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

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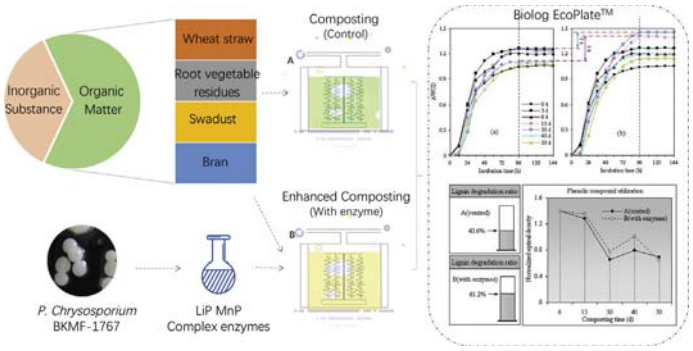
HIGHLIGHTS

- 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



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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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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.

2.2. Preparation of complex fungal enzymes

Fungal cultures were maintained on potato dextrose agar (PDA) slants at 4 °C, and then transferred to PDA plates at 37 °C for 5–7 days until the medium was full of spores. The spores on the agar surface were gently scraped and blended in the sterile distilled water as spore suspension. The spore was measured by a microscope with a blood cell counting chamber and adjusted to 2.0×10^6 CFU per mL. Then added 8% (v/v) spore suspension into fluid medium, and shake cultured for 7 days at 30 °C. After centrifugation and filtration, the supernatant was collected to prepare the complex fungal enzymes by using two steps salting out with $(NH_4)_2SO_4$ and dialysis method.

2.3. Composting set-up and sampling

The experimental apparatus used for this research consisted of a lab-scale square reactor (76 × 55 × 45 cm) with a 75% filling level under indoor conditions. The pile was turned once every three days in the first 2 weeks and then once every six days afterwards. The composting experiment was performed in three replicates (done simultaneously), and each lasted 50 days. Two identical sets of experimental apparatuses were prepared and labeled as Reactors A and B. Traditional composting method was adopted in reactor A (control) with the addition of inactivated complex fungal enzymes. While the complex fungal enzymes were added into reactor B (with enzymes) after 7 days composting, and the pile temperature was plunged to below 38 °C at the same time. Three parallel samples were set both in A (control) and B (with enzymes).

2.4. Physicochemical parameters analysis

The temperatures were monitored every day. The moisture content of samples was determined by oven-drying at 105 °C for 24 h. The dried samples were analyzed for total organic carbon (TOC) by dry combustion overnight at 550 °C before reweighting (Jiménez and García, 1992). OM values were commonly calculated by a conversion factor of 1.724 to convert total organic carbon: OM (%) = TOC (%) × 1.724. Total organic nitrogen (TN) was measured by the Kjeldahl method (Martins and Dewes, 1992). C/N ratios were determined by the quotient values of TOC and TN. Fourier transform infrared spectrometry (FT-IR) was used to identify the functional groups and analyze the structures of the product after lignocellulosic waste composting. The samples from A (control) and B (with enzyme) were freeze-dried, then were fully ground and mixed with equal amounts of KBr (spectral purity). After that the FT-IR spectra of the compost samples were obtained from a Nicolet 5700 Spectrometer (Nicolet, USA), with the scanning wavelength ranged from 4000 to 500 cm^{-1} (Porras et al., 2016).

2.5. Lignin content analysis

The contents of lignin, including cellulose and hemicellulose, were analyzed by acidizing. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined as demonstrated by Soest et al. (Van Soest et al., 1991). Hemicellulose was estimated as the difference between NDF and ADF. Cellulose was estimated as the difference between ADF and ADL content. Lignin was estimated as the difference between ADL and ash content. Selective

Parallel sample 1 (32 micropores) — sample 2 and sample 3

Control Water	**Carbon Source 8** Glucoside	**Carbon Source 16** D-Galactonic Acid γ-Lactone	**Carbon Source 24** L-Arginine
Carbon Source 1 Pyruvic Acid Methyl Ester	**Carbon Source 9** D-Xylose	**Carbon Source 17** D-Galacturonic	**Carbon Source 25** L-Asparagine
Carbon Source 2 Tween 40	**Carbon Source 10** i-Erythritol	**Carbon Source 18** 2-Hydroxy Benzoic Acid	**Carbon Source 26** L-Phenylalanine
Carbon Source 3 Tween 80	**Carbon Source 11** D-Mannitol	**Carbon Source 19** 4-Hydroxy Benzoic Acid	**Carbon Source 27** L-Serine
Carbon Source 4 α-Cyclodextrin	**Carbon Source 12** N-Acetyl-D-Glucosamine	**Carbon Source 20** γ-Hydroxybutyric	**Carbon Source 28** L-Threonine
Carbon Source 5 Glycogen	**Carbon Source 13** D-Glucosaminic Acid	**Carbon Source 21** Itaconic Acid	**Carbon Source 29** Glycyl-L-Glutamic Acid
Carbon Source 6 D-Cellobiose	**Carbon Source 14** Glucose-1-Phosphate	**Carbon Source 22** α-Ketobutyric Acid	**Carbon Source 30** Phenylethylamine
Carbon Source 7 α-D-Lactose	**Carbon Source 15** D,L-α-Glycerol-phosphate	**Carbon Source 23** D-Malic Acid	**Carbon Source 31** Putrescine

1,13,17,20-23 were carboxylic acid.

