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

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

Keywords: Enhanced Composting; Ligninolytic Microorganisms; Complex Enzymes; Degradation; Carbon Metabolic Diversity; Biogeochemical cycle.

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

80 germination of plant (Aslam et al. 2008; Gong et al. 2017). These wastes are valuable
81 for soil erosion control and soil nutrient replenishment, but will harm the environment
82 if applied without proper treatment such as composting (Bustamante et al. 2008;
83 Huang et al. 2018; Gong et al. 2018). The degradation and transformation of
84 lignocellulosic waste is attributed to the metabolism of indigenous microorganisms
85 during composting. Therefore, it is very important to improve the practicality and
86 efficiency of the composting technology. Among a variety of enhanced composting
87 methods, the combination with microorganisms and enzymes is a more popular and
88 efficient one (Xu et al. 2017; Sun et al. 2017).
89 Lignin is a kind of cross-linked phenolic polymer, which was rigidity and do not
90 rot easily. The degradation of lignin in composting mainly depends on the ligninolytic
91 enzymes, which was also a kind of extracellular enzyme secreted by ligninolytic
92 microorganisms. White rot fungi (WRF) are capable of degrading lignin and most of
93 lignin structure analogues efficiently, via unique extracellular oxidative enzyme
94 systems with a low substrate specificity, and intracellular enzyme systems (Huang et
95 al. 2017a; Huang et al. 2017b; Huang et al. 2017c). Vividly, WRF also has been
96 referred to "externalized stomachs" that secrete hydrolytic enzymes and organic acids
97 into the extracellular conditions and transport metabolite and chelates pass through
98 into the cell wall. Lignin peroxidases (LiP), manganese peroxidases (MnP) and
99 copper-based laccases (Lac) are three kinds of typical ligninolytic enzymes secreted
100 by WRF. LiP oxidize non-phenolic lignin, whereas MnP only oxidize the phenolic
101 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-$C_ç$ 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 et al. 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 is 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 10-20mm. 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 ℃, and then transferred to PDA plates at 37 ℃ 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 ℃. 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.

165 *2.3 Composting set-up and sampling*

166 The experimental apparatus used for this research consisted of a lab-scale square
167 reactor (76 x 55 x 45 cm) with a 75% filling level under indoor conditions. The pile
168 was turned once every three days in the first 2 weeks and then once every six days
169 afterwards. The composting experiment was performed in three replicates (done
170 simultaneously), and each lasted 50 days. Two identical sets of experimental
171 apparatuses were prepared and labeled as Reactors A and B. Traditional composting
172 method was adopted in reactor A (control) with the addition of inactivated complex
173 fungal enzymes. While the complex fungal enzymes were added into reactor B (with
174 enzymes) after 7 days composting, and the pile temperature was plunged to below
175 38 ℃ at the same time. Three parallel samples were set both in A (control) and B
176 (with enzymes).

177 *2.4 Physicochemical parameters analysis*

178 The temperatures were monitored every day. The moisture content of samples
179 was determined by oven-drying at 105 ℃ for 24 h. The dried samples were analyzed
180 for total organic carbon (TOC) by dry combustion overnight at 550 ℃ before
181 reweighting (Jiménez and García 1992). OM values were commonly calculated by a
182 conversion factor of 1.724 to convert total organic carbon: OM (%) = TOC (%) ×
183 1.724. Total organic nitrogen (TN) was measured by the Kjeldahl method (Martins
184 and Dewes 1992). C/N ratios were determined by the quotient values of TOC and TN.
185 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 4000-500 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 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.

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

249 which inhibits the degradation of cellulose and hemicellulose by microorganisms.
250 These results indicated that the addition of complex enzymes in composting was
251 benefit to the growth and reproduction of microbial communities, and the carbon
252 metabolic capacity was promoted by adding complex enzymes.

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

257 *3.2 Functional diversity of microbial community during composting*

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

267 $$H = - \sum_{i=1}^{R} p_i \ln p_i \ ,$$

268 $$p_i = n_i \ / \mathrm{N} \qquad (2)$$

269 $$\mathrm{D_{mc}} = \frac{N-U}{N-\sqrt{N}} \ ,$$

$$U = \sqrt{\sum_{i=1}^{S} n_i^2} \quad (3)$$

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.

