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Research Paper

Influence of inoculating white-rot fungi on organic matter transformations and mobility of heavy metals in sewage sludge based composting

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HIGHLIGHTS

- Sewage sludge composted with straw, bran and *Phanerochaete chrysosporium*.
- The transformation of organics were associated to bioavailability of heavy metals.
- Stepwise linear and quadratic curve indicated relationship between indexes.
- The white-rot fungi passivate heavy metals by provoking the formation of humus.

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ABSTRACT

White-rot fungi, *Phanerochaete chrysosporium* was inoculated to sewage sludge composting. Its effect on transformation of organic matter and mobility of heavy metals (Zn, Pb, Cu, and Ni) was studied. Detailed sampling was performed to measure C contents in humic extracts (HE), humic acids (HAs), fulvic acids (FAs), humin and distribution of heavy metals, including acid exchangeable fraction (AE), reducible fraction (RED), oxidization fraction (OXI) and residual fraction (RES). In our study, it is evident that the HE, HAs increased obviously and hydrolyzed humin decreased markedly in inoculation. The stabilization rate ((OXI + RES)/(AE + RED)) of Zn, Pb, Cu, and Ni was 20.31%, 7%, 14.3% and 19.79% higher in inoculating reactor. Additionally, the changes of heavy metals fractions could be explained by the organic variables. The results of this study demonstrated that *Phanerochaete chrysosporium* passivates the heavy metal by provoking the formation of humus.

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1. Introduction

Sewage sludge, which generated from the metabolism of microbe in municipal wastewater treatment, contains plenty of organic matters and is rich in nutrients. It could be used as organic amendment to improve soil fertility. Unfortunately, high load of heavy metals existing in sewage sludge would tend to accumulate along with the food chain and do harm to the environment and ecosystem [1]. The toxicity and mobility of heavy metals depend mainly on the form of the metals. Heavy metals could be immobilized and stabilized into OXI and RES fractions, the AE and RED fractions, on the other hand, are mobile in solid and could be a threat to environment. Composting, one of the most promising technologies, can not only biodegrade organic matters but also leads to the formation of humic substances that might be mainly associated to transformations of heavy metals [2].

Microbial activity plays a significant role on biodegradation of organic matters and improves the quality of the compost [3]. During the composting, lignocellulosic substances constitute an important fraction of the total organic matter, resulting in the adequate part of lignocellulolytic microorganism in humification process [4]. White-rot fungi, a representative lignocellulolytic microorganism, can not only have positive effect on compost maturity [5], but also has been used to immobilize heavy metals by intracellular uptake or chelation in wastewater [6]. Most of the previous studies focused on the direct effect of inoculating microbes in single parameter. Zeng et al. studied the effect of inoculating white-rot fungus on composting in different aspects, including the compost maturity of agricultural wastes during different phases [7] and its effects on metal behavior [8]. However, they failed in associating the heavy metal with maturity which might result from white-rot-fungi inoculation. Some studies concentrated on the bioremediation of specific substances

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by microbes or the effect of white-rot fungi on fungal communities [9–11]. Additionally, the accumulation of heavy metals by white-rot fungi itself was also presented [12]. Whereas, there are fewer investigations which dig out the feasibility of heavy metals passivation by the direct influences caused by inoculating microbes. Herein, it is necessary to study the differences of humification between inoculating and non-inoculating composting treatments as well as the changes of heavy metals resulted from the maturity by stepwise linear regressions and quadratic curve estimation.

Therefore, the objectives of this work were to examine the mobility of heavy metals between two composting reactors with or without inoculation, and the changes in the organic matter content, especially humus fractions. Because Zn and Cu dominate the amount of heavy metals in sewage sludge from many places [13], and Pb which was confirmed that could be immobilized by white-rot fungi without the comprehensive mechanism of the immobilization [8,14], it is meaningful to focus on Zn, Cu and Pb. In addition, Ni was used as evidence the relationship between heavy metals and organic matter since there was a small number of Ni in sewage sludge generally and Ni was lacking of studying [15]. It would be potential to extension the observation of this study to other kind of heavy metals if all of these heavy metals could be explained. Meanwhile, it also presented the analysis of the influence of inoculating white-rot fungi on the transformation of organic matter and explored the indirect mechanism of heavy metals stabilization.

2. Materials and methods

2.1. Composting heaps

Sewage sludge which was dewatered in LiJiao wastewater treatment plant, Guangzhou, Guangdong Province, China, was mixed with straw and bran, in the ratio 30:9:5 (on a wet basis). The characteristics of the sewage sludge were 83.87% moisture content, pH 7.41, 55%TOC, and 8.0% total N, C/N 6.89. In the sludge, the total Zn, Cu, Ni, Pb contents was 241.55, 19.28, 9.91, and 13.29 mg kg^{-1} respectively (all results expressed in dry basis). The straw of which TOC and total N was 89.74% and 0.7% respectively, was dried and cut to 3–5 cm lengths. Bran was used to increase the initial C/N ratio of composting and was used as bulking agent as well.

