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Effects of ratio of manganese peroxidase to lignin peroxidase on transfer of ligninolytic enzymes in different composting substrates

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ABSTRACT

The transfer of ligninolytic enzymes in composting substrates (soil, vegetable leaf, rice straw and chaff) was investigated using a series of column elution experiments. Environmental scanning electron microscope (ESEM) and Fourier transform infra-red spectroscopy (FTIR) were used to analyze the mechanism of ligninolytic enzymes adsorption onto those substrates. The significant differences were found between substrates with adsorption of enzymes and those without enzymes by FTIR. Hydrophobic groups (methylene and alkane groups) and active groups (—CH_2—CO— or —CH_2—NH—) contents decreased in the substrates with adsorption of enzymes. The transfer abilities of total protein in the four composting substrates (soil, vegetable leaf, rice straw and chaff) were weakened as the ratio of manganese peroxidase (MnP) to lignin peroxidase (LiP) increased from 4 to 6, and were enhanced when the MnP/LiP ratio increased to 8. The transfer abilities of LiP in the four composting substrates were enhanced continually with the increasing MnP/LiP ratio. The transfer of MnP was similar to that of total protein with the change of MnP/LiP ratio. These results indicated that the change of MnP/LiP ratio could affect the transfer of ligninolytic enzymes in different composting substrates.

1. Introduction

Currently, composting has been widely used as a sustainable alternative to manage and recycle organic solid wastes to provide relative stable product as a fertilizer [1–3]. Lignocellulose is the most abundant component in organic wastes, and probably responsible for limiting degradation during composting process [4,5]. Lignin slows down biodegradation of cellulose and hemicellulose in lignocellulosic substrates because it acts as a physical barrier protecting the carbohydrates. Therefore, lignin degradation can be considered as a key process during composting of lignocellulosic substrates.

Lignin degradation is catalyzed by a group of hemoperoxidases secreted by fungi in response to nutrients limitation during their secondary metabolism, namely lignin peroxidase (LiP) and manganese peroxidase (MnP) [6–8]. In addition, these enzymes also catalyse the degradation of a wide variety of organic pollutants, such as dye, carbamazepine, diclofenac and so on [9–11]. As we know, ligninolytic enzymes (LiP and MnP) secreted by *Phanerochaete chrysosporium* (*P. chrysosporium*) could catalyze the degradation of lignin to accelerate the compost process [12]. Also the addition of ligninolytic enzymes in composting could enhance the lignin degradation and improve carbon utilization [13]. So LiP and MnP can be considered as crucial enzymes involved in the process and efficiency of composting. The cooperation and inhibition between LiP and MnP were important in enzymolysis [11,14]. Wang et al. [15] reported the cooperation between ligninolytic enzymes in the process of biological degradation of lignin, and the results indicated that the ratio of MnP/LiP activity was a key factor affecting the degradation rate of lignin. However, composting is a very complicated process involving intensive microbial activity and enzymolysis reaction. Adsorption and transfer of ligninolytic enzymes, which deemed to be physicochemical processes, have occurred before the biodegradation of lignocellulose by enzymes. The degradation of deeper layer component would be hindered because enzymes mainly remain in the shallow layer. Therefore, it is important to transfer ligninolytic enzymes into all over the composting substrates. Moreover, the transfer of high efficient enzymes to the deeper layer of composting substrates would be influenced

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Table 1
The physico-chemical properties of composting substrates.

Substrates	True density (g/mL)	Organic matter content (%)	pH	Moisture content (%)
Soil	2.3491	6.34	7.05	2.79
Vegetable leaf	1.5819	82.23	6.56	11.58
Rice straw	1.7633	76.36	7.46	11.02
Chaff	1.4869	60.90	6.17	9.90

Table 2
The enzymes activity and the protein content consisted in different crude enzymes solution.

MnP/LiP ratio	MnP activity (U/L)	LiP activity (U/L)	Protein content (μg/mL)
4	250.15	62.02	35.10
6	345.38	57.48	45.29
8	465.11	58.23	59.30

by different physical and biochemistry factors which related to ratios of MnP to LiP. Consequently, it was necessary and meaningful to study the transfer of ligninolytic enzymes at different ratios of MnP to LiP. However, little attention has been devoted to research on factors affecting the transfer of ligninolytic enzymes in composting substrates.

The main objective of this study was to get deep insight into the transfer of ligninolytic enzymes at different ratios of MnP to LiP in different composting substrates, which could offer some useful information for further understanding lignocellulose enzymatic degradation mechanism and promoting composting by addition of enzymes.

2. Materials and methods

2.1. Composting substrates

The composting substrates (soil, vegetable leaf, rice straw and chaff) were collected from the suburb of Changsha, China. These materials were air-dried for 3 days at an average temperature of 35 °C before used. They were then ground to powder using a jar mill to pass through a 100-mesh sieve (0.15 mm). The physico-chemical properties of these substrates are shown in Table 1.