6-12, 14-16 were carbohydrate.

24-29 were amino acid.

2-5 were polymer.

18,19 were phenolic compound.

30,31 were amine.

Fig. 1. Carbon sources in Biolog Eco-plate.

Table 1
Analysis procedures for carbon source utilization by microbial community based on Biolog.

Order	Analytical Procedures
1	Adding 36 mL saline solution into 4 g composting sample. After shocking 1 h with the speed of 160 r min^{-1} in convoluted shaker, 18 mL saline solution was added into 2 mL supernatant of the sample to prepared the inoculum with the dilution of 10^{-3}.
2	Adding 150 μL inoculums above-mentioned inoculum into the microscopic holes of Biolog Eco-plate by using eight-channel spreader. After 7-day incubation at 28 °C in dark, the absorbance of Biolog EcoPlate can be read every 12 h by Biolog automatic tester at 590 nm.
3	The AWCD value can be calculated by followed formula: $AWCD = \frac{\sum_{i=1}^{93}(OD_i - OD_0)}{93}$ Where OD_0 represent the average absorbance of 3 control groups, if the value of OD_i-$OD_0 < 0$, the value will be adjusted to 0.
4	After a standardized processing, the standardized absorbance value of every microscopic holes in Biolog EcoPlate will be used in clustering analysis. The standardized formula was showed as follow: $\overline{OD_i} = \frac{OD_i - OD_0}{AWCD}$

index was calculated as the ratio of lignin/lignocellulose degradation efficiency. Degradation efficiencies of lignin (D_n) were calculated by the following formula:

$$D_n = \frac{m_p - m_n}{m_p} \times 100\% \quad (1)$$

where m_p and m_n represent the total amount of lignin, cellulose or hemicellulose in fermentation substrate at the previous sampling time and that at the nth day, respectively.

2.6. Analysis of carbon metabolisms

The potential metabolisms of carbon of microbial community in different composting stage were assessed using Biolog EcoPlate™ (Biolog Inc., California, USA) (Weber et al., 2007; Gomez et al., 2006). There are 96 microholes in one plate which was segmented into three parallel samples. Each parallel group was comprised of 31 kinds of carbon sources and 1 control sample which instead water into carbon. The carbon sources include seven kinds of carboxylic acid, four kinds of polymer, ten kinds of carbohydrate, two kinds of phenolic compound, six kinds of amino acid and two kinds of amine. The details were showed in Fig. 1. The average absorbance (average well color development, AWCD) was then calculated for each plate at each reading time (Zak et al., 1994). The specific analysis procedures and formulas were showed in Table 1.

2.7. Data analysis

The results to be presented were the mean values of three replicates, and the standard deviations were used to analyze experimental data. Statistical analyses were performed to obtain more comprehensive and useful information, using the software package SPSS 13.0 for Windows (SPSS, Germany). Data on the AWCD, degradation efficiency of OM and lignin, and carbon sources metabolic properties were subjected to one-way analysis of variance (ANOVA) tests, followed by Duncan's test ($p < 0.05$), to determine the significance of the differences between A (control) and B (with enzymes). Cluster analysis was used to classify experimental treatment and control groups based on the calculation of distance measures between the values of carbon source metabolic indexes in Biolog Eco-plate in one group and those in another group.

