting with dewatered sludge (Jurgens et al., 2014).

In this study, the degradation of OPEs in aerobic composting and anaerobic digestion with sewage sludge was investigated. To the best of our knowledge, there are still no studies that systematically evaluate the impact of microbial communities on the degradation of OPE compounds during the aerobic composting and anaerobic digestion in sewage sludge. Therefore, batch experiments were conducted and combined with high-throughput sequencing. The objectives of this study were to (1) evaluate the degradation of OPEs with different treatments; (2) investigate the bacterial community compositions, abundance, and diversity in each treatment; (3) confirm the variance of bacterial community structure using Shannon index, PCA, network analysis, and heat map; (4) identify the potential bacterial strains responsible for the biodegradation of OPE compounds in each treatment.

2. Methods

2.1. Materials and devices

Dewatered sewage sludge was taken from a wastewater treatment plant (domestic sewage/industrial sewage = 7/3) in Zhengzhou, China. Fine sawdust (< 2 mm) was provided by a sewage sludge treatment plant and pig manure was obtained from a local farm. Initial characteristics are shown in Table 1.

Aerobic composting was performed by using a bench-scale composting bioreactor, which was made from unplasticized polyvinyl chloride (UPVC) with outside diameter of 620 mm and inside diameter of 600 mm. The height of the bioreactor was 1400 mm. An insulating layer padded with cotton with a thickness of 20 mm was wrapped around the wall and top cap. Temperature sensor was immobilized in the center axis of the bioreactor, approximately 200 mm from the bottom. The oxygen sensor vertically immobilized in the reactor was 150 mm away from the center and 800 mm away from the bottom. The aerobic composting was carried out through static forced-aeration process, controlled by CTB auto-control technology. A stainless-steel wire plate was installed at 200 mm above the bottom of bioreactor for ventilation. According to the temperature and oxygen consumption rate, Compsoft 3.0 (GreenTech Environmental Engineering Ltd., Beijing, China) was used to automatically adjust the aeration rate at different composting stages (Fig. 1).

The anaerobic digestion experiments were conducted as described in the literatures with minor modifications (Sunyoto et al., 2016; Xiang et al., 2016). Briefly, anaerobic digestion was performed in a bucket made of stainless steel. The diameter of the bucket was 250 mm with the height of 300 mm. The bottom was drilled with several holes for discharging the water generated from the digestion process. Before digestion, the headspace of the bucket was aerated using nitrogen gas for 5 min to remove the oxygen. Afterwards the bucket was sealed immediately by a cap and was incubated at 37 °C in a shaking air bath.

Table 1
Initial chemical characteristics of the materials.

Material	Organic carbon (%)	Total nitrogen (%)	Moisture content (%)	C/N ratio	Particle size (mm)	Volatile solid (%)
Sewage sludge	30.5	2.05	79.8	14.9	–	59.4
Sawdust	52.8	0.40	8.23	132	< 2	–
Pig manure	43.6	2.19	73.7	19.9	–	–

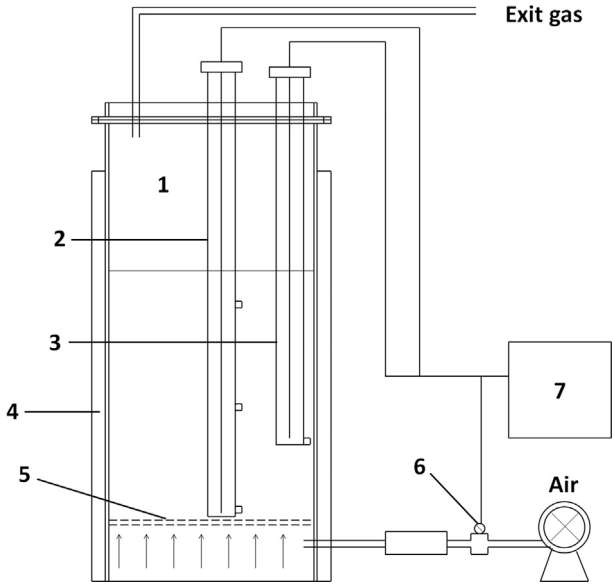

Fig. 1. Scheme of the composting device and its components. 1. bioreactor, 2. temperature sensor, 3. oxygen sensor, 4. Insulating layer, 5. stainless steel wire plate, 6. air volume flow meter, 7. control system.

2.2. Experimental design

Four individual treatments were carried out with sewage sludge, including Treatment 1 (T1): aerobic composting by using sewage sludge combined with pig manure (sludge: sawdust: pig manure = 13:3:4, w/w); Treatment 2 (T2): aerobic composting by using sewage sludge without pig manure (sludge: sawdust = 3:1, w/w); Treatment 3 (T3): anaerobic digestion by using sewage sludge combined with pig manure (sludge: sawdust: pig manure = 13:3:4, w/w); Treatment 4 (T4): anaerobic digestion by using sewage sludge without pig manure (sludge: sawdust = 3:1, w/w).

2.3. Sampling and analytical methods

Temperature and oxygen were automatically recorded by Compsoft 3.0. The collection of temperature data was in a medium frequency of every 30 min during the entire process. Oxygen concentration was detected in the period of one ventilation cycle for aerobic composting, but was not monitored in anaerobic digestion. The sludge samples were collected from three points along the center of vertical axis and then mixed thoroughly for further quantitative analysis of OPEs. The aerobic composting process can be finished within 15 d in this study, but the anaerobic digestion process usually continued for several ten days. To facilitate the comparison, similar treatment time was applied for all treatments. Thus, the mixtures were collected on days from 0.25 day to 14.25 day for T1 and T2, and from 0.25 day to 10.75 day for T3 and T4.

In our previous study, an analytical method for the determination of OPEs in composts was developed by using accelerated solvent extraction (ASE) coupled with solid phase extraction (SPE) prior to UPLC-MS/MS (Pang et al., 2017). Details on the sample preparation, UPLC-MS/MS analysis, quantitation and quality control were provided in the Supporting Information.

2.4. DNA extraction and high-throughput pyrosequencing

Sludge samples were obtained from each treatment to analyze microbial community variation. DNA was extracted using an E.Z.N.ATM Mag-Bind Soil DNA Kit (OMEGA, USA) according to the manufacturer's