Science of the Total Environment 643 (2018) 539–547

Contents lists available at ScienceDirect

Science of the Total Environment

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

Responses of microbial carbon metabolism and function diversity induced by complex fungal enzymes in lignocellulosic waste composting

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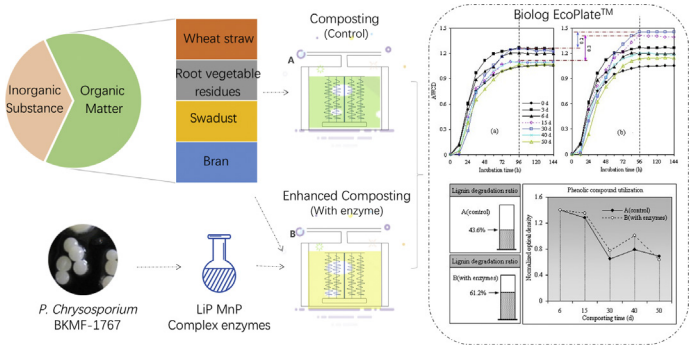
HIGHLIGHTS

- Composting limited by the recalcitrance of lignin and phytotoxic substance release.
- LiP-MnP enhanced composting is efficient for organic matter biogeochemical cycle.
- Biolog EcoPlate™ method was applied to test the microbial carbon metabolism.
- Shannon and McIntosh index quantify the functional diversity of microbial community.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 April 2018
Received in revised form 8 June 2018
Accepted 8 June 2018
Available online xxxx

Editor: Frederic Coulon

Keywords:
Enhanced composting
Ligninolytic microorganisms
Complex enzymes
Degradation
Carbon metabolic diversity
Biogeochemical cycle

ABSTRACT

Composting is an economic and effective technology for solid waste treatment, which is an essential method to promote the biogeochemical cycle of contaminants. However, the application of this technology was limited by the bio-degradative recalcitrance of lignin and other kind of phytotoxic substances release. The combination with microorganisms and enzymes is a popular and efficient way to enhanced composting. This study, referring to metabolic mechanisms, fungal molecular and biogeochemical cycles, was performed to investigate the effects of lignin degradation, carbon metabolic diversity, as well as the related changes induced by these two kinds of complex enzymes in composting. The biological diversity is important indicator in ecosystem, which concerns the environmental applicability of one technology. The carbon metabolism diversity reflected the biogeochemical cycles of organic matter, which was also an essential input to analyze the effects of composting. The changes on the diversity characteristics of carbon are essential to comprehensively understand the deep mechanisms of this process, and extended the application of complex enzymes in the field of enhanced composting. The analysis of Biolog revealed that the utilization of pyruvic acid methyl ester, α-Cyclodextrin, D-Mannitol, D-Galacturonic, Itaconic acid and L-asparagine were deeply promoted, and that of D, L-α-Glycerol-phosphate, L-Threonine, Glycyl-L-Glutamic acid and putrescine were depressed by adding the complex enzyme in composting. Moreover, according to the data, the addition of complex enzymes improved the degradation efficiency and the metabolic capacity of carbon in composting. These findings undoubtedly contribute to the development of enzyme-based

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https://doi.org/10.1016/j.scitotenv.2018.06.102

technologies and the applications of complex enzymes in composting, which is of great benefit to eliminate the limitation and extend the application of composting.

1. Introduction

The biogeochemical cycle of contaminants is an essential issue nowadays. Composting is a recovery and innoxiousness treatment method for contaminants, especially organic solid waste. Composting is also considered to be one of the most attractive technologies applied on municipal solid waste or sewage sludge on account of low environmental impact and cost. The pollutants can be decomposed and recycled as fertilizers and soil amendments (Lu et al., 2008). Comparing to other methods, such as advanced nanomaterials, the distinct advantage of composting is that this technology does not cause any secondary contamination. In addition, there is no disadvantage about the recycling of the material from the environment during this process, because this method is indeed a harmless treatment which was dominated by microorganisms. For example, with the wide application of nanotechnology, many researchers focus on the remediation of soil pollution by using nanomaterials. Unquestionably, carbon-based nanomaterials, iron nanoparticles and photocatalytic material can efficiently degrade antibiotics, polycyclic aromatic hydrocarbon and other kinds of organic pollutants (Ghiyasiyan-Arani et al., 2016; Mazloom et al., 2016). But people have to pay more attention to the ecological impact of it account for its high toxicity for ecological environment and soil communities (McKee and Filser, 2016; Shareghi et al., 2016). On the contrary, the environment impacts of composting is comparatively small. However, the possible presence of phytotoxic substances in the traditional compost, such as biodegradative recalcitrance of lignin, heavy metals release and other secondary metabolites by microorganisms, may inhibit germination of plant (Aslam et al., 2008; Gong et al., 2017). These wastes are valuable for soil erosion control and soil nutrient replenishment, but will harm the environment if applied without proper treatment such as composting (Bustamante et al., 2008; Huang et al., 2018; Gong et al., 2018). The degradation and transformation of lignocellulosic waste is attributed to the metabolism of indigenous microorganisms during composting. Therefore, it is very important to improve the practicality and efficiency of the composting technology. Among a variety of enhanced composting methods, the combination with microorganisms and enzymes is a more popular and efficient one (Xu et al., 2017; Sun et al., 2017).

