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Roles of bacterial community in the transformation of dissolved organic matter for the stability and safety of material during sludge composting

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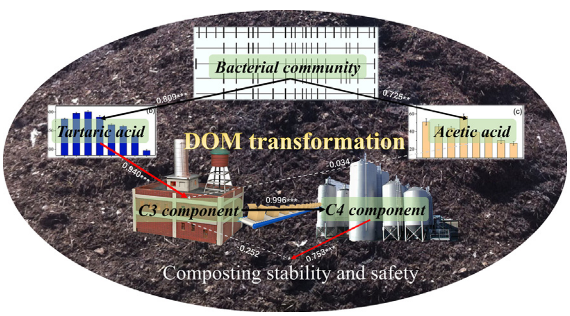
GRAPHICAL ABSTRACT



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ABSTRACT

This study was conducted to assess the roles of bacterial community in the dissolved organic matters (DOM) transformation during sludge composting. The relationship among the bacterial community, organic acids, diverse components of DOM as well as the indexes of the phytotoxin level and stability of materials was analyzed by regression and redundancy analysis. The results showed that there were significant correlations between the parameters for evaluating compost phytotoxicity and maturity including GI, C/N, $SUVA_{254}$, $SUVA_{280}$, $E_{253/203}$, and $A_{240\text{-}400}$, which led to a new index (PC1) by principal component analysis. PC1 was significantly affected by four components of DOM, acetic and tartaric acids that were correlated with the bacteria community shift, especially seven key bacteria. Based on structural equation modeling, the key bacteria with the ability to degrade tartaric acid exerted more important roles in regulating the transformation of DOM components, which was helpful for the stability and safety of compost.

1. Introduction

Sludge is the by-product of wastewater treatment process. It was reported that more than 7 million metric tons of sludge were generated annually in China with a 13% increase each year as a result of intensive wastewater treatment (Yang et al., 2015). If not properly treated, sludge can cause serious environment pollution due to the large amount of toxic substances such as pathogens, heavy metals and some organic

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contaminants. However, considering that sludge also contains a high proportion of biodegradable organic compounds with high density and diversity of microbial flora (Su et al., 2015), it is important to find an effective and economical method for the treatment of sludge. Composting is a biotransformation process of organic materials into stable and relatively nontoxic complex macromolecules under the action of microbe and corresponding enzyme, through which the volume and mass of sludge is reduced and pathogens are removed in the high temperature process (Plachá et al., 2013; Xi et al., 2016; Zhao et al., 2017). Therefore, more and more attention was paid on composting to effectively manage and recycle urban sewage sludge for improving soil properties (Su et al., 2015).

Dissolved organic matters (DOM) are a mixed organic continuum with a series of different molecular sizes, whose structure and composition are complex and difficult to determine due to its wide range of chemical compounds and variety of decomposed and synthesized products (Wei et al., 2014a). The DOM may consist of low molecular weight organic acids, tannic acid, aromatic acids, amines, polysaccharides as well as various types of macromolecular humic acid, fulvic acid, and numerous other mixtures (Guppy et al., 2005). Understanding the characteristic of the DOM formed during composting is important, because most of the biologic activities occur in solid-liquid interface and the changes in DOM during composting can reflect transformation progress of organic matter and compost stability (Gómez-Brandón et al., 2008; Said-Pullicino et al., 2007). Usually, analysis of DOM includes composition, functionalities, and structural, chemical and spectroscopic characteristics by different techniques such as elemental assays, Ultraviolet–visible (UV–Vis) spectroscopy, Fourier transform infrared, and fluorescence spectra, etc. (Caricasole et al., 2010; He et al., 2014; Marhuenda-Egea et al., 2007; Santos et al., 2010; Wei et al., 2014b). There are many parameters used to assess the degree of humification in DOM with UV–Vis and fluorescence spectra, for example, $SUVA_{254}$, $SUVA_{280}$, E_{253}/E_{203}, S_R, A_4/A_1, I_{470}/I_{360}, $r_{(A,C)}$, and so on, moreover, fluorescence regional integration has been used to quantitatively analyze all wavelength-dependent fluorescence intensity data from Excitation emission matrix (EEM) spectra (Chen et al., 2003; He et al., 2011; Helms et al., 2008; Nishijima and Speitel, 2004). Considering that using a single technique to assess the highly heterogeneous properties of DOM is insufficient, the integration of various techniques is needed to comprehensively characterize different DOM fractions. Two-dimensional correlation spectroscopy (2DCOS) can resolve issues regarding peak overlap in traditional chromatography by extending the spectra toward a second dimension, which enable the accurate identification of the sequential order of spectral changes and is often used to investigate the correlation between bands in two different types of chromatography (Lasch and Noda, 2017; Yu et al., 2018). However, to our knowledge, few studies have characterized the transformation process of DOM by 2DCOS analysis using EEM spectra with parallel factor (PARAFAC) analysis and UV spectrophotometry during composting combining to the evaluation of the phytotoxin level and stability of composting.