2.2. Enzymes solution

P. chrysosporium strain BKM-F-1767 was obtained from China Center for type Culture Collection (Wuhan, China). Fugal cultures were maintained on potato dextrose agar (PDA) slants at 4 °C, and then transferred to PDA plates at 30 °C for several days. *P. chrysosporium* was grown in an immobilized and nonimmersed liquid culture system. The culture medium was modified on the basis of the method described by Tien and Kirk [16], which was considered advantageous for the formation of ligninolytic enzymes. Spore suspensions were prepared in sterile distilled water and subsequently adjusted to the concentration of 2.0×10^6 CFU/mL. Those suspensions in same concentration were inoculated into cultures at various volume ratios to get different ratios of MnP to LiP. In detail, 2, 4 and 6 mL spore suspensions were inoculated to 200 mL cultures in 500 mL Erlenmeyer flask to get values of 4, 6 and 8 respectively for the ratio of MnP to LiP. After that, the prepared samples were incubated at 30 °C in a rotary shaker with agitation at 120 rpm with a 2.5 cm-diameter throw. The cultures were harvested at the day of the maximum activities of the ligninolytic enzymes and centrifuged at 9000 rpm, 4 °C for 10 min. The solution supernatant was employed as crude ligninolytic enzymes for the following sorption and transfer experiments. The enzymes activity and protein content consisted in the different crude enzymes solutions are shown in Table 2. All reagents used were of analytical grade.

2.3. Transfer procedure

As shown in Fig. 1, the experimental apparatus consists of eight elution columns, a distribution installation, a multipass, a filtering flask, a rotormeter and a vacuum air pump. Standard syringes for medical purpose packed with one-millimeter filter papers in the bottom were used as elution mini-columns and mounted to a vacuum air pump, equipped with a rotormeter and a multipass. Composting substrates (soil, vegetable leaf, rice straw and chaff) were prepared equally in quality as porous media and put into columns until 10 mL scale-position, respectively. The media equipped were sterilized before elution test. The protocol for the enzymes injection experiment consisted of rinsing the media in the column with 19.6 mL sterile deionized water which was no less than threefold pore volume of the individual substrate to ensure media completely moistened, adding 20 mL enzymes solution, rinsing with another 19.6 mL sterile deionized water and then maintaining the vacuum until the column appeared to be dry. All solutions were added to the top face of the media. The rotormeter was set to produce a superficial velocity at 0.3 Nm^3/h. The experimental temperature was controlled at room temperature. The control experiment was composed of the same procedures, except that 20 mL enzymes solution was replaced by 20 mL sterile deionized water.

The column was then cut at the bottom and sliced into 2 cm slices while it was extruded using the syringe plunger through the top of the tube. Then the five parts of substrates were putted to 100 mL erlenmeyer flasks, respectively. And then 20 mL sterile water was added to each flask. The extract of each substrate was obtained by severe oscillation at 250 rpm for 30 min, followed by centrifugation at 10,000 rpm, 4 °C for 15 min. The solution

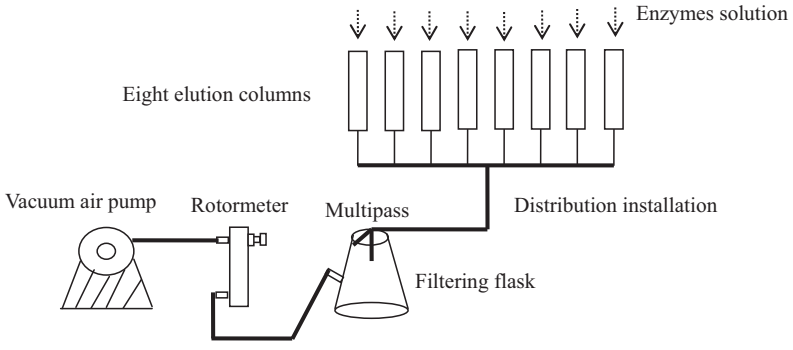

Fig. 1. A schematic diagram of the experimental system. Vacuum air pump (vacuum degree 0.098 MPa), rotormeter (air, 25 °C, 101.3 kPa).

