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Effect of the addition of exogenous precursors on humic substance formation during composting

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ABSTRACT

The aim of this work was to explore the effect of the addition of exogenous precursors on humic substance (HS) formation during composting. HS formation is a complex biochemical process that occurs during composting. In addition, HS precursors and bacterial communities were recognized as the key factors that affect HS formation. The addition of exogenous precursors can promote the humification process during composting, but few studies have explored the potential relationships between the proportion of additional exogenous precursors, the bacterial community and HS formation. Jointly adding benzoic acid (BA) and soybean residue after extracted oil (SR) treatment can promote HS formation, especially humic acid formation. In addition, the increase in the proportion of exogenous precursors added could strengthen the relationship among different precursors, thereby changing the bacterial community composition and further promoting the humification process during composting. In addition, a structural equation model (SEM) showed that precursors were the key factors to regulate HS formation and certain bacteria as the direct drivers to affect HS formation. This model provides more possibilities to regulate HS formation during composting and enhances its potential applicability under real conditions.

1. Introduction

Composting is an economical and environmentally friendly treatment method that provides a potential sustainable way to treat various organic solid wastes, which could reduce carbon emissions and improve land use (Li et al., 2014; Jiang et al., 2015; Xi et al., 2015; Zhao et al., 2016a). Many studies suggested that compost added to polluted soils could affect heavy metal mobility through different mechanisms and interaction, like the formation of insoluble metal organic matter complexes (Gadepalle et al., 2008; Silvetti et al., 2017). Stable and mature compost can be applied to soil as an organic amendment to improve plant growth and soil fertility, as well as enhance the function of soil for carbon sequestration (Piccolo et al., 2004). Composting is the process of destroying of pathogens and recycling nutrients to generate a final stable product, such as humic substances (HS) which can be used as a soil conditioner or fertilizer (Rawoteea et al., 2017; Wu et al., 2017b). In addition, HS, as the most important by-product of composting, play a key role in predicting the product efficiency of compost during applications.

The HS formation is a complex biochemical process. Many hypotheses regarding the formation of HS include the lignin-protein, phenol-protein and sugar-amine condensation theories. However, it is well known that these humification pathways do not occur separately but rather interact with each other (Jokic et al., 2004; Zhang et al., 2015). The core of these theories is that precursors are polymerized to form HS in various manners. It has been showed that precursors play a key role in the process of HS formation (Wu et al., 2017a). To date, many studies investigated the spectral characteristic and evolution of HS with various raw materials and under conditions during composting. Guo et al. (2016) observed that the aromatization and nitrogenous compounds of HS significantly increased during the co-composting of dairy manure and sugarcane press-mud with the addition of flue

Abbreviations: BA, benzoic acid; SR, soybean residue after extracted oil; MS, maize straw; CM, chicken manure; HS, humic substance; HA, humic acid; FA, fulvicacid; RDA, redundancy analysis; NMDS, non-metric mufti-dimensional scaling; PCoA, principal coordinate analysis; CCA, canonical correspondence analysis; DGGE, denaturing gradient gel electrophoresis; *H'*, Shannon-Wiener biodiversity indices; AAs, amino acids; RS, reducing sugars; SEM, structural equation model; MSG, residue of monosodium glutamate; TOC, total organic carbon; TN, total nitrogen; TP, the total phosphorus; TK, total potassium; DW, dry weight.

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gas desulphurization gypsum. However, there are few studies on the influence of the addition of exogenous precursors on the characterization and formation of HS components during composting.

Organic wastes, such as soybean residue after extracted oil (SR), contain abundant polysaccharides and protein in addition to their moisture content. Such wastes can be used for microbial metabolism. Alternatively, these wastes can be degraded to produce a large number of precursors (polysaccharides, reducing sugars and amino acids). Tan (2014) have proposed that the production of precursors is closely related to the bacteria. Previous studies have found that *Clostridiales*, *Bacillales* and *Actinomycetales* were the main and typical group of degrading organic matter and they may decompose and transform organic matter into HS precursors during the heating phase and the thermophilic phase of composting, which will polymerize to form HS (Antunes et al., 2016; Tian et al., 2013; Wu et al., 2017a). Otherwise, as an aromatic functional group, benzoic acid (BA) may influence the change of precursors such as phenol and carboxyl groups during humification and it has been reported that BA is also an important intermediate product during HS synthesis (Tan, 2014). Nevertheless, excessive phenol could cause a negative effect on soil, and phenol compounds also found in olive mill solid wastes were tested to determine the individual contribution to phytotoxicity (Ines et al., 2017). Therefore, we hypothesized that jointly adding a small amount of BA and a certain amount of SR could affect the humidification process during composting and alter the bacterial community. Many researchers showed that microorganisms play key roles in all of the events related to the bio-transformation of organic matter. In particular, the bacteria among them are more influential due to their metabolic versatility (Tan, 2014; López-González et al., 2015; Antunes et al., 2016). Although the key bacterial communities that influence precursors formation have been investigated during previous research (Wu et al., 2017b), it is not clear if the addition of exogenous precursors can alter the bacterial community composition during composting.

