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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

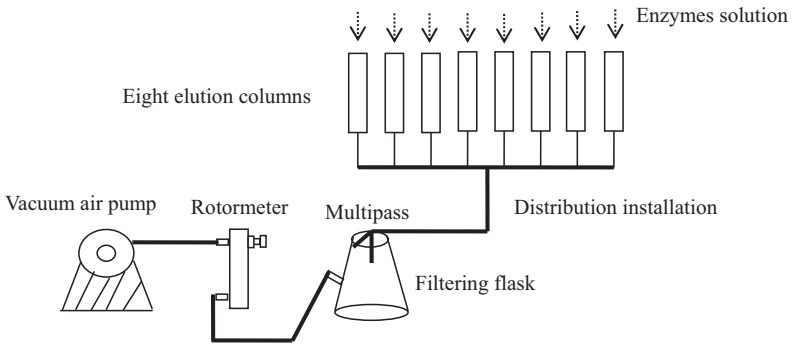

Fig. 1. A schematic diagram of the experimental system. Vacuum air pump (vacuum degree 0.098 MPa), rotormeter (air, 25 °C, 101.3 kPa).