Low molecular weight organic acids, approximately 2–10% in the total DOM, have been hypothesized to perform many functions in soil including the detoxification of pollutants, improving the bioavailability of heavy metals, root nutrient acquisition and mineral weathering (Guppy et al., 2005; Jones et al., 2003). As for composting, organic acids were mainly produced by the decomposition of organic matter and the release from microorganisms. Many studies have shown that organic acids accumulated during the early stage of composting, which may cause a decrease in pH, reduce the effectiveness of composts and finally affect the safety of composting products (Plachá et al., 2013; Wei et al., 2018). Relatively high concentrations of organic acids can have a toxic effect on the activity of microorganisms (Cheung et al., 2010), however, organic acids also suffer a number of fates such as sorption, metal complexation, especially biodegradation in consideration of the rich bacteria biomass and diversity with different enzymatic capabilities in composting ecosystem (Jones et al., 2003; López-González et al., 2015). Therefore, given that the stability and safety of composting material associated with the transformation of DOM may be severely affected by organic acids, it is essential to understand the influence of bacterial community on the transformation of DOM based on the organic acids biodegradation and their roles in the stability and safety of material during composting.

In this study, our intent was (1) to obtain the organic acids, the composition of DOM, and phytotoxic characteristics during sludge composting, (2) to evaluate the relationship between different organic acids, the bacterial community as well as the indexes of the phytotoxin level and stability of composting, and (3) to identify the key species affecting the transformation of organic acids and DOM fractions. This research will provide useful information about the transformation process of DOM during composting and lead to new perspectives on strategies to improve the stability and safety of composts.

2. Methods and materials

2.1. Composting materials and experimental design

Composting materials were mainly sewage sludge from a municipal wastewater treatment plant in Beijing and rice hulls that was used to adjust the initial C/N ratio. The sludge was taken and mixed with rice hulls after ultrasonication at 100 Hz for 20 min. The ratio of water content is approximately 60% and C/N ratio is about 22:1. The composting experiment was performed in a 34 L laboratory reactor for 42 days, which were replicated three times. Updraft ventilation is achieved by providing an air flow at the bottom of the reactor with an air pump. In order to reduce the effect of ambient temperature on the temperature of the reactor, the reactor is placed in an incubator. The composting materials were turned and mixed every three days in case of stratification, which would bring about poor ventilation. Homogeneous samples of compost were collected using the multipoint sampling method, and were mixed thoroughly before analysis on days 0, 3, 6, 12, 18, 26, 34 and 42 following the initiation of composting. The solid samples were then divided into two parts, and one was stored at −20 °C. The other part was air dried, and then ground to 0.25 mm for chemical analysis.

2.2. Analysis of physicochemical indexes and organic acids

Temperature was monitored using a digital thermometer throughout the composting period by inserting the thermometer into the compost reactor. The fresh compost samples (n = 3) were mixed with distilled water (1:10 w/w ratio) and shaken for 0.5 h. The pH of the composts was determined by a pH meter (MP-521). Dissolved organic carbon (DOC) and total organic carbon (TOC) were tested using an organic carbon analyzer (TOC-Vcp, Shimadzu, Japan). The content of organic matter (OM) of the composting samples were determined by chemical oxidation with 5 mL $K_2Cr_2O_7$ (0.2 M) and 5 mL H_2SO_4 at 100 °C for 90 min in a thermostat. Total nitrogen was measured by Kjeldahl method. The C/N ratio was then computed based on the concentration of TOC and total nitrogen. The germination index (GI) was assayed according to Zucconi et al. (1981) using the following formula:

$$\mathrm{GI}(\%) = \frac{\text{Seed Germination} \times \text{Root Length of Treatment (mm)}}{\text{Seed Germination} \times \text{Root Length of Control (mm)}} \times 100$$

Cabbage seeds were determined utilizing as a test species. All the determinations for seeds germinations were carried out by triplicate.

Organic acids in composting samples were extracted according to the method described by Nakasaki et al. (2013). The amount and composition of organic acids were analyzed by high performance liquid chromatography (HPLC) as described by Wei et al. (2018). Composting