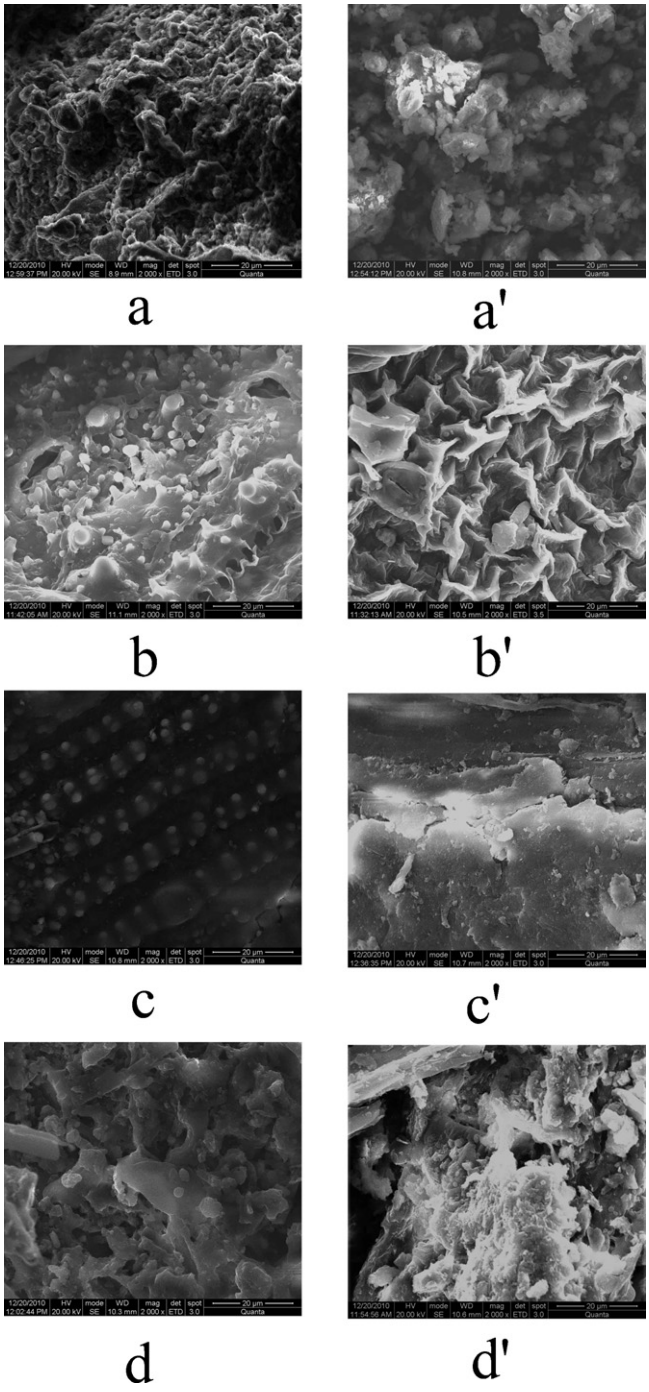

Fig. 2. ESEM images of the soil with adsorption of enzymes (a), vegetable leaf with adsorption of enzymes (b), rice straw with adsorption of enzymes (c), chaff with adsorption of enzymes (d) and the soil without enzymes (a′), vegetable leaf without enzymes (b′), rice straw without enzymes (c′), chaff without enzymes (d′).

supernatants were used as samples for calculating protein retention and enzyme activity.

The transfer ability was defined that the amount of enzyme transferred relative to the depth of the composting substrate column at some time point.

2.4. Protein determination

Protein concentration in the solution was measured by the method of Coomassie brilliant blue G-250 with bovine serum albumin as a standard [17].

2.5. Enzyme assays

Part of the supernatant fluid was filtered through a 0.45-μm filtering film for ligninolytic peroxidases activities analyses. In this study, two main ligninolytic peroxidases (LiP and MnP) were measured with an ultraviolet spectrophotometer (UV-2250, SHIMADZU, Japan) [18,19].

LiP activity was determined by monitoring the conversion of veratryl alcohol to veratryl aldehyde at 30 °C in the presence of hydrogen peroxide at 310 nm. The final reaction mixture (total volume, 3 mL) contained 1.2 mL of sodium tartrate (250 mM, pH 3.0), 0.6 mL of veratryl alcohol (10 mM) and 1.2 mL of enzyme sample. The reaction was initiated by adding 60 μL of H_2O_2 (10 mM) at 30 ± 1 °C. One unit of enzyme activity was defined as the amount of the enzyme which can produce 1 μM veratryl aldehyde from the oxidation of veratryl alcohol per minute [20].

MnP activity was determined according to the method described by Huang et al. [21], which was based on the oxidation of Mn^{2+} to Mn^{3+}. $MnSO_4$ (0.2113 g) was added into 1 L of sodium succinate (50 mM, pH 4.5). The final reaction mixture contained 2.4 mL of sodium succinate (50 mM, pH 4.5) and 0.6 mL of enzyme sample. The reaction was initiated by adding 60 μL of H_2O_2 (10 mM) at 30 ± 1 °C. The rate of Mn^{3+}-succinate complex formation was monitored by measuring the increase in absorbance at 290 nm. One unit of enzyme activity was defined as the amount of the enzyme which can produce 1 μM Mn^{3+} from the oxidation of Mn^{2+} per minute.

2.6. Environmental scanning electron microscope (ESEM) and Fourier transform infra-red spectroscopy (FTIR) characterization

Adsorption experiments were carried out by agitating 1 g of individual composting substrate (soil, vegetable leaf, rice straw or chaff) and 10 mL of ligninolytic enzymes (at MnP/LiP ratio of 4) at 25 °C in a magnetic stirrer operating at 500 rpm. Samples were withdrawn after 30 min and centrifuged at 5000 rpm for 15 min. The composting substrates without adsorption of enzymes were taken as control samples. After that, all samples were freeze dried to water content of 5%, then pounded into powder for ESEM and FTIR characterization.

