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Degradation of bamboo-shoot shell powder by a fungal consortium: Changes in chemical composition and physical structure

Jian-Guo Zhang [a], Qiao-Liang Li [a], Zhao-Jun Wei [a, b, *]

[a] School of Food Science and Engineering, Hefei University of Technology, Hefei 230009, People's Republic of China
[b] Agricultural and Forestry Specialty Food Processing Industry Technological Innovation Strategic Alliance of Anhui Province, Hefei 230009, People's Republic of China

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ABSTRACT

Co-culturing microorganism to degrade lignocellulose has been adapted by various researchers globally. The decomposition of bamboo-shoot shell powder (BSSP) by a two-fungus (*Pleurotus ostreatus* and *Aspergillus niger*) together was examined. During the degradation, there was a rise in the activities of enzymes carboxymethylcellulase (catalyzing cellulose degradation by the hydrolysis of β-1, 4 glycosidic bonds in cellulosic materials) and laccase (phenol oxidase catalyzing lignin degradation) responsible for metabolism of lignocellulosic material resulting into decrease in acid detergent fiber and lignin contents of BSPP. In addition, the physical properties of the shell fiber were changed from smooth and flexible to rough and crisp. The products of the decomposing fiber were complex, and most of them contained methyl or ethyl groups. The fungi decomposed native silicon compounds simultaneously during biodegradation. The net result of decomposition was a decrease in biomass as well as production of carboxymethylcellulase and laccase, two biotechnologically relevant extracellular enzymes with multiple industrial uses.

1. Introduction

Bamboo is perennial herb rich in lignocellulosic materials such as cellulose, hemicellulose and lignin (Nirmala et al., 2014). The estimated annual production of moist bamboo shoot shell (BSS) in China exceeds millions of tons (Qing et al., 2016). While a small amount of BSS is used as functional food or natural fibers (Jia et al., 2011; Nirmala et al., 2014), most are discarded as agronomic waste (Jia et al., 2011). Therefore, there is a lot of interest in not only converting BSS into products of added economic value but also benefit environment by decreasing agri-waste load. Numerous studies have reported safe and environmentally friendly methods for lignocellulose degradation using fungi (Okano et al., 2005; Boonyuen et al., 2014; Knezevic et al., 2014; Mathews et al., 2016).

One of the key problems hindering the effective utilization of lignocellulose by microorganism is the low susceptibility of lignocellulose to hydrolysis, which is due to the crystalline structure of cellulose fibers surrounded by hemicellulose and lignin seal (Taniguchi et al., 2005). Thus, the ideal microorganism used for lignocellulose degradation should possess a battery of enzymatic system that can decompose both crystalline cellulose and lignin. To this end, white rot fungi have been extensively studied for biodegradation lignocellulose (Daassi et al., 2016; Lomeli-Ramirez et al., 2009; Schmidt et al., 2016). Among these white rot fungi, *Pleurotus ostreatus* has been proved to be capable of degrading both lignin fraction and holocellulose component due to its unique enzymatic system, (Bari et al., 2015a, 2015b). *Aspergillus niger,* a filamentous fungus produces a wide spectrum of polysaccharide hydrolytic enzymes (Chen et al., 2006; de Souza et al., 2011; Hanif et al., 2004). The use of *P. ostreatus* or *A. niger* alone, however, does not seem to be an attractive strategy for effective degradation of lignocelluloses for the following reasons. While *P. ostreatus* growth rate is relatively slow, *A. niger* growth rate is fast but it can only degrade isolated cellulose and hemicellulose. However, the use of a mixed cultures of different microorganisms has been reported by others to be more effective in degradation of lignocelluloses (Ahamed and Vermette, 2008; Haruta et al., 2002; Taha et al., 2015).

* Corresponding author.
E-mail addresses: zhangjianguo@hfut.edu.cn (J.-G. Zhang), 46607391@qq.com (Q.-L. Li), zjwei@hfut.edu.cn (Z.-J. Wei).

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The objective of the present research was to explore whether a combination of *P. ostreatus* and *A. niger* could be more effective in decay of lignocellulosic materials in the bamboo-shoot shell powder. The results of the studies show decay BSS into short branches of hemicellulose and lignin and production of Carboxymethylcellulase (Endo-β-1,4-glucanase, EC 3.2.1.4) and Laccase (Benzenediol: oxygen oxidoreductase, EC 1.10.3.2) — two biotechnologically relevant extracellular enzymes with multiple industrial uses (Abdel-Fatah et al., 2012; Singh et al., 2014).

2. Materials and methods

2.1. Preparation of inoculant and substrate

Both *P. ostreatus* (CCTCC AF 92003) and *A. niger* (CCTCC AF 91005) were procured from China Center for Type Culture Collection and cultured on potato dextrose agar (PDA) slants. A mycelial suspension of *P. ostreatus* was obtained by grinding slant culture in sterile saline. A conidial suspension of *A. niger* was prepared by adding 20 mL of sterile saline to the *A. niger* slant and the final concentration was adjusted to 10^6 spores/mL.