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

292 and the structure of microbial community was abundant to make the metabolic
293 diversity indexed pick up after 40 d of composting. That's one of the reasons why a
294 little negative effect can be found after adding the complex enzyme.

295 *3.3 Effect of complex enzymes on carbon utilization*

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

305 Some kinds of carbon divided into the same group both in A(control) and B
306 (with enzymes), including (i) the carbon 16 and carbon 23 were all belong to group 2,
307 (ii) the carbon 6, 14 and 19 were all belong to group 3, and (iii) the carbon13, 18 and
308 30 were all belong to group 4. This phenomenon indicated that the addition of
309 complex enzymes has almost little impact on the metabolism of D-Galactonic Acid
310 γ-Lactone, D-Malic Acid, D-Cellobiose, Glucose-1-Phosphate, 4-Hydroxy Benzoic
311 Acid, D-Glucosaminic Acid, 2-Hydroxy Benzoic Acid and Phenylethylamine. While
312 the carbon 1, 3, 4, 5, 7, 8, 11, 12, 15, 17, 20, 21, 22, 25, 28, 29 and 31 were divided

313 into different group in A(control) and B (with enzymes), which indicated that these
314 kinds of carbon were influenced considerably after adding the complex enzymes in
315 composting.

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

325 A comprehensive analysis of the total carbon metabolic capacity in Fig.4 shows
326 that the addition of complex enzymes has deeply promoted the utilization of pyruvic
327 acid methyl ester, α-Cyclodextrin, D-Mannitol, D-Galacturonic, Itaconic acid and
328 L-asparagine by microorganisms in 15-50 d, and depressed the utilization of D,
329 L-α-Glycerol-phosphate, L-Threonine, Glycyl-L-Glutamic acid and putrescine. That
330 may due to the addition of compound enzymes that affect the composition of
331 microbial communities. It is means the species which possess the high ability to
332 degrade pyruvic acid methyl ester and et al. were increased, and the group which was
333 benefit to use the D, L-α-Glycerol-phosphate and et al. were decreased during this
334 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 ℃, and the MnP present the highest activity under the condition of pH 4-7 and temperature 40-60 ℃, 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 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

400 complex enzymes, but was promoted mildly at a later stage of composting.

401 The results of cluster analysis and dynamic analysis of metabolic capacity in a
402 certain kind of carbon source were revealed in this study, which indicated that the
403 metabolic capacities of 17 kinds of carbon in Eco-plate were impacted remarkable by
404 the complex enzymes at 15-50 d. Especially on 15 d, the utilization of pyruvic acid
405 methyl ester, α-Cyclodextrin, D-Mannitol, D-Galacturonic, Itaconic acid and
406 L-asparagine were deeply promoted, and that of D, L-α-Glycerol-phosphate,
407 L-Threonine, Glycyl-L-Glutamic acid and putrescine were depressed by adding the
408 complex enzyme in composting. The average metabolic rate of carboxylic acid was
409 increased in B (with enzymes), and average metabolic capability of amine was
410 decreased under the same condition. The related reason is the addition of complex
411 enzyme changed the microbial communities structure during the process of
412 composting, which were benefit to the utilization of pyruvic acid methyl ester and et
413 al, and had negative effect to D, L-α-Glycerol-phosphate et al. Furthermore, the
414 degradation efficiency of lignin was significantly promoted by adding the complex
415 enzymes, and the value was arrived at 61.2%. The main reason is the addition of
416 complex enzymes accelerated the utilization of phenol and carboxylic acids.

417 To summarize, this study revealed characteristic and changes about the metabolic
418 way of microbial community in composting. These results undoubtedly contribute to
419 the understanding and developing of composting treatment which was strengthened
420 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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560 **Table 1**

561 Functional diversity of microbial community during composting

Samples	Incubation time				
	6 d	15 d	30 d	40 d	50 d
Shannon diversity index *H*					
A (control)	4.5035	4.4961	4.4732	4.4941	4.4630
B (with enzymes)	4.5084	4.4571	4.4442	4.5044	4.4652
McIntosh diversity index D_{Mc}					
A (control)	1.0470	1.0466	1.0460	1.0465	1.0448
B(with enzymes)	1.0470	1.0446	1.0448	1.0471	1.0452

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Figure captions:

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

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).