The surface morphology of sample was scanned by an ESEM. The coating Au film was applied to the samples as sputter coater, followed by the coated samples examined and imaged with an ESEM (QUANTA 200, FEI, America). The magnification applied in ESEM images for different substrates was 2000 times.

FTIR analysis was conducted between 4000 and 400 cm^{-1}, using a WQF-410 spectrometer. KBr pellets for FTIR spectroscopy were prepared using a PerkinElmer pellet die (2 mg of composting substrate samples in 40 mg of KBr).

2.7. Statistical analysis

All presented results were average values of triplicate, and the relative standard deviation values were always lower than 5%. Statistical analysis was carried out using the software package SPSS for Windows (version 16.0, SPSS, Chicago, IL). The data showed in Figs. 4–6 used to assess the transfer abilities of total protein and ligninolytic enzymes were corrected by subtracting the blank value of relative controlled tests.

3. Results and discussion

3.1. ESEM and FTIR spectroscopy analysis

ESEM was employed to observe the morphologies at the micron level of different composting substrates with adsorption of enzymes and those without enzymes, respectively (Fig. 2). The

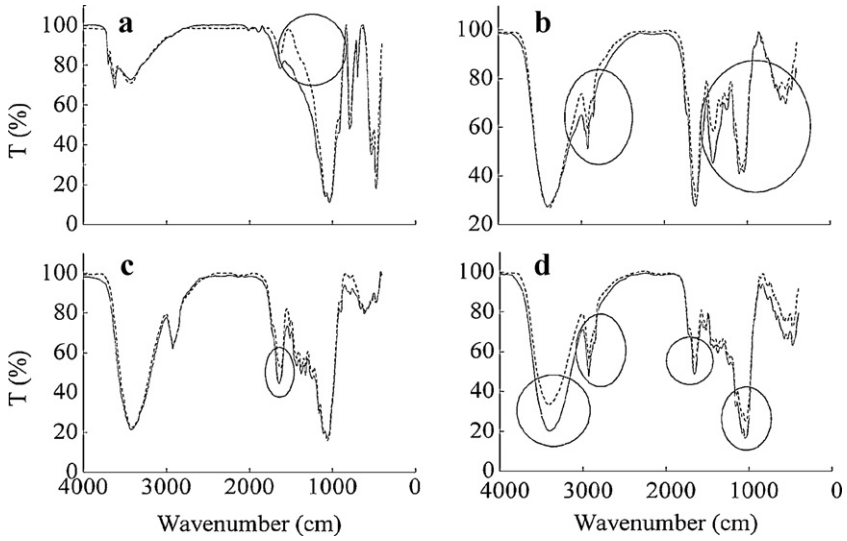

Fig. 3. FTIR spectra of different composting substrates including soil (a), vegetable leaf (b), rice straw (c) and chaff (d). In each figure, the dotted line represents substrate with adsorption of enzymes and the solid line represents substrate without enzymes. The circles in figures (a), (b), (c) and (d) presented the parts of significant difference in substrates with and without adsorption of enzymes.

surface of soil without adsorption of enzymes looked like dispersing and was covered by large particles (Fig. 2a′), while the surface of soil with adsorption of enzymes seemed to be more compact than that of the forerunner and adherence of many small particles to the reticular structure can be found on it (Fig. 2a), which was attributed to the enzymes bonding to some parts of soil. It can be seen from Fig. 2b′ that there were grooves arranged in disordered orientation for the vegetable leaf without adsorption of enzymes, showing rough and wrinkled surface. From Fig. 2b the surface of vegetable leaf with adsorption of enzymes was shown more smooth and covered by more particles, and the grooves became a litter shallower than that of those without enzymes: ligninolytic enzymes might be permeated to the grooves of vegetable leaf in the adsorption process. Compared with Fig. 2c′, there were prominent tenting in the surface of rice straw with adsorption of enzymes and slots by column permutation appeared on it (Fig. 2c), which indicated that some enzymes are adsorbed onto the surface of rice straw. Fig. 2d and d′ showed the ESEM micrographs of chaff with adsorption of enzymes and that without enzymes, from the micrographs it is clear that more small particles existed in the surface of chaff with adsorption of enzymes. According to the above discussions, it could be concluded that the four substrates had different styles of adsorption of ligninolytic enzymes.