Moist BSS were obtained from Maosheng Foodstuffs Co., Ltd. in Ningguo City, Anhui Province, P. R. China, washed thoroughly in running tap water and dried in a hot-air oven at 60 ± 5 °C to a constant weight. Dried shellswere milled to powder, passed through a 40-mesh sieve and labeled BSSP (bamboo-shoot shell powder).

2.2. Biodegradation of BSSP by fungal consortium

The basal growth medium was prepared as described by Vane (2003). 8 g of BSSP was mixed with13 mL growth medium in Petri dish, autoclaved at 121 °C for 30 min and then cooled to room temperature. The sterilized BSSP was inoculated with 1 mL of mycelial suspension of *P. ostreatus* and incubated at 28 °C at relative humidity 60% for 4 days. After 4 days, the solid state culture was also inoculated with 1 mL of conidial suspension of *A. niger* and incubation continued for another 8 days at 28 °C at relative humidity 60%.More than 40 replicates were prepared, and 3 replicates were used for each measurement.

2.3. Determination of enzymatic activity

The degraded powder was extracted with 25 mL distilled water at 30 °C for 2 h, and the mixture was centrifuged at 12,000 g for 10 min. The supernatant was used to measure carboxymethylcellulase (CMCase) and laccase activities. CMCase and laccase activity were determined as described elsewhere (Li et al., 2014). CMCase activity (1 U) was expressed iterature **.expressed as the amount of enzyme that produced 1 mg of reducing sugar in 1 h per mL of extraction solution. One unit of laccase activity was defined as the amount of oxidation of ABTS (3-ethylbenzothiazoline-6-sulfonic acid) followed by 0.1 increase in absorbance at 420 nm.

2.4. Measurement of composition of decomposed BSSP

Decomposed powder was extracted with boiling water for 20 min. The mixture was filtrated, and the residue was dried in a hot-air oven at 105 °C until a constant weight. The lignin and acid detergent fiber (ADF) contents were determined according to the modified method proposed by the NREL, USA (Sluiter et al., 2012). ADF refers to the fibers that could be dissolved by sulfuric acid. The weight of ADF (W_A) and loss of total biomass (W_L) were calculated using Eq. (1) and Eq. (2).

$$W_A = W_1 - W_{LG}, \quad (1)$$

$$W_L = W_0 - W_1 \quad (2)$$

Where, W_0 was the dry weight of the initial powder, W_1 was the dry weight of the decomposed powder and W_{LG} was the weight of the lignin.

2.5. IR analysis

Decomposed powder was soaked in acetate buffer (pH 4.8) for 3 h. The suspension was filtrated, and the residue was oven-dried at 105 °C until a constant weight. Dried residue was analyzed with FTIR as described earlier (Zhang et al., 2007). The FTIR spectra were obtained using a Nicolet 67 Fourier-transform infrared spectrometer (FT-IR) (Nicolet Instrument Co., USA).

2.6. GC-MS analysis

Decomposed powder was soaked in distilled water for 1 h. Then, the suspension was centrifuged at 12,000 g for 10 min, and the supernatant was extracted three times with dichloromethane. All the dichloromethane solvent was combined and concentrated. The concentrated solvent was filtrated through a 0.45 μm filter (Millipore, USA) and the filtrate was analyzed by a combination of gas chromatography and mass spectrometry (SCION SQ GCMS, Bruker Daltonics Inc. USA) with a capillary column (DB-5MS, 60 m × 0.25 mm, Agilent) where helium (64 kPa) was used as the carrier gas. The column temperature was 50 °C (2 min) → 100 °C, 5 °C min^{-1} → 280 °C (3 min), 15 °C min^{-1}.

2.7. SEM analysis

Decomposed powder was washed sequentially with distilled water, 95% ethanol, absolute ethanol and acetone. The washed powder was dried in a freeze drier. Dry powder was placed on carbon glue and plated with platinum. The surface morphological structures were investigated with a scanning electron microscope (JSM-6490LV, JEOL Ltd., Japan) at a voltage of 15 kV. The native powder was treated following the same procedures as the control sample.

3. Results and discussion

3.1. Changes in CMCase and laccase activity during biodegradation

CMCase and laccase play important roles for the degradation of lignocellulose. As shown in Fig. 1, the CMCase and laccase activities

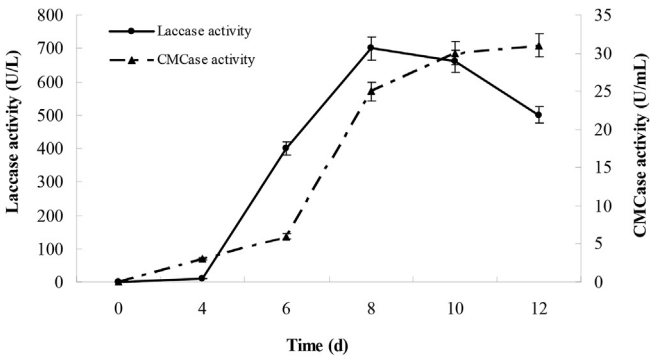

Fig. 1. Extracellular Laccase and CMCase activities during biodegradation of BSSP.