The pilot-scale composting system were set up in two 50L capacity open type lab-scale reactors without drainage or forced ventilation systems. Moisture content in both piles were adjusted to about 70%. The composts were turned in the first 25 days and then once a week afterwards to avoid the loss of heat [16]. In this experiment, two compost reactors were conducted and covered by quilts after mixing. In Reactor A, the heap was uninoculated with *Phanerochaete chrysosporium* as control. While the Reactor B was inoculated with *Phanerochaete chrysosporium* from the start of composting. Two reactors was monitored for 60 days under the same circumstance.

2.2. Microbial inoculum

The white-rot fungi *Phanerochaete chrysosporium* was obtained from Guangdong Institute of Eco-environment and Soil Science and it was stored at 4 °C on potato dextrose agar (PDA). *Phanerochaete chrysosporium* mycelium from a culture grown on PDA plate at 37 °C for 7 days was suspended in sterile water to reach the desired concentration of 2×10^6 colony-forming unit's mL^{-1} ($CFUmL^{-1}$). Considering the moisture of raw materials, sterile water was added into Reactor A to adjust the moisture to 70%. As for Reactor B, the spore suspension diluted further by sterile water at weight ratio of 6% was used to adjust the moisture of compost to 70% at the beginning of fermentation. After inoculated, all the composting materials in B Reactor were turned to spread the microbe consortium. Both of two reactors were mixed and stirred before composting.

2.3. Physico-chemical analysis

The temperature in the center of the composting heap was monitored at 9:00 and 16:00 every day. Samples were taken at 0, 5, 10, 20, 40, 60 days of composting. In order to obtain representative samples for the physic-chemical analysis, homogenization must be ensured. Samples were taken from five different spots at half height of the heap and mixed up. Three replicates were set up for later analysis. The moisture content was determined after drying at 105 °C for 6 h. Then the dried samples were used to analyze the total organic carbon by dry combustion [7]. pH was measured in 1:2.5 sample-H_2O suspension with HACH pH meter according to Zeng et al. [8].

The extraction method of humic substances followed the methods Huang et al. mentioned [17]. One gram fresh sample was washed with 20 mL H_2O to avoid interfering sugars and proteins [7]. Though it might also remove some of humic substances [18], it would not hinder the analysis of the passivation of heavy metals by humic substances. The extraction was soaked by 20 mL pyrophosphate-NaOH solution (0.1 M $Na_4P_2O_7{\cdot}10H_2O$ + 0.1 M NaOH, pH 13.0) in centrifuge tubes for 1 h and shaken at 200 rpm for 30 min, and then the samples were conserved for 13–14 h at 4 °C. The mixture was centrifuged at 15,000 rad for 15 min and the supernatant was filtered for humic extracts (HE) analysis. The alkaline extracts were then acidified with 1 M H_2SO_4 to pH1, left standing for 24 h to allow the separation of humic acids (HAs) and fulvic acids (FAs). By means of centrifugation, the solution was used for FAs analysis, while the insoluble fraction contained HAs which could be calculated by subtracting FAs from HE. Since the alkali-insoluble humin fraction may contain humic-like substances, such as decomposed lingo-cellulosic materials, it can be determined as the hydrolyzed humin [19]. The hydrolyzed humin which was considered as a part of the humus in the compost was obtained after acidification of the pyrophosphate-NaOH residue retained in the filter paper with 6 M HCl for 10 h, filtration and washing with deionized water. All fractions of humic substances were measured by TOC-VCPH and results are presented as carbon content per unit dry weight compost each fraction.

There are many methods used for extract heavy metal step by step. For drawing on each other's strength and unfirming standards, European Community Bureau of Reference (BCR) [20] came up with continuous extraction method which has been widely using in whole area. At the same time, it was the best method to measure the mobility of heavy metals by determined fractions of metals. Therefore, each fraction of Zn, Cu, Ni and Pb was determined according to BCR. One gram of dry sample was treated by 0.11 M acetum and H_2O at ratio 1/4, shaking 16 h at 25 °C. The extractant which was used to measure as acid exchangeable fraction of heavy metals, was collected by centrifuge at 4000r/min for 20 min and flited through 0.45 μm filter. 20 mL H_2O was used to washed the residue after solid-liquid separation. The sequence of reagents application to collect the extractant was 40 mL mixture by 0.5 M $NH_4OH{\cdot}HCl$ and 0.05 M HNO_3 for reducible fraction; 20 mL H_2O_2 and 50 mL NH_4OAc for oxidizable fraction. Finally, the residue was digested with HNO_3: HF: $HClO_4$ as residual fraction. Each concentration was determined through ICP-MS (Prodigy Spectrum).

2.4. Statistical analysis

Statistical analyses were performed with the SPSS v18.0 statistical software. T-test was used to detect the significant effect of inoculating white-rot fungi on transformations of organic mat-

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