The FTIR spectra of composting substrates with adsorption of enzymes indicated great changes of peaks compared with composting substrates without enzymes (Fig. 3). These changes were different among different substrates. The interpretation of the spectra was based on numerous works, notably Fialho et al. [4], Rovira et al. [22] and Jouraiphy et al. [23]. The changing main bands found in this study could be assigned as follows. The band at 1630 cm^{-1} was assigned to antisymmetric stretching vibration of carboxyl which can be mainly attributed to carboxyl in enzymes single-tooth coordinating with metal M in soil (Fig. 3a). Both the methylene asymmetric stretching vibration and the methylene symmetric vibration of vegetable leaf with adsorption of enzymes respectively at 2922 cm^{-1} and 2854 cm^{-1} decreased to a certain degree. The prominent peaks at 1246–1101 cm^{-1} represent alkane groups, and the adsorption of alkane groups decreased. The amount of —CH_2—CO— or —CH_2—NH— (1410 cm^{-1}) decreased significantly (Fig. 3b). In summary, the results indicated that the hydrophobic groups (methylene and alkane groups) of vegetable leaf could bond alternatively with hydrophobic groups of enzymes, and the active groups (—CH_2—CO— or —CH_2—NH—) of vegetable leaf could form hydrogen bonds with —NH— or —CO— of protein. The study was in accordance with hydrophobic fiber contained in vegetable leaf described by Annunciado et al. [24]. The band at 1644 cm^{-1} (amide carboxyl stretching) decreased in the rice straw with adsorption of enzymes compared with the sample without enzymes (Fig. 3c). The peak at 3396 cm^{-1} and 2922 cm^{-1} was assigned to —OH stretching of carboxylic acid-dimer and methylene symmetric vibration, respectively. The methyl rocking vibration occurred at 1051–920 cm^{-1} (Fig. 3d). The changes of absorbance for these bands indicated that large amounts of organic groups existed in rice straw and chaff could combine with some groups of enzymes to enhance its absorption onto those substrates.

3.2. Analysis of total protein transfer abilities in different composting substrates

The transfer abilities of total protein at different ratios of MnP to LiP in different composting substrates are shown in Fig. 4. The transfer abilities of total protein in the four composting substrates (soil, vegetable leaf, rice straw and chaff) were significantly different at MnP/LiP ratio of 4. During the first 6 cm depth, the content of total protein amount in soil increased as the depth increased, while decreased after the depth of 6 cm. The transfer of total protein in vegetable leaf had the similar tendency with that in soil except for the discrepancy in the extent of increase and decrease. Compared with the transfer abilities of total protein in aforementioned two substrates, total protein showed better transfer abilities in rice straw and chaff. Protein transferred to the deeper until the depth of 8 cm without a hitch, and little protein was detected at the first 6 cm depth in rice straw and chaff (Fig. 4a). The transfer abilities of total protein in the four composting substrates were all changed at MnP/LiP ratio of 6 in contrast to the results showed in Fig. 4a. The transfer abilities of total protein were weakened with increasing MnP/LiP ratio in the four substrates. The results showed that 336.51 μg and 322.68 μg protein were held up in the depth of 4 cm and only 106.22 μg and 97.90 μg protein transferred to the depth of 10 cm in soil and vegetable leaf, respectively. A similar tendency in transfer of total protein was observed at MnP/LiP ratio of 6 in rice straw and chaff, and the largest protein amount just transferred to the depth of 6 cm (Fig. 4b). Compared with the above conditions, the transfer abilities of total protein

in the four composting substrates were all enhanced at MnP/LiP ratio of 8. About 363.28 μg, 361.97 μg, 341.75 μg, 354.54 μg protein were detected at the depth of 10 cm in soil, vegetable leaf, rice straw and chaff, respectively (Fig. 4c). The results indicated that the transfer abilities of total protein were different in the four substrates, and different ratios of MnP to LiP had various effects on the transfer of total protein.

The adsorption behavior of protein onto substrate could affect the protein transfer in it. In general, the more protein adsorbed onto substrate, the less protein could transfer to the deeper of substrate column. It is generally considered that the interactions of protein–protein, protein–water molecules, and proteins-interfaces exist in the protein adsorption process onto solid surface, including hydrophobic interactions, electrostatic forces, van der Waals force and so on. However, many phenomena, such as the overshooting effects, cooperative adsorption, and protein aggregation, are still controversially discussed in the scientific community [25]. The results shown in Fig. 4 may be attributed to differences in physicochemical properties among the four composting substrates. The transfer ability of total protein in soil was relatively weaker. This result might be due to reasons as follows. In soil, there are a lot of clays, iron and manganese oxides. Many groups with variable charges exist on these matters' surface. These charges value would change with pH value. Protein is with charges in non-isoelectric point solution. Thus, protein could form steady complexes with these groups on soil surface and adsorb onto soil because of opposite charges attracting effects [26]. Adsorption capacity increases as hydrophobicity increases. Patterson et al. [27] have reported that the matter with more hydrophobic organic cations, the greater adsorption of enzymes onto it. It could be concluded that, the weak transfer ability of total protein in vegetable leaf may be attributed to hydrophobic fibers contained in it, whose hydrophobic parts can combine with hydrophobic parts of protein. That enhanced the affinity of protein for the substrate surface. In addition, the better transfer abilities of total protein in rice straw and chaff may be resulted from larger true density of rice straw and low content of organic material in chaff, respectively.

It was found that the transfer abilities of total protein in the four composting substrates were weakened lightly when MnP/LiP ratio increased to 6, but were enhanced when the ratio increased to 8 (Fig. 4a–c). To the best of our knowledge, little research has been done about this phenomenon formerly. Further works should be preformed for the deep understanding of the transfer mechanism of protein. In this paper, a possible reason for the phenomenon could be attributed to the two mechanisms among proteins, i.e. competition and cooperation mechanisms. Promoting or hindering transfer of protein in composting substrates depends on which mechanism playing a dominant role.