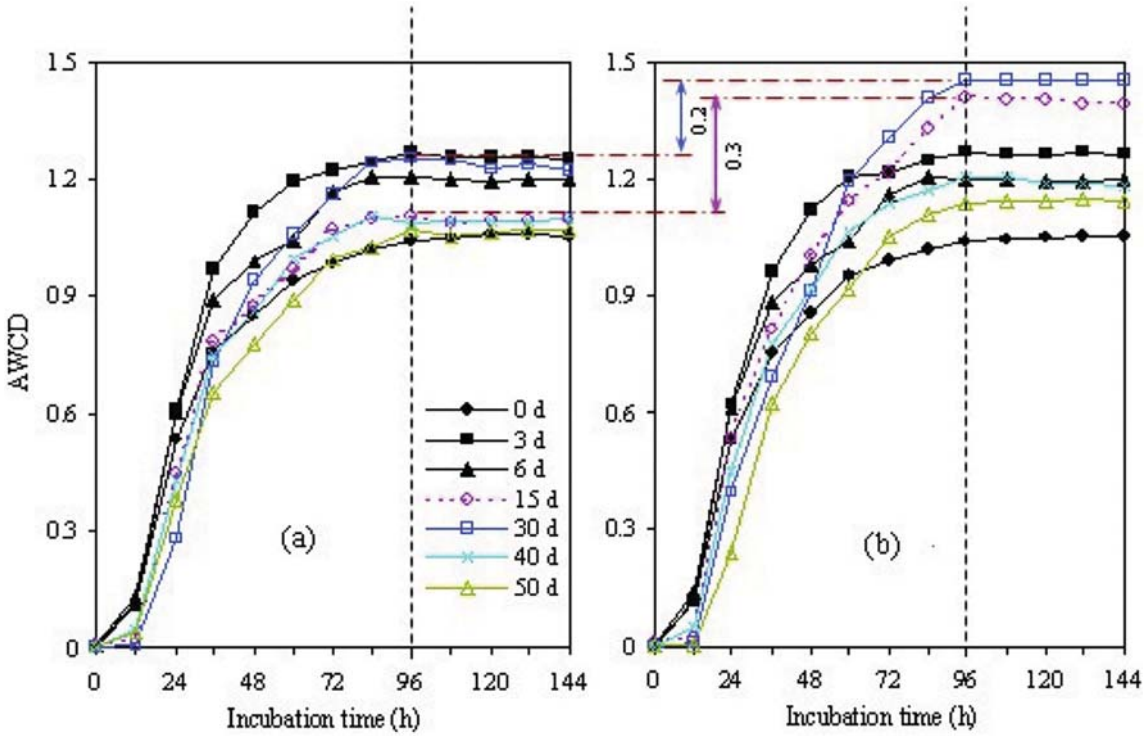

Fig. 2. Changes in AWCD with incubation time in A (control) and B (with enzymes) during composting. Results are mean values of triplicate, and the standard deviations are below 3% (n = 3).

3. Results and discussion

3.1. Carbon metabolism and total organic matter degradation of microbial community in composting

AWCD values determined by Biolog assessment were always used to evaluate the carbon metabolism of microbial community in composting. A higher number and growth rate of AWCD indicate a better carbon metabolism of microbial community (Cheng et al., 2016). As shown in Fig. 2, the AWCD value of A (control) in 3 d and 6 d was much higher than that in other sampling time, which indicated that the carbon metabolism ability of microbial community was strong in the initial stage of traditional composting. This phenomenon was mainly caused by the following reasons: (i) readily biodegradable OM was abundant at the initial time, which make the microbe grows rapidly, and (ii) the degradable OM substantial decrease to restrict the growth of microbe. The data in B (with enzymes) presented different trend from A (control). The AWCD value of B (with enzymes) in 15 d and 30 d was higher than that in 3 d and 6 d, which was diametrically opposite to the phenomenon in A (control). Furthermore, in 15 d and 30 d, the increase between A (control) and B (with enzymes) was 0.3 and 0.2 respectively. After 50 d compost treatment, the degradation efficiency of OM in A (control) and B (with enzymes) was 54.5% and 65.3% respectively. The implications of this phenomenon were the addition of complex enzyme can promote the degradation of lignin, and alleviate barrier action of lignin which inhibits the degradation of cellulose and hemicellulose by microorganisms. These results indicated that the addition of complex enzymes in composting was benefit to the growth and reproduction of microbial communities, and the carbon metabolic capacity was promoted by adding complex enzymes.

Both in A (control) and B (with enzymes), the AWCD value of Ecoplate has grown rapidly at the initial time, and after 96 h, the value drove to stability which indicated that the carbon metabolic ability was stable at that time. Therefore, the point of 96 h was selected to represent the carbon metabolic ability in further trials.

3.2. Functional diversity of microbial community during composting

A diversity index is a quantitative measure that reflects how many species there are in all samples, and simultaneously considers how evenly the individuals are distributed among those types. Shannon and McIntosh diversity index have been two kinds of popular diversity index in the ecological literature (Sun and Liu, 2004; Xue et al., 2017). Based on the typical sampling investigation, the metabolic diversity of carbon in composting was evaluated by application of Shannon and McIntosh diversity index in this study. The Shannon entropy (H) and McIntosh index (D_{mc}) quantify the uncertainty associated with this prediction, which are most often calculated as follows (Xi et al., 2015):

$$H = -\sum_{i=1}^{R} p_i \ln p_i, \quad p_i = n_i/\mathrm{N} \tag{2}$$

$$D_{mc} = \frac{N-U}{N-\sqrt{N}}, \quad U = \sqrt{\sum_{i=1}^{S} n_i^2} \tag{3}$$

Where p_i is the proportion of individuals belonging to the species in composting, S is the number of all species, n_i is the number of species i, and N is the number of all individuals.

As we can see according to these diversity indexes, the functional diversity of microbial community, both in A (control) and B (with enzymes), decreased gradually in 6–30 d, and rose again after 40 d. What's more, the functional diversity in B (with enzymes) was a little lower than that in A (control) before the initial 30 d, but the trend was opposite with an indistinctive difference ($P > 0.05$) after 40 d. These results indicated that the addition of complex enzymes had no obvious effects on the functional diversity of microbial community in composting. Precisely in the earlier-stage of composting, the complex enzymes were adverse to the metabolic diversity, but played the positive role to promote the diversity in the later-stage of composting.