Lignin is a kind of cross-linked phenolic polymer, which was rigidity and do not rot easily. The degradation of lignin in composting mainly depends on the ligninolytic enzymes, which was also a kind of extracellular enzyme secreted by ligninolytic microorganisms. White rot fungi (WRF) are capable of degrading lignin and most of lignin structure analogues efficiently, via unique extracellular oxidative enzyme systems with a low substrate specificity, and intracellular enzyme systems (Huang et al., 2017a, 2017b, 2017c). Vividly, WRF also has been referred to "externalized stomachs" that secrete hydrolytic enzymes and organic acids into the extracellular conditions and transport metabolite and chelates pass through into the cell wall. Lignin peroxidases (LiP), manganese peroxidases (MnP) and copper-based laccases (Lac) are three kinds of typical ligninolytic enzymes secreted by WRF. LiP oxidize non-phenolic lignin, whereas MnP only oxidize the phenolic structures. Lac takes a significant role both in these two reactions during the process of lignin degradation (De et al., 2016; Huang et al., 2016; Huang et al., 2015). Previously study indicates that H_2O_2 was related the reactions catalyze by these two kinds of enzyme. Side chain epoxidation, demethylation and the broken of C_α-C_ς and β-O-4 were the main approaches in the reaction (Zhou et al., 2017).

As a clean and efficient catalyzer, enzyme was often used as the enhancer in composting or often immobilized by advanced material for environmental remediation (Liu et al., 2012). The isoenzyme of LiP was obtained from *phanerochaete sordida* YK-624 by Hirai et al., which efficiently decomposed the dimers of lignin and catalyzed the oxidation of phenolic compound (Hirai et al., 2005). Hofrichter researched the degradation of pine sawdust by using MnP, and the results analyzed by size exclusion chromatography indicated that the original material can be transform into the fiber fragment, and the non-phenolic can be oxidized by MnP (Hofrichter, 2002). However, the degradability of single enzyme still limited. Hatakka proved that the complex enzyme of LiP and MnP showed the high performance in lignin degradation (Hatakka, 1994). Kluczek-Turpeine isolated the complex ligninolytic enzymes from *paecilomyces inflatus*, and discovered that 15.5% of lignin was converted to the hydrosoluble fragment by analyzing ^{14}C labeled lignin (Kluczek-Turpeinen et al., 2003). Most of the current researches focus on the degradation efficiency of lignin by using complex enzymes. However, it is seldom reported that applying the complex enzyme extracted by microorganisms to the process of composting, and seldom focus on the diversity characteristics of carbon induced by complex fungal enzymes in lignocellulosic waste composting.

Apparently, these changes on the diversity characteristics of carbon are very important for us to comprehensively understand the deep mechanisms of functional complex enzymes, and extended application of complex enzymes in the field of enhanced composting. Herein, the present study was conducted with the aim of investigating the addition of complex enzymes in composting. To observe the dynamic changes of carbon metabolic diversity, the Biolog method was applied in this study. Furthermore, the effects of organic matter (OM) and lignin degradation by adding the functional complex enzymes were discussed in detail. These results not only promote the further development of enzyme technology, but also provide new ideas for the improvement and development of composting technology, which make sense of the theoretical basis and technological innovation.

2. Materials and methods

2.1. Materials

Wheat straw, root vegetable residues, bran, sawdust and soil, which were adopted in this experiment, were collected from a suburb of Changsha, China. Wheat straw and root vegetable residues were air-dried and cut to 10–20 mm. Soil was air-dried and ground to pass through a 2 mm nylon screen, offering native microorganisms and some necessary nutriments. Bran was used to adjust the ration of carbon to nitrogen, and finally make the ration reached 32:1. The original ratio of the soil: root vegetable residues: straw: the bran: sawdust was 54:16:33:9:7, which was aimed to control the organic-matter content of this mixture was 63.0% (dry weight), the lignocellulose content was 47.3% (dry weight) and the moisture content was maintained at 65%.

The fungus *P. chrysosporium* strain BKMF-1767 was selected to produce complex fungal enzymes, which was obtained from China Center for type Culture Collection (Wuhan, China). The fluid medium was composed by analytical reagent grade $MgSO_4$, $FeSO_4$, $ZnSO_4$, $CuSO_4$, NaCl, $CaCl_2$, $CoCl_2$, KH_2PO_4 and $AlK(SO_4)_2 \cdot 12H_2O$, which were obtained from Sinopharm Chemical Reagent Co., Ltd. China. Ammonium tartrate, D-glucose and nitrilotriacetic acid (NTA) were purchased from Aladdin Chemistry Co., Ltd. China. Ultrapure water (18.3 MΩ cm) was used in all the batch experiments.