3.3. Comparison of LiP and MnP transfer performances in different composting substrates

Figs. 5 and 6 presented the relationship between ratio of MnP/LiP and ligninolytic enzymes (LiP and MnP) activities percentage at different depth in different composting substrates, respectively. Different peaks were observable from the changes of LiP activity percentage in soil at different ratios of MnP to LiP. In particular, one peak was present at the depth of 4 cm, and LiP activity percentage increased firstly and decreased afterward in soil during whole transfer procedure at MnP/LiP ratio of 4. The tendency of falling after rising in soil at MnP/LiP ratio of 6 was similar with that at MnP/LiP ratio of 4, but the peak was present at the depth of 6 cm. However, one peak of LiP activity percentage was detected at the depth of 10 cm in soil at MnP/LiP ratio of 8 (Fig. 5a). The results indicated that the transfer ability of LiP in soil was enhanced as MnP/LiP ratio increased continuously. Fig. 5b presented the change

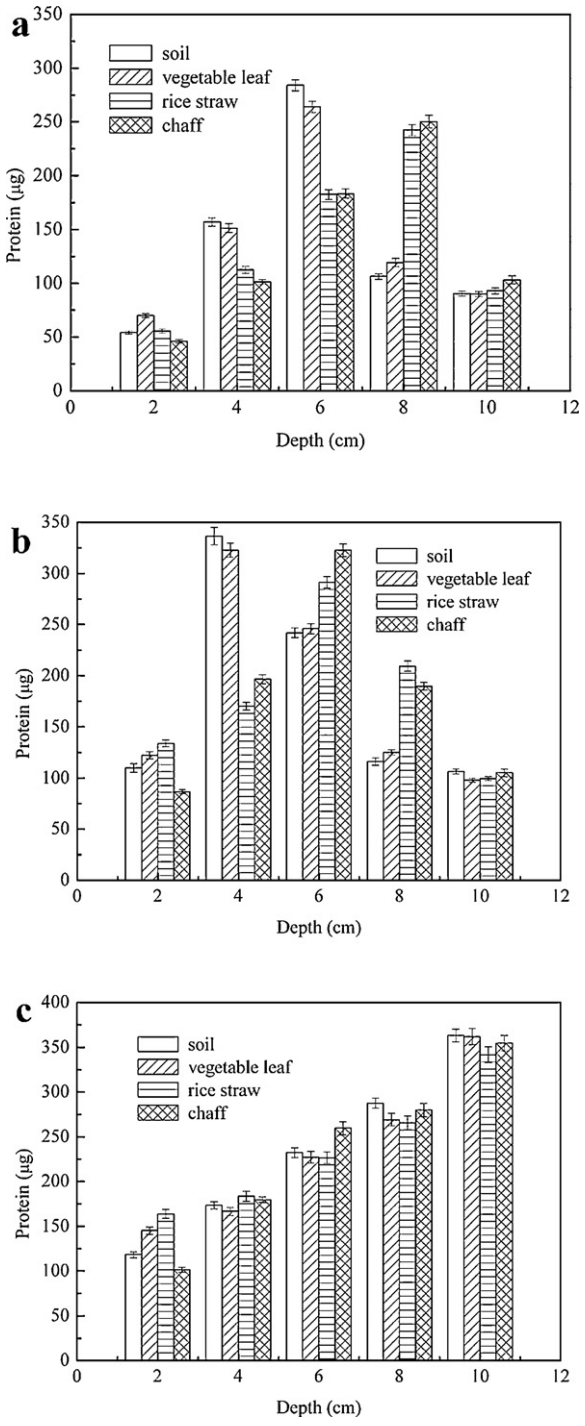

Fig. 4. Total protein contents at different depth of composting substrates measured at different ratios of MnP to LiP. The MnP/LiP ratio of 4 (a), the MnP/LiP ratio of 6 (b) and the MnP/LiP ratio of 8 (c). Results are means of triplicate, and bars represent standard deviations.

of LiP activity percentage in vegetable leaf at different ratios of MnP to LiP. The tendency of LiP transfer was similar with that in soil as the MnP/LiP ratio changed. Thus it could be indicated that the influence performance model of ratios of MnP to LiP on LiP transfer in vegetable leaf was basically similar with that in soil. In Fig. 5c, the largest percentage of LiP appeared from at the depth of 6 cm to 8 cm with the ratio changed from 4 to 6, and it appeared at the deepest part at the MnP/LiP ratio of 8. In Fig. 5d, the transfer abilities of LiP in chaff at different MnP/LiP ratios were almost the same with that