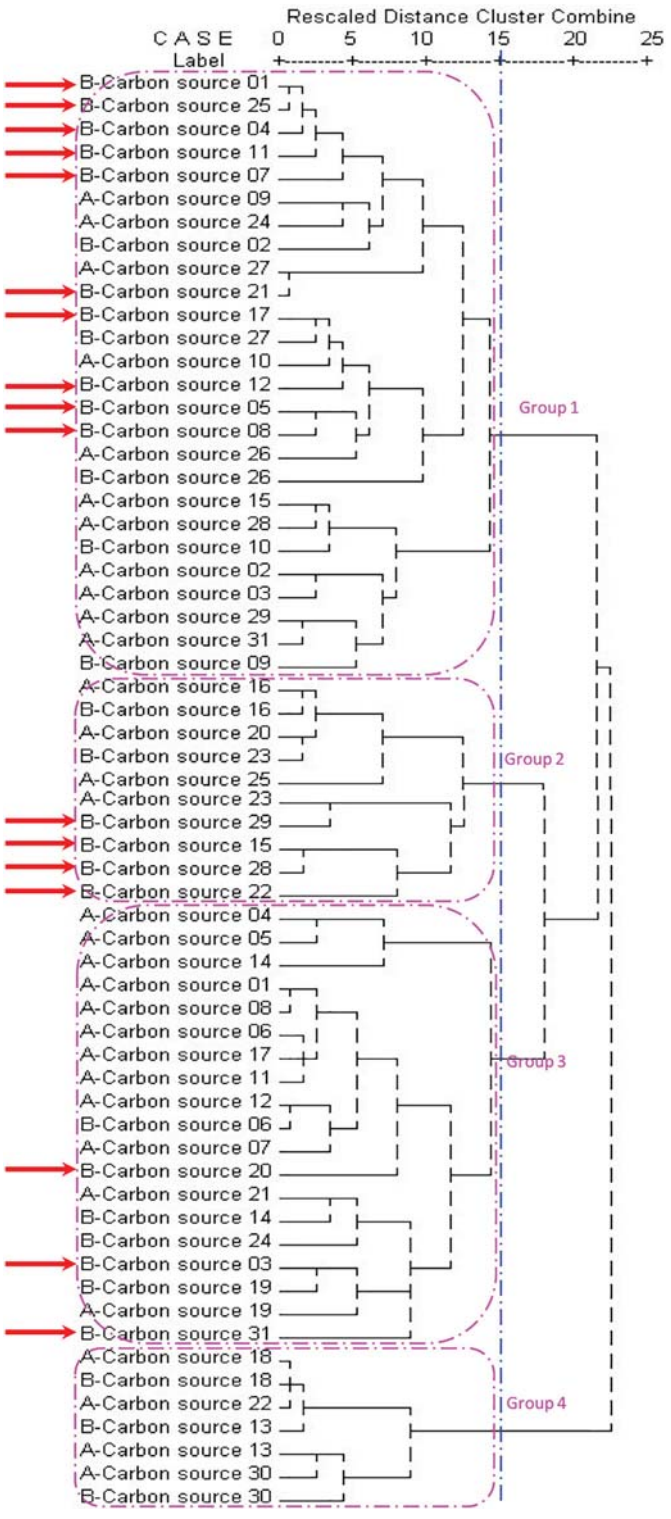

Fig. 3. Cluster analysis of carbon utilization on Biolog EcoPlate in A(control) and B (with enzymes).

The microbial community analysis showed that the lignin degrading fungi, actinomycetes and bacteria has become the dominant strains in 6–30 d of composting, which were benefit to degrade lignin. And after 40 d, the microbial community structure became more and more diversified. These results were corresponding to the trends of diversity indexes. Due to the limitation of available carbon, the more adaptable species play a main role in composting, which cause the decrease of metabolic diversity indexed. But after a period, lignin was decomposed to the organic matters with lower molecular weight, so that the quantity of microbial was increase and the structure of microbial community was abundant to make the metabolic diversity indexed pick up after 40 d of composting. That's one of the reasons why a little negative effect can be found after adding the complex enzyme.

3.3. Effect of complex enzymes on carbon utilization

Clustering analysis was used in this study to understand the effects of complex enzymes on metabolic capacity of different carbon sources. Fig. 3 showed that the 31 carbon sources in Eco-plate can be divided into 4 groups by analyzing their carbon source metabolic properties in A (control) and B (with enzymes). According to the data, the 2, 9, 10, 24, 26 and 27 carbon sources (Tween 40, D-Xylose, i-Erythritol, L-Arginine, L-Phenylalanine and L-Serine) were alike and went together to group 1. That means that the metabolic capacities of these carbon sources were similar both in A (control) and B (with enzymes). These results indicated that the addition of complex have almost no influence on these carbons in composting.

