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Fungal pretreatment of switchgrass for improved saccharification and simultaneous enzyme production

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HIGHLIGHTS

- An efficient method for pretreatment of switchgrass is developed.
- The fungus *Pycnoporus* sp. SYBC-L3 selectively degraded lignin over cellulose.
- Great enhancement of hydrolysis was achieved for cellulose and hemicellulose.
- An extract containing high enzyme activities was obtained as a co-product.
- Pretreatment of biomass with simultaneous enzyme production was achieved.

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ABSTRACT

This study investigates fungal pretreatment of switchgrass involving solid state fermentation (SSF) to improve saccharification and simultaneously produce enzymes as co-products. The results revealed that the fungus *Pycnoporus* sp. SYBC-L3 can significantly degrade lignin and enhance enzymatic hydrolysis efficiency. After a 36-d cultivation period, nearly 30% reduction in lignin content was obtained without significant loss of cellulose and hemicellulose, while a considerable amount of laccase, as high as 6.3 U/g, was produced. After pretreatment, pores on switchgrass surface were observed using scanning electron microscopy (SEM). The enzymatic hydrolysis efficiency for the switchgrass with 36-d pretreatment was about 50% greater than the untreated one. Our results suggest that solid state fungal cultivation may be a good method for switchgrass pretreatment, which can simultaneously achieve high efficiency of enzymatic hydrolysis and production of some useful enzymes for other industrial utilization.

1. Introduction

As known petroleum reserves are gradually depleted, the need of new alternatives to petroleum-based fuels becomes increasingly urgent. Renewable bioenergy sources have drawn the attention of researchers due to the abundance of available raw materials and their reduced environmental impact. Biofuels, such as bioethanol and biodiesel, have become a research hot spot among researchers worldwide. Biofuels can play an important role in helping lessen the impacts of climate change, improving national security, and protecting the environment, and thus have become increasingly important to the global energy supply. One of the most promising bioenergy strategies is to produce bioethanol and biodiesel using grain crops, such as corn and canola. However, this approach will compete with the food supply, potentially leading to a global food crisis (Li and Khraisheh, 2010). To avoid a conflict over farm land, food, edible oil and sugars, some alternatives such as lignocellulosic biomass or agro-wastes can be used as feedstocks for renewable energy production (Balan et al., 2008).

Large quantities of biomass produced every year can greatly contribute to bioenergy production. At present, some lignocellulosic biomasses have been employed for potential bioenergy production, e.g., agricultural residues, hard/soft wood, waste papers, and switchgrass (Limayem and Ricke, 2012). Among lignocellulosic biomasses used for biofuel, switchgrass has become the material of choice due to its high yield, high nutrient-use efficiency, and wide geographic distribution (McLaughlin and Walsh, 1998). In addition, switchgrass stands out for its great advantages in conservation of water and soil and grassland improvement, and thus can be viewed as one of the bioenergy feedstocks with a great potential.

Lignocellulosic biomass is the most abundant renewable resource on the earth, consisting mostly of agricultural wastes, forestry residues and energy crops. It is mostly composed of

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cellulose, hemicellulose, and lignin. Cellulose is a polysaccharide linked by beta-1,4-glycosidic bonds which can be digested by cellulase and beta-glucosidase to glucose, a fermentable sugar for bioethanol production. Hemicellulose, however, is a highly branched short polymer composed of xylose, arabinose, glucose, galactose, and mannose. Unlike cellulose, hemicellulose is more easily hydrolyzed into monomeric sugars, of which xylose can also be utilized for bioethanol production (Matsushika et al., 2009). Lignin is a complex polyphenolic polymer in lignocellulose which can greatly impede enzymatic hydrolysis for fermentable sugars. The main procedure for bioethanol production from biomass can be divided into the following three steps: (1) pretreatment of biomass; (2) enzymatic hydrolysis to produce fermentable sugars; (3) anaerobic fermentation of sugars to bioethanol and subsequent distillation (Faga et al., 2010; Pessani et al., 2011). Bioethanol yields are directly dependent on the yield of fermentable sugars available from hydrolysis of pretreated biomass. Thus, a pretreatment method capable of disrupting recalcitrant lignocellulosic structures is critical for bioethanol production (Pallapolu et al., 2011).

Various physical, chemical, or combined methods have been used to date for the pretreatment of lignocellulosic biomass to disrupt recalcitrant structures (Faga et al., 2010; Limayem and Ricke, 2012; Pessani et al., 2011). These pretreatment methods, while disrupt recalcitrant structures, often alter biomass chemical composition, and further improve subsequent hydrolysis. Most of these methods are performed under severe reaction conditions or using acid and alkali, which are often energy consuming and/or generate new environmental pollutants. Utilizing white-rot fungi with solid state fermentation (SSF) is a promising new pretreatment method, effective in degrading lignin and improving biomass saccharification (Hatakka, 1983; Taniguchi et al., 2005, 2010). White-rot fungi are the only known microorganisms to date with the capability of effectively degrading lignin due to their powerful extracellular lignin-degrading enzymes (Eggert et al., 1997; Floudas et al., 2012; Higuchi, 1993).