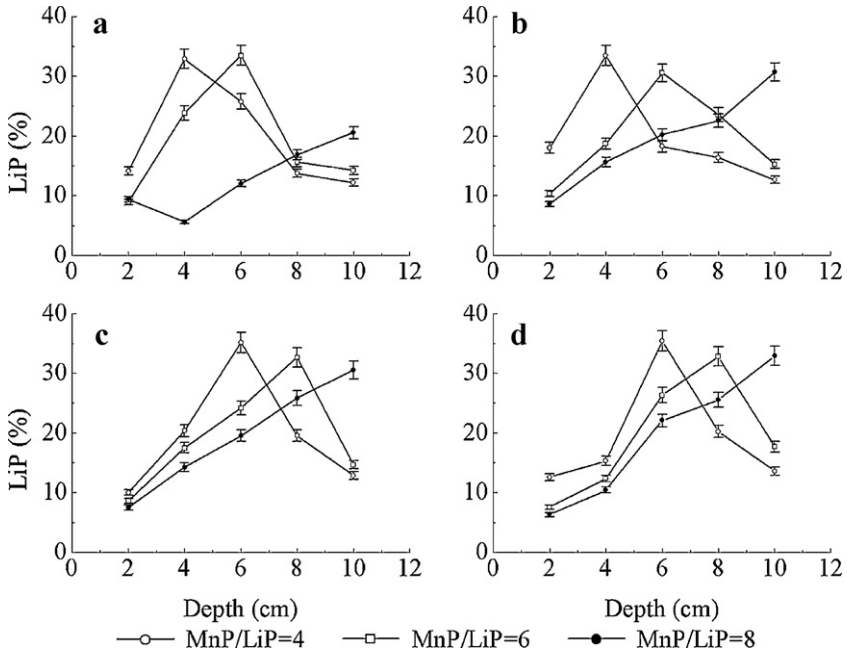

Fig. 5. Effect of MnP/LiP ratio on the LiP activity percentage measured at different depth of soil (a), vegetable leaf (b), rice straw (c) and chaff (d) columns. Results are means of triplicate, and bars represent standard deviations.

in rice straw, and it indicated that the change of ratio had positive effect on the transfer of LiP in chaff.

In Fig. 6a–d, the peak of MnP activity percentage appeared earlier at MnP/LiP ratio of 6 and latter at MnP/LiP ratio of 8 than that at MnP/LiP ratio of 4. These phenomena could be explained by the fact that more enzyme molecules would gather together, which leaded to MnP retention in the shallow layer. However, the pressure formed by a large increase of enzymes may breakthrough the apertures and make MnP transfer to deeper layer as they would. The results suggested that the transfer abilities of MnP in the four substrates were weaken as MnP/LiP ratio increased from 4 to 6, but enhanced sharply as MnP/LiP ratio increased to 8.

The transfer abilities of the two kind of enzymes were found better in rice straw and chaff than those in soil and vegetable leaf at the MnP/LiP ratio of 4 (Figs. 5 and 6). These results were in good accordance with the findings of FTIR spectroscopy study. More enzymes can be adsorbed by soil and vegetable leaf through all kinds of adsorption patterns, and thereby enzymes cannot transfer to the deeper layer of substrates columns. In addition, the transfer abilities of MnP were better in the four composting substrates than that of LiP at MnP/LiP ratio of 4. It could be concluded that the adsorption of LiP was predominant in competitive adsorption between LiP and MnP at the ratio level. But this kind of a position of prominence did not maintain as the ratio increased. The explanation of

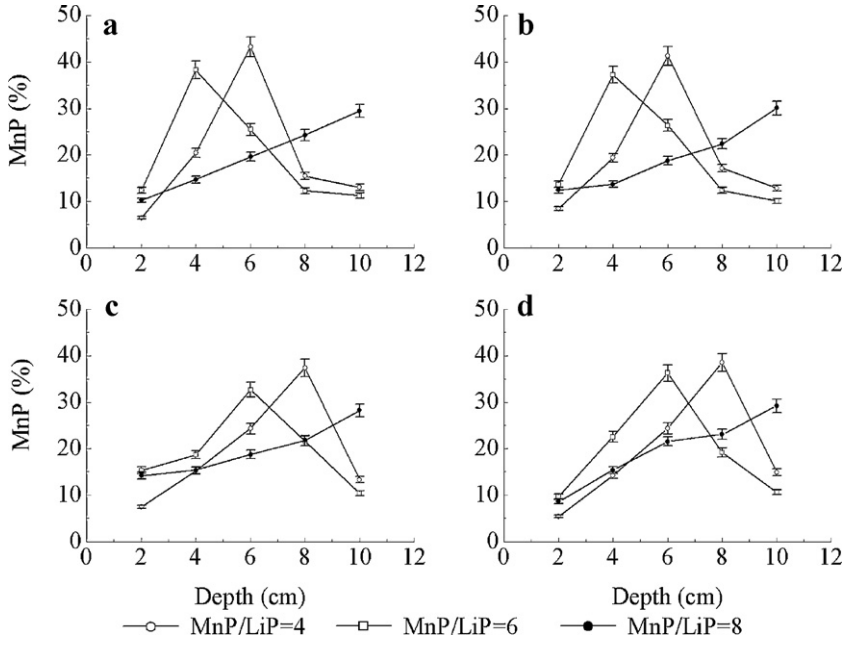

Fig. 6. Effect of MnP/LiP ratio on the MnP activity percentage measured at different depth of soil (a), vegetable leaf (b), rice straw (c) and chaff (d) columns. Results are means of triplicate, and bars represent standard deviations.