Some kinds of carbon divided into the same group both in A(control) and B (with enzymes), including (i) the carbon 16 and carbon 23 were all belong to group 2, (ii) the carbon 6, 14 and 19 were all belong to group 3, and (iii) the carbon13, 18 and 30 were all belong to group 4. This phenomenon indicated that the addition of complex enzymes has almost little impact on the metabolism of D-Galactonic Acid γ-Lactone, D-Malic Acid, D-Cellobiose, Glucose-1-Phosphate, 4-Hydroxy Benzoic Acid, D-Glucosaminic Acid, 2-Hydroxy Benzoic Acid and Phenylethylamine. While the carbon 1, 3, 4, 5, 7, 8, 11, 12, 15, 17, 20, 21, 22, 25, 28, 29 and 31 were divided into different group in A(control) and B (with enzymes), which indicated that these kinds of carbon were influenced considerably after adding the complex enzymes in composting.

The details about the utilization of these 17 kinds of carbon, which were influenced considerably after adding the complex enzymes, were showed in Fig. 4. Data on 15–50 d displayed that the Putrescine was the carbon that possess the highest utilization efficiency in A (control). And the metabolic capability of Tween 80, Pyruvic Acid Methyl Ester, γ-Hydroxybutyric, Itaconic Acid, L-Asparagine and Glycyl-L-Glutamic Acid were better among all kinds of carbon source. The Itaconic Acid possesses the highest utilization efficiency in B (with enzymes). These results indicated that the addition of complex affect the metabolic capability of these 17 kinds of carbon in composting, especially in 15 d.

A comprehensive analysis of the total carbon metabolic capacity in Fig. 4 shows that the addition of complex enzymes has deeply promoted the utilization of pyruvic acid methyl ester, α-Cyclodextrin, D-Mannitol, D-Galacturonic, Itaconic acid and L-asparagine by microorganisms in 15–50 d, and depressed the utilization of D, L-α-Glycerol-phosphate, L-