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

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)

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 4% (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)

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

585 **Fig. 1.**

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 and 19 were phenolic compound.
30 and 31 were amine.

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590 **Fig. 2.**

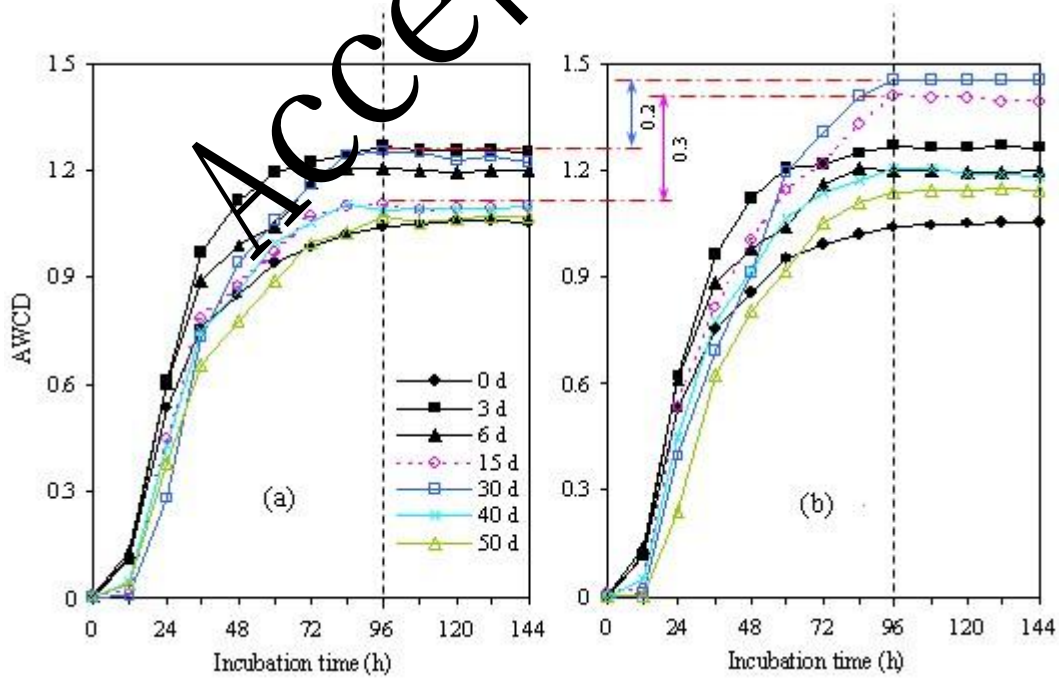

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Fig. 3.