Some typical white-rot fungi, such as *Pleurotus ostreatus* (Taniguchi et al., 2005) and *Ceriporiopsis subvermispora* (Wan and Li, 2010) have been successfully employed to pretreat rice straw and corn stover for improving saccharification and selective lignin degradation. Lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase are the three main ligninolytic enzymes involved in delignification among various fungi (Eggert et al., 1997; Sun et al., 2011; Wan and Li, 2010). Fungal cultivation with high ligninolytic activities thus has the potential for effective biomass pretreatment. For example, genus *Pycnoporus* has been shown effective for the pretreatment of wheat straw as early as in 1983 (Hatakka, 1983). It is believed that laccase plays an significant role in delignification by *Pycnoporus cinnabarinus* (Eggert et al., 1997). Indeed, laccase is an important industrial enzyme that has broad applications (Couto and Herrera, 2006). Studies evaluating the efficiency of fungal pretreatment by genus *Pycnoporus* on other biomass, such as switchgrass, remain scarce. Furthermore, most of the published works relating to fungal pretreatment of biomass were focused on investigation of ligninolytic activities but the possibility of extracting enzymes as useful co-products was not explored (Sun et al., 2011; Wan and Li, 2010). Considering that some biomass loss always occurs during the fungal pretreatment, generation of some co-products during pretreatment may be a compensating or value-adding mechanism to make it practical by reducing the overall cost of bioethanol production.

We have in our recent study obtained a fungus belonging to *Pycnoporus* with great ability to produce laccase (Liu et al., 2013). We in the study reported herein, evaluated this fungus, *Pycnoporus* sp. SYBC-L3, for both pretreatment of switchgrass biomass and enzyme production. The examined factors included disruption of recalcitrant structures, changes in chemical composition, amounts of various enzymes obtained in the crude extracts as co-products, and the efficiency of enzymatic hydrolysis after pretreatment. The main objectives of this study were: (1) to evaluate the efficiency of enzymatic hydrolysis of switchgrass biomass after fungal pretreatment and (2) to evaluate the potential to produce useful enzymes as co-products during fungal treatment of switchgrass biomass.

2. Methods

2.1. Chemicals, biomass, enzymes and operation process

The chemicals used in this study (including standard sugars for HPLC) were from Sigma unless stated otherwise. Cellulase from *Trichoderma viride* (Lot# 110M1456V, Japan) and cellulysin cellulase from *T. viride* (Cat# 219466, Japan) were purchased from Sigma. Filter paper (No. 1 quality for determination of cellulase activity) was purchased from Whatman™ (W & R Balston Limited, England). 4-Nitrophenyl β-D-glucopyranoside (*p*-NPG, N7006) and 2,6-dimethoxyphenol (DMP, D135550) were obtained from Sigma as a chromogenic substrate for determination of β-glucosidase and laccase activity, respectively. The switchgrass biomass (PI 422000) was harvested from the field at the University of Georgia, Griffin Campus in 2010, air dried and stored at room temperature prior to use. Syringeless filter device (Mini-UniPrep™, 0.45 μm Pore Size) from Whatman™ (GE Healthcare UK Limited) was used to prepare samples for HPLC. The general procedure used in this study is illustrated in Fig. S1 (Supporting Information).

2.2. Fungal strain for pretreatment of switchgrass biomass

The white-rot fungus *Pycnoporus* sp. SYBC-L3 (18S rRNA sequence was deposited in GenBank with an accession number GU182936) was used in this study for biological pretreatment of switchgrass and simultaneous enzymes production. The strain was identified in our previous study as an effective laccase producer (Liu et al., 2012), and its culture stock was stored at the Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, (Wuxi, China). The fungus was maintained on potato dextrose agar slants at 4 °C and subcultured on plates at intervals of every two weeks.

2.3. Fungal pretreatment of switchgrass biomass

Solid state fermentation was used for fungal pretreatment of switchgrass biomass in this study. Air-dried switchgrass biomass was cut into small pieces (1.5–2 cm long) and 5 g of the biomass were transferred into a 200 ml flask supplemented with 15 g distilled sterile water (pH 7.0). Each flask was autoclaved at 120 °C for 30 min and cooled down to room temperature prior to inoculation. Then five cut disks with a diameter of 0.5 cm from the margin of fungal mycelia on potato dextrose agars were transferred into each flask. Fungal growth was carried out under static condition in the flasks at 30 °C for various periods of time. The cultivation was terminated at 18-d, 36-d, 54-d or 72-d, respectively, for crude enzyme extraction, determination of ligninolytic and hydrolytic activities, compositional analysis and subsequent enzymatic hydrolysis. Each fungal pretreatment was performed with two replicates.

2.4. Enzyme extraction and activity determination

After each period of solid state fermentation, 100 ml buffer (0.1 M citrate phosphate, pH 4.8) was added into the flask to fully soak the switchgrass for 24 h at room temperature before