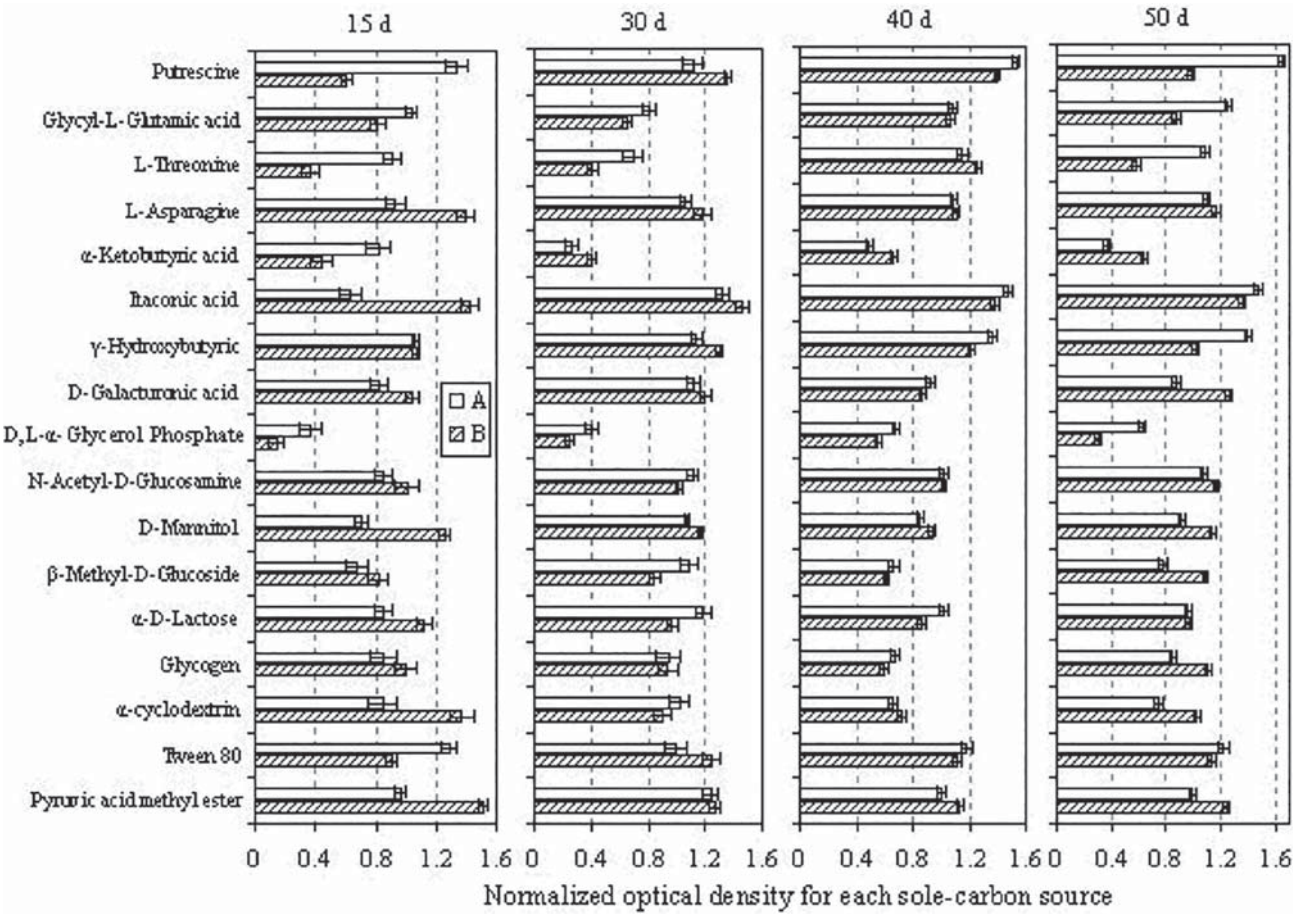

Fig. 4. Utilization of 17 carbon sources on Biolog EcoPlate for samples in A (control) and B (with enzymes) during composting. The bars represent the standard deviations of the means (n = 3).

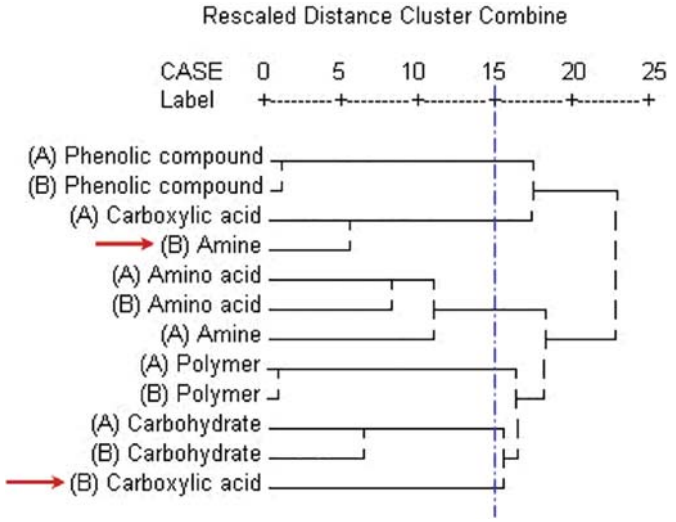

Fig. 5. Cluster analysis of utilization profiles of six classes of carbon source on Biolog EcoPlate in A (control) and B (with enzymes).

Threonine, Glycyl-L-Glutamic acid and putrescine. That may due to the addition of compound enzymes that affect the composition of microbial communities. It is means the species which possess the high ability to degrade pyruvic acid methyl ester and et al. were increased, and the group which was benefit to use the D, L-α-Glycerol-phosphate and et al. were decreased during this process. Therefore, the decomposition and utilization of carbon in composting can be promoted or inhibited these positive or negative effects.

3.4. Carbon utilization and lignin degradation analysis

The results of cluster analysis of 6 kinds of carbon in Eco-plate showed that the average metabolic capability of carboxylic acid and amine in A (control) and B (with enzymes) were in different group, which means a visible difference in composting after adding complex enzyme (Fig. 5). The dynamic changes of carboxylic acid and amine metabolism in Eco-plate by microorganisms during the process of composting were shown in Fig. 6. The results showed that the average metabolic rate of carboxylic acid increased by adding complex enzymes, and the average metabolic capability of amine decreased under the same condition. In the details of the phenomenon, that is since the complex enzymes can encourage the utilization of Pyruvic Acid Methyl Ester, D-Galactonic Acid and Itaconic Acid, and inhibit the utilization of putrescine, thereby further changing the average metabolic capacity of these two kind of carbon source in the Eco-plate by composting. Based on the above-mentioned research results, there might have been two main reasons: (i) the addition of complex enzyme changed the community composition of soil system, which means this new microbial group was benefit to the utilization of carboxylic acid, and had negative effect to amine, and (ii) the activity of LiP will reach the optimum level with the pH of 2–5 and the temperature of 35–55 °C, and the MnP present the highest activity under the condition of pH 4–7 and temperature 40–60 °C, therefore the acid environment is more propitious to the complex enzyme reaction (Asgher et al., 2008).

After 50 d of composting, the degradation rate of lignin in A(control) and B (with enzymes) were 43.6% and 61.2% respectively (Fig. 7), which indicated that the addition of complex enzymes in composting can obviously promote the degradation of lignin ($P = 0.001$). Previous studies have confirmed lignin is a complex phenolic polymer containing carboxyl, carbonyl, hydroxyl, methyl and other kind of side chain. According to Fig. 8, the change in FT-IR spectra of the samples in A (control) and B (with enzyme) are found. Some kinds of functional group have obviously changed. The stretching vibration of OH was distinctly decreased at 3450–3350 cm^{-1}. The stretching vibration of saturated methylene was decreased at 2923–2850 cm^{-1}. The antisymmetric stretching vibration of organic carboxylic acid was slightly enhanced at 1650–1635 cm^{-1}. The asymmetric stretching vibration of ether bond C—O—C was greatly weakened at 1150 cm^{-1}. The vibration of O—H in methyl catechol was disappeared at 1045 cm^{-1}. The absorption peak of monosubstituted aromatic hydrocarbons was enhanced 898 and 694 cm^{-1}. The vibration of substituted benzene was obviously increased at 790 cm^{-1}, which indicated that substitution reaction was one of the major processes during the lignin degradation by complex enzyme in composting.