Currently, many techniques have been increasingly used to analyze microbial community composition and identify the primary factors influencing microbial communities with special biological capacity, such as methanogenic communities (Lin et al., 2012), and cellulose-decomposing actinomycetes (Zhao et al., 2017b). The unconstrained ordination such as non-metric multidimensional scaling (NMDS) or principal coordinate analysis (PCoA) was used to analyze species distribution. Direct multivariate analysis (constrained ordination) such as redundancy analysis (RDA) or canonical correspondence analysis (CCA) provides the appropriate ways to consider the correlation between changes in the microbial community composition and environmental variables (Wei et al., 2016). In addition, the structural equation model (SEM) as a *priori* approach, allows for an intuitive graphical representation of the causal relationships between variables and it was conducted to verify casual hypotheses by fitting data (Eisenhauer et al., 2015). In this study, the SEM was conducted to explore the main factors of the influence of HS formation on the condition of adding precursors.

Thus, the goals of this study were to: (i) analyze the effect of adding exogenous precursors on HS formation during composting, (ii) compare the influence of different amount of addition on HS formation and bacteria community composition, and (iii) identify the main factors affecting HS formation on the addition of precursors by using SEM. This research will lead us to re-examine the effects of precursors and bacteria on HS formation and provide new perspectives on strategies to improve HS formation by adding exogenous precursors.

2. Materials and methods

2.1. Composting trial and sample collection

Composting trials were carried out at a lab-scale reactor with a height of 40 cm and a base diameter of 33 cm. The working volume was 34L. The reactor has a temperature control system, gas supply and metering systems, and leachate collection device, which was described by Zhao et al. (2016b). The analogous experimental reactor was easier to control and widely used in substantial studies (Wang et al., 2015; Zeng et al., 2010; Zhao et al., 2017b, 2016b). The control of compost temperature was based on the changes of natural composting and reactor composting. The actual temperature of compost was basically the same as reactor. The changes of reactor temperature were shown in Supplementary information (Fig. S3).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.wasman.2018.08.025.

The organic material was mainly composed of maize straw (MS), and chicken manure (CM) was used to adjust the C/N ratio at 20:1. Approximately 10 kg dry weight (DW) of mixture of material was composted in the reactor and the ratio of the MS to CM in the mixtures was 6:1. It is better to explore the impact of exogenous precursors during composting by using relatively pure materials. Considering that the composition of SR and BA is relatively simple, the additives selected were dry SR and BA. However, the cost of SR and BA were very high to directly apply in composting. Therefore, natural organic wastes containing more precursors will be added in composting to replace pure materials in next experiments.

To explore the potential relationships between the proportion of additional exogenous precursors and HS formation, the composting experiments were divided into five groups which were showed in Table 2. One control and four treatments were set up in this study. Control group (CK) was no adding BA and SR and different proportion of dry SR and BA was added in four treatments (R1, R2, R3 and R4) in the initial of composting (day 0). MS and CM were obtained from the Xiangfang farm in Harbin (China). SR was obtained from the dining hall at the Northeast Agricultural University (China) and BA was taken from the laboratory. The basic physical-chemical characteristics of the raw materials were shown in Table 1. All of the characteristics were adjusted to the optimum condition for composting. The particle diameter of the raw material was reduced to approximately 1.0–2.0 cm, and the initial moisture content and C/N ratio were adjusted to 60% and about 20, respectively. During the composting process, the moisture content was maintained at about 60% by the addition of distilled water. The entire aerobic composting process was carried out in 62 days, during which the piles were turned every 7 days during compost-

Table 1
Physical-chemical characteristics of the raw materials.

	MS	CM	SR
Moisture content (%)	4.75 ± 0.02	56.5 ± 0.87	84.56 ± 1.07
TOC (%)	46.94 ± 1.21	47 ± 1.14	53.01 ± 1.65
TN (%)	1.17 ± 0.01	4.69 ± 0.03	5.74 ± 0.03
TP (%)	0.37 ± 0.03	2.43 ± 0.08	2.01 ± 0.06
TK (%)	0.83 ± 0.05	1.65 ± 0.04	2.13 ± 0.06
EC (mS/cm)	1.12 ± 0.06	4.03 ± 0.04	1.22 ± 0.03
pH	6.99 ± 0.02	8.01 ± 0.03	6.14 ± 0.02
C/N	21.63 ± 0.5	10.02 ± 0.06	9.24 ± 0.02

TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; MS, maize straw; CM, chicken manure; SR, soybean residue after extracted oil.