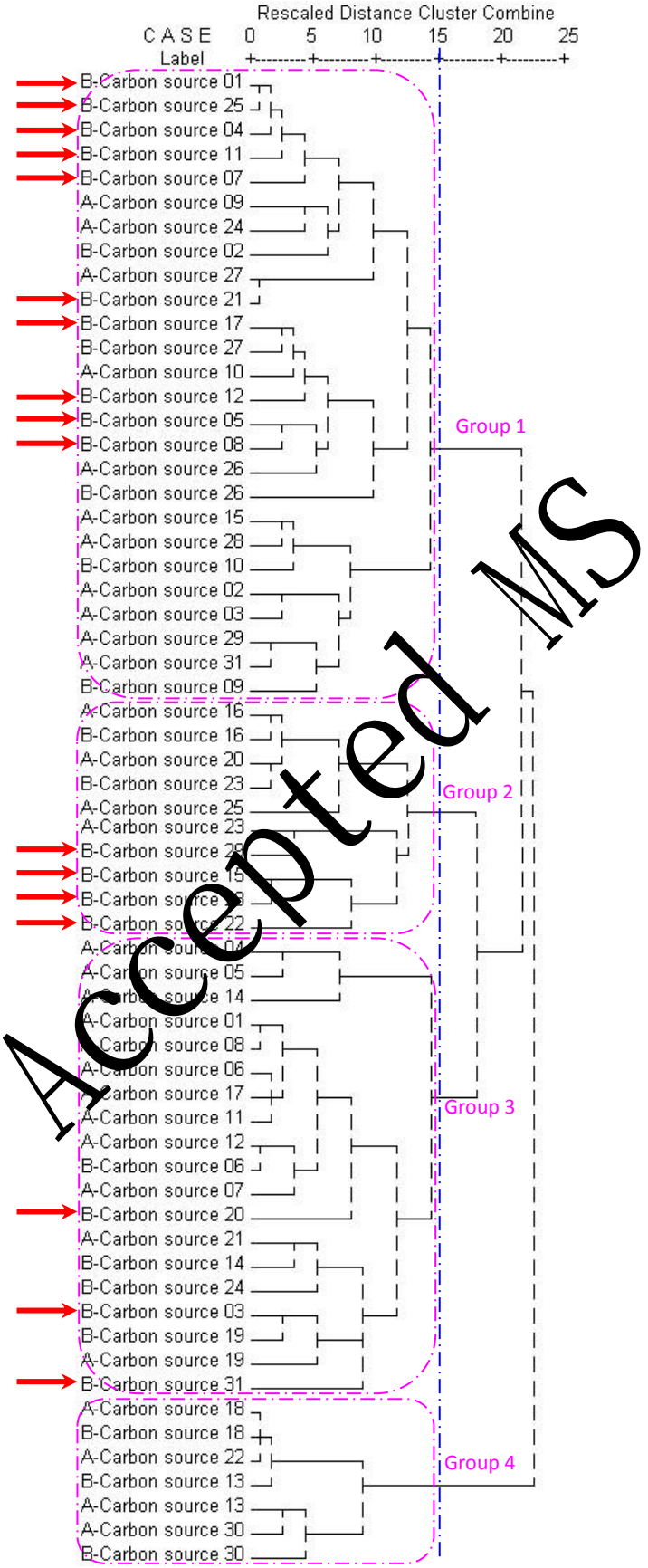


Fig. 4.