These changes of function groups indicated that, during the lignocellulosic waste degradation by adding complex enzyme, organic carboxylic acids and amide compounds were continually break down, and the bonds of large molecules can be cut off into small ones. The changes or removal of replaceable group in benzene ring can be replaced by hydroxide radical during the process of the decomposition. At the same time, a variety of intermediate products such as 4-hydroxybenzoic acid and carboxylic acids were released in lignin decomposition (Sachan et al., 2010; Cañas and Camarero, 2010). The promotions of phenolic compound and carboxylic acids degradation by adding the

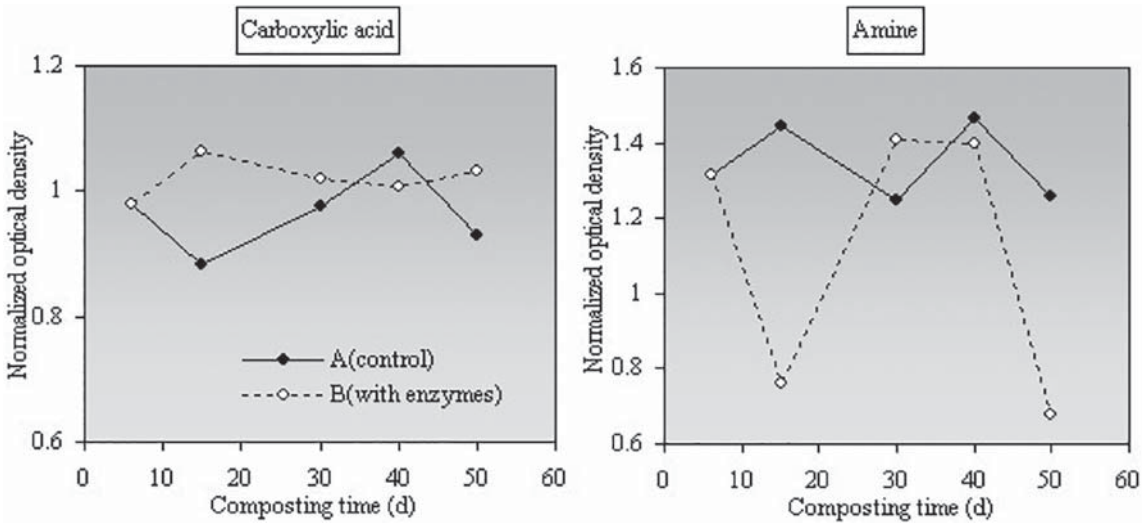

Fig. 6. Carboxylic acid and amine utilization on Biolog EcoPlate in A(control) and B (with enzymes) during composting. Results are mean values of triplicate, and standard deviations are below 1% ($n = 3$).

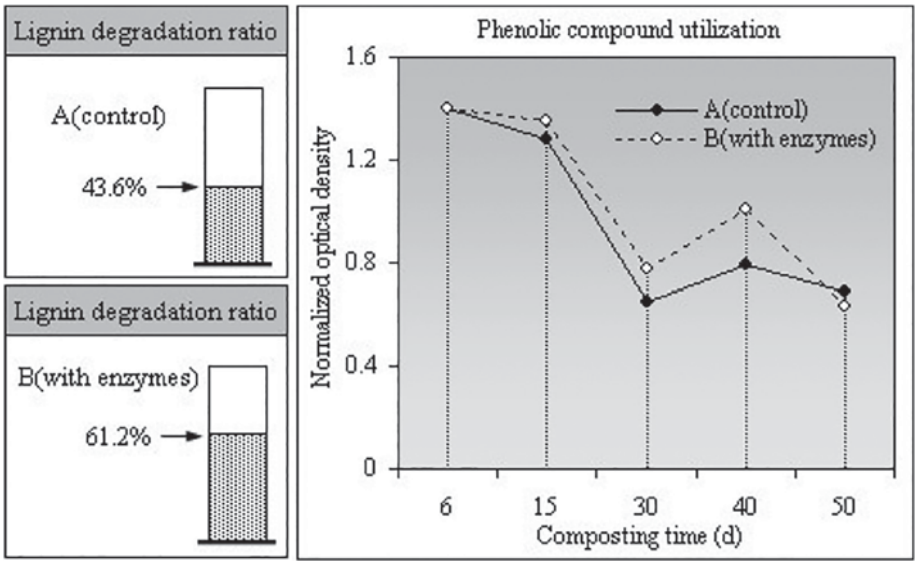

Fig. 7. Phenolic compound utilization on Biolog EcoPlate and lignin degradation in A (control) and B (with enzymes). Results are mean values of triplicate, and standard deviations are below 2% (n = 3).

complex enzymes in composting were benefit to the complete oxidation of these intermediates, which can be oxidized into CO_2 and H_2O (Tanaka et al., 2009; Mester and Ming, 2000). Therefore, the following two reasons could lead to the high degradation efficiency of lignin in composting: (i) the complex enzymes can promote the decomposition of lignin, and (ii) the high utilization of phenolic compound and carboxylic acids in composting were revealed by adding complex enzyme.