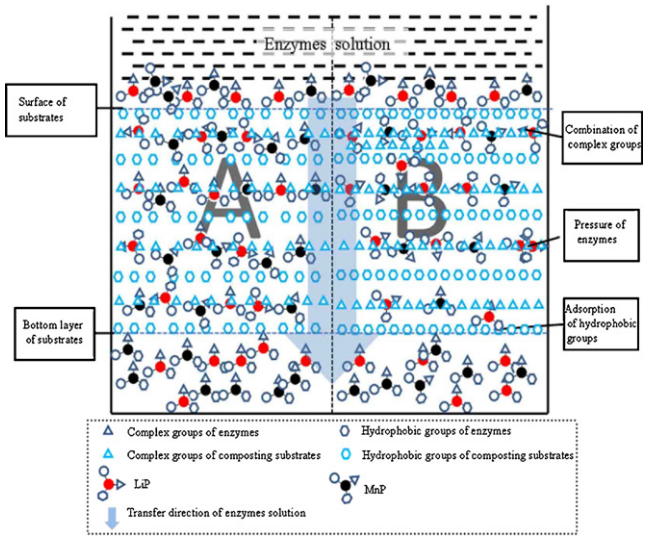

Fig. 7. The proposed transfer mechanisms of ligninolytic enzymes (LiP and MnP) in composting substrates. True density: situation A > situation B, LiP and MnP included in enzymes solution, Carboxyl, amide carboxyl and methyl and so on included in complex groups, Methylene and alkane included in hydrophobic groups.

the phenomena might be that the amount of MnP increased with the increasing MnP/LiP ratio, and the almost same amount of LiP was always kept at different MnP/LiP ratios.

3.4. Transfer mechanisms of LiP and MnP

The mechanisms of ligninolytic enzymes transfer affected by different factors were interpreted in Fig. 7. Firstly, by comparison of Fig. 7A and B, better transfer abilities of ligninolytic enzymes were showed when the true density was large. The reasons may be that small specific surface area was provided by substrates with large true density, and less active groups existed in small specific surface area. The less active groups would react with enzymes so as to advance the transfer of ligninolytic enzymes to deeper layer of substrate columns. Secondly, the enzymes transfer was usually characterized by one single enzyme molecule, or mass molecules, or both in the transfer process of ligninolytic enzymes in composting substrates. For instance, one single LiP molecule could transfer to the deeper layer as well as gathered LiP molecules, and so did MnP. Thirdly, because the hydrophobic groups and complex groups of enzymes could combine with that of composting substrates, respectively, the transfer abilities of ligninolytic enzymes in composting substrates were weak. Consequently, it was concluded that the composition of substrate and organic matter content of substrate might influence the transfer of ligninolytic enzymes in composting substrates. So the transfer of ligninolytic enzymes in composting would become effective by reasonably selecting and mixing the organic wastes as composting materials. Fourthly, some enzyme molecules gathered together and that might block the holes in substrate, which made the enzymes retain the shallow layer of substrate columns. While more and more enzyme molecules gather to be mass enzymes, this mechanism could increase physical pressure which leaded to better transfer abilities of enzymes. Finally, the transfer of MnP was slightly better than that of LiP at the MnP/LiP ratio of 4, contrary to the result found at the MnP/LiP ratio of 6. And in the presence of the MnP/LiP ratio of 8, the transfer abilities of LiP and MnP were almost the same. This was probably because that the changes of transfer abilities were attributed to the amount of enzymes. The amount of LiP was almost the same in the whole process, while the amount of MnP increased gradually with the increasing ratio. Enzyme playing the main role in adsorption onto substrate depended on the different ratios of MnP/LiP, thus leading to variations of transfer abilities of ligninolytic enzymes in composting substrates. Consequently, the changes of the transfer of ligninolytic enzymes took place in composting substrates at different ratio of MnP/LiP.

4. Conclusions

The transfer of ligninolytic enzymes in different composting substrates were related to the physico-chemical properties of composting substrates, including true density of substrates, contents of organic matter in substrates, complex formed by groups of enzymes and composting substrates, physical pressure formed by enzymes and so on. Furthermore, the physico-chemical properties of these four composting substrates (soil, vegetable leaf, rice straw and chaff) were responsible for the transfer mechanisms, however which one playing a dominant role is different in different composting substrates. Since the changes of MnP/LiP ratio could affect the actions of above-mentioned properties to different extents, the transfer of ligninolytic enzymes in different substrates changed as the MnP/LiP ratios changed. The present findings revealed the transfer abilities of ligninolytic enzymes in different composting materials and the varied factors affecting the transfer of ligninolytic enzymes in composting substrates, which could contribute to transfer of ligninolytic enzymes by optimizing the ratio of MnP/LiP and be used as references for promoting lignocellulose degradation by composting with addition of ligninolytic enzymes.

Acknowledgments

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