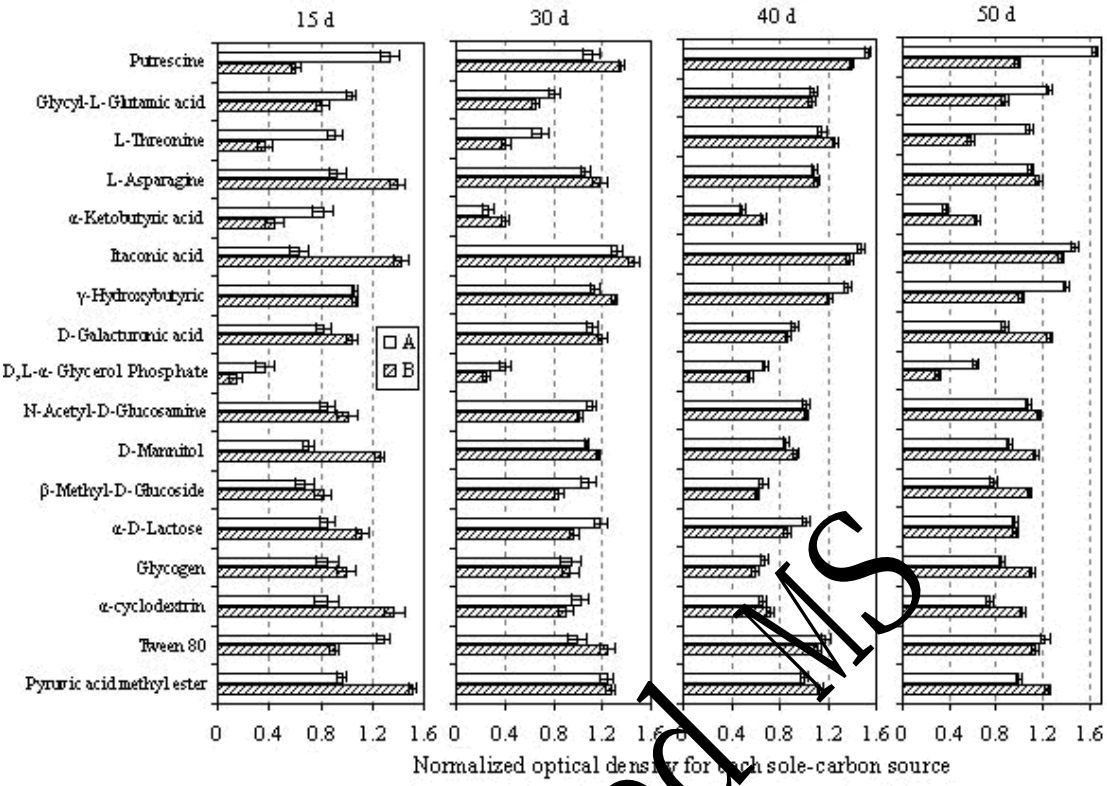
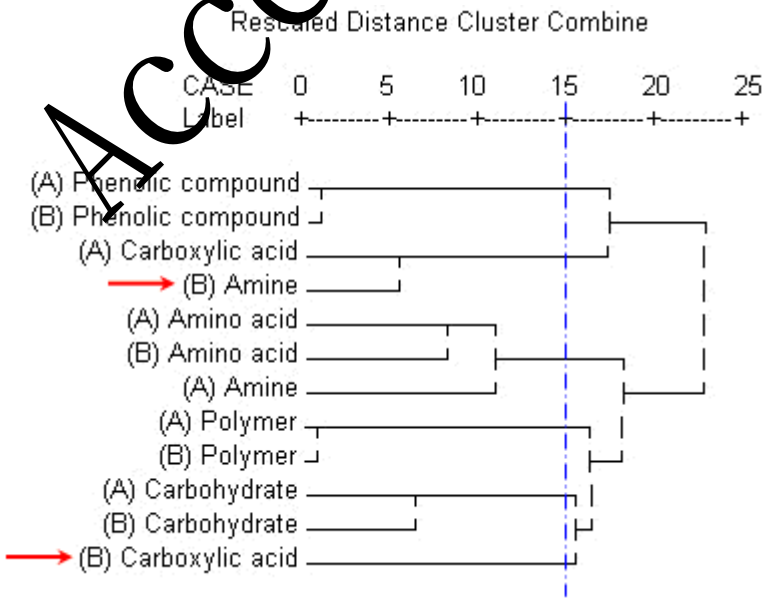
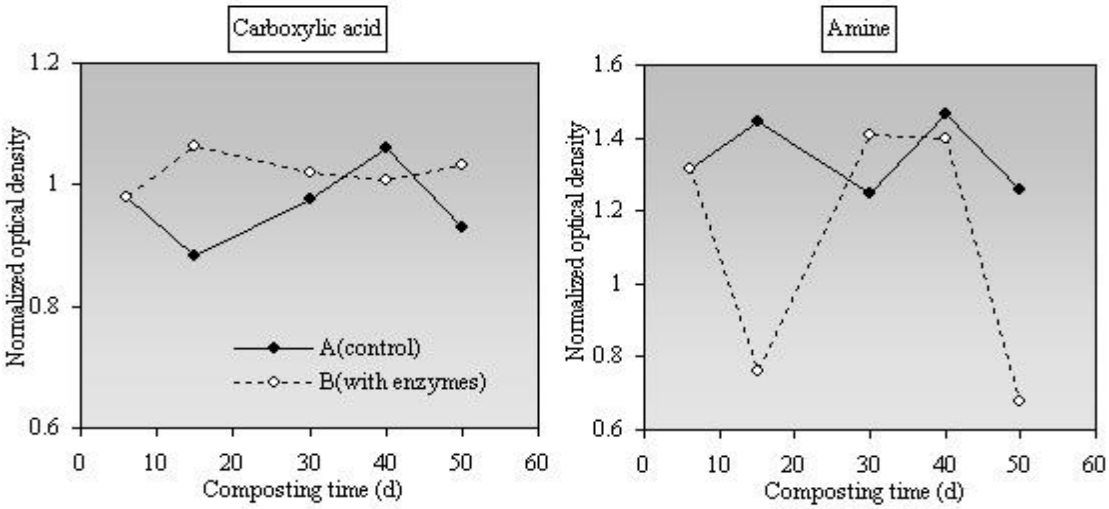


Fig. 5.