4. Conclusions

A novel composting system which was added the functional complex lignin degradation enzyme after 7 d composting was set up. Based on these results obtained in the present study, it is concluded that the addition of complex enzymes in composting could not only improved degradation efficiency of OM and other hazardous materials in composting, but also provide extensive carbon sources to promote the growth of microorganisms. The degradation efficiency of OM arrived at 65.3% after 50 d in B (with enzymes), and the AWCD value of which was higher than A (control) in 15 d and 30 d when adding the complex enzyme into the composting process. However, the addition of compound enzyme had no significant effect on the metabolic diversity of microbial community during composting ($P > 0.05$). The metabolic diversity of carbon was inhibited slightly before 30 d by adding the complex enzymes, but was promoted mildly at a later stage of composting.

The results of cluster analysis and dynamic analysis of metabolic capacity in a certain kind of carbon source were revealed in this study,

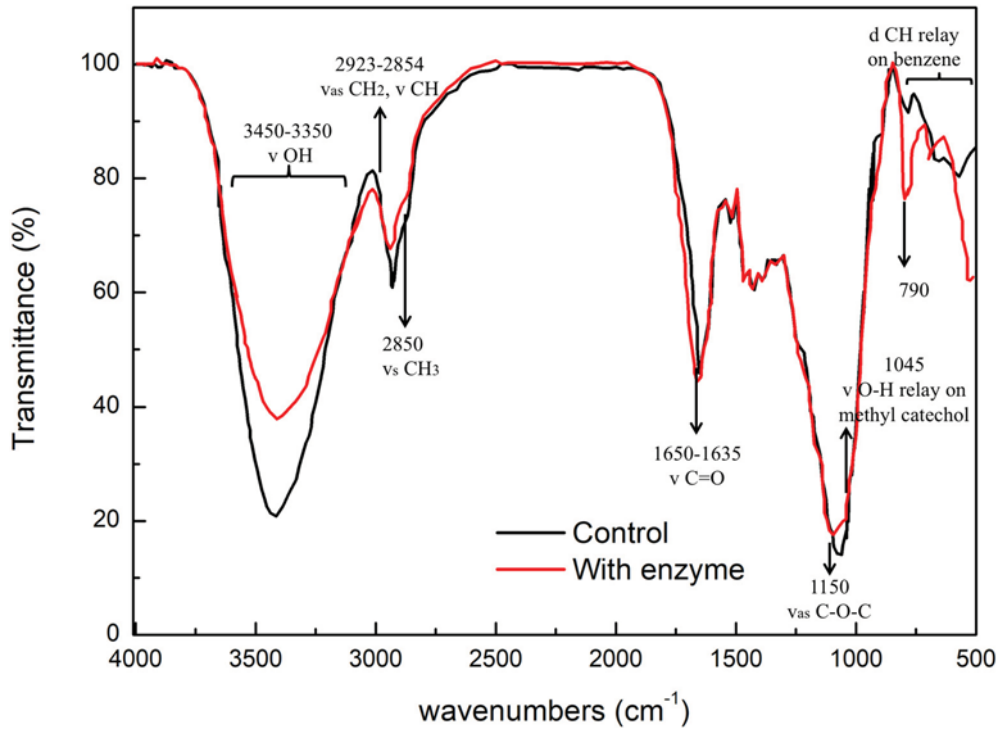

Fig. 8. FT-IR spectrum in A (Control) and B (with enzymes) after 60 days composting.

which indicated that the metabolic capacities of 17 kinds of carbon in Eco-plate were impacted remarkable by the complex enzymes at 15–50 d. Especially on 15 d, 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. The average metabolic rate of carboxylic acid was increased in B (with enzymes), and average metabolic capability of amine was decreased under the same condition. The related reason is the addition of complex enzyme changed the microbial communities structure during the process of composting, which were benefit to the utilization of pyruvic acid methyl ester and et al., and had negative effect to D, L-α-Glycerol-phosphate et al. Furthermore, the degradation efficiency of lignin was significantly promoted by adding the complex enzymes, and the value was arrived at 61.2%. The main reason is the addition of complex enzymes accelerated the utilization of phenol and carboxylic acids.

To summarize, this study revealed characteristic and changes about the metabolic way of microbial community in composting. These results undoubtedly contribute to the understanding and developing of composting treatment which was strengthened by the complex enzymes. Such data provided the theoretical basis and the application possibility of enzymes strengthened composting, which will be of great benefit to the understandings of the trends in enzyme biotechnology of composting, which would benefit to shift public's focus to the ecological impacts of composting.

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