608 **Fig. 6.**



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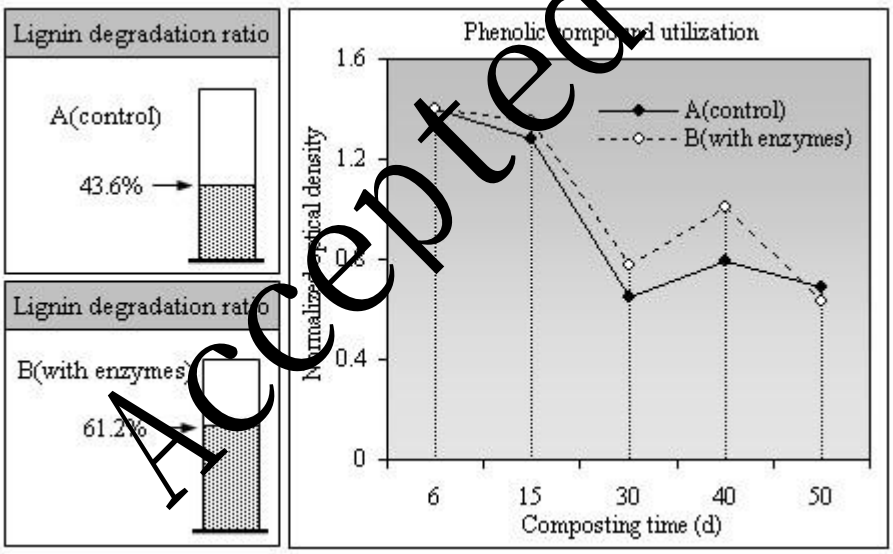
614 **Fig. 7.**



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618 **Fig. 8.**

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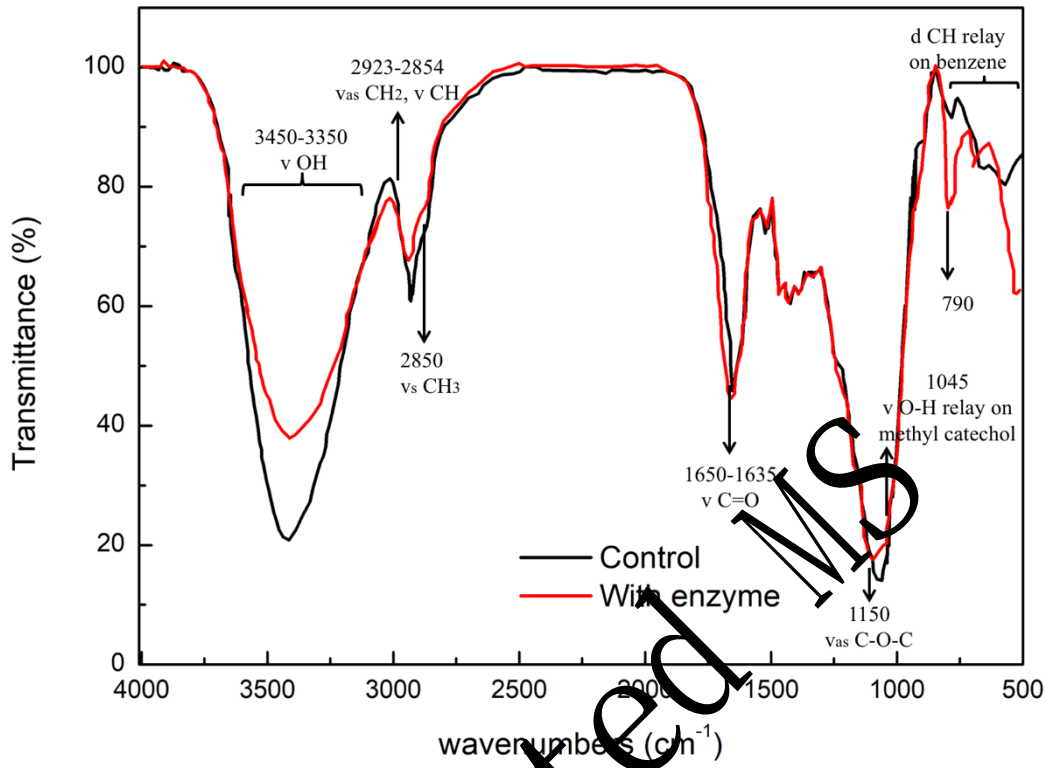

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