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Biological Pretreatment of Rice Straw by *Phenarocheate chrysosporium* for the Production of Cellulases and Xylanases using *Asperigillus niger* Isolate

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ABSTRACT

Rice straw was pretreated biologically with *Phenarocheate chrysosporium* and subsequently used in the production of cellulases and xylanases employing *Asperigillus niger* isolate. The FTIR spectrum of pretreated rice straw was compared with standard cellulose library. The spectra of 1048 cm^{-1} for C-O stretch in cellulose and hemicellulose were observed in rice straw. The enzymatic activity produced by *A. niger* on biologically Pretreated Rice Straw (PRS) was higher than that of the control treatments, which were carried out with Untreated Rice Straw (URS) and Cellulose (CEL). For medium supplemented with PRS, the peak activities obtained were 1.03±0.06, 1.54±0.08, 0.82±0.14 and 1.02±0.08 U mL^{-1} for FPase, endoglucanase, β-glucosidases and xylanases, respectively. For the cellulose control, values of 0.68±0.04, 1.24±0.11, 0.54±0.16 and 0.90±0.06 U mL^{-1} and URS values of 0.48±0.08, 0.94±0.10, 0.46±0.12 and 0.64±0.14 U mL^{-1} were obtained, respectively. This was substantiated by higher contents of N-acetylglucosamine and fungal protein in PRS samples than the cellulose control and untreated rice straw samples.

Key words: Biological pretreatment, *Phenarocheate chrysosporium, Asperigillus niger* isolate, submerged fermentation, N-acetylglucosamine

INTRODUCTION

Lignocellulosic biomass, such as bioenergy crops and trees (e.g., switch grass, aspen wood) or agricultural and forestry wastes (e.g., wood residues, corn stover, straw) are potential low cost sources of fermentable sugars. Lignocellulosic material consists of mainly three different types of polymers namely cellulose, hemicellulose and lignin which are associated which each other (Fengel and Wegener, 1989). Many factors like lignin content, crystallinity of cellulose and particle size limit the digestibility of the hemicellulose and cellulose present in the lignocellulosic biomass. Pretreatments have as a goal to improve the digestibility of the lignocellulosic biomass. Each pretreatment has its own effect(s) on the cellulose, hemicellulose and lignin, the three main components of lignocellulosic biomass (Hendriks and Zeeman, 2009). However, the polysaccharides (cellulose and hemicellulose) present in the lignocellulose biomass need to be hydrolyzed with acids or enzymes in order to liberate fermentable sugars. For the enzymatic conversion of lignocellulose biomass to ethanol and other chemical products, a pretreatment stage is required to break the lignin structure and to partially solubilize the polysaccharides (Keller *et al.*, 2003).

An extensive research was carried out on the methods of pretreatments to enhance the digestibility of lignocellulosic material. Several pretreatment methods have been investigated, such as steam explosion, solvent extraction and thermal pretreatment using acids or bases (Mosier *et al*., 2005) along with biological pretreatments with whiterot fungi (Itoh *et al*., 2003). Many pretreatment processes require expensive equipment and large quantities of energy. In the physical processes, energy expenditure can make the conversion of holocellulose economically unviable (Wingren *et al*., 2004). The pretreatments carried out by steam explosion, the glucose liberated from the cellulose can be degraded to the 5-hydroxymethyl furfural and levulinic and formic acids, while the pentoses liberated from the hemicellulose can be converted to furfural and formic acid. These products act as inhibitors in the later stages of enzymatic hydrolysis and fermentation (Klinke *et al*., 2003).

Rice straw, consisting of cellulose (35-40% w/w) and hemicellulose (25-30% w/w) in close association with lignin (10-15% w/w) is one of the most abundant lignocellulosic waste materials in the world (Thygesen *et al*., 2003). It has been estimated that 650-975 million tons of rice straw is produced per year globally and a large part of this is going as cattle feed and rest as waste. The options for the disposition of rice straw are limited by the low bulk density, slow degradation in the soil, harboring of rice stem diseases and high mineral content. Nowadays, field burning is the major practice for removing rice straw, but it increases the air pollution and consequently affects public health (Mussatto and Roberto, 2004). As climate change is extensively recognized as a threat to development, there is growing interest in alternative uses of agro-industrial residues for energy applications.

Biological pretreatment offers some conceptually important advantages such as low chemical and energy use, but a controllable and sufficiently rapid system has not yet been found. Chemical pretreatments have serious disadvantages in terms of the requirement for specialized corrosion resistant equipment, extensive washing and proper disposal of chemical wastes. Biological pretreatment is a safe and environment friendly method for lignin removal from lignocellulose. Lignolytic enzymes consist of mainly LDPs (Lignin degrading peroxidases) such as lignin peroxidases (LiPs; E.C.1.11.1.14), manganese peroxidases (MnPs; E.C.1.11.1.13) and versatile peroxidases (E.C.1.11.1.16) and lignin-degrading enzyme such as laccases (E.C.1.10.3.2). Some or all of these enzymes and their isozymes can be produced by a number of woodrotting fungi including white-rot basidiomycetes, brown-rot basidiomycetes and soft-rot ascomycetes or deuteromycetes fungi (Singh and Chen, 2008). According to Singh *et al*. (2011) the biodegradation of wheat straw by *P. chrysosporium* was investigated and the result showed ~30% loss of total lignin within three weeks of biopretreatment.

In the present study, Rice Straw (RS) was biologically pretreated with the white rot fungus *P. chrysosporium* NCIM-1197 by solid state fermentation. *P. chrysosporium* is one of the best studied whiterot fungi for the biological pretreatment of lignocellulosic biomass, particularly since it selectively degrades lignin and organopollutants more rapidly than other microbes (Reddy and D'Souza, 1994). It has the ability to produce lignin peroxidases (LiPs), manganese peroxidases (MnPs) and esterases (ESTRs) (Martinez *et al*., 2004). This fungus also produces H_2O_2 generating enzyme systems such as Copper Radical Oxidases (CROs), Aryl Alcohol Oxidases (AAOs), Glyoxal Oxidases (GOs) and other oxidases, which play key roles during the biomass lignin degradation or modification process (Chen *et al*., 2010; Singh and Chen, 2008). Subsequently, the use of this biologically Pretreated Rice Straw (PRS) was evaluated for the production of cellulases and xylanases by the fungus *A. niger* isolate.

MATERIALS AND METHODS

Microorganisms and culture media: The fungus used for biological pretreatment in the present study was *P. chrysosporium* NCIM-1197 procured from National Collection of Industrial Microorganisms (NCIM), Pune, India. Stock cultures were maintained on potato dextrose agar slants at 4°C. The *Asperigillus niger* strain was isolated from soil samples and was maintained on potato dextrose agar slants at 4°C.

Preparation of inoculum: The *P. chrysosporium* NCIM-1197 culture was maintained on potato-dextrose agar slants. For preparation of inoculum, the fungus was grown in a liquid medium (pH 4) containing (g L^{-1}): Glucose, 10.0; KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 0.5; $CaCl_2$, 0.1; NH_4Cl, 0.12 and thiamine, 0.001. After inoculation with *P. chrysosporium*, incubation was carried out at 37°C (150 rev min^{-1}) for 3 days to get a homogeneous conidial suspension.

Preparation of rice straw powder: The rice straw was collected in local paddy fields was washed with water twice and dried in oven at 60-70°C for 6 h. The oven dried rice straw was milled through 5 mm mesh .The resultant rice straw was collected in polythene bags and preserved.

Biological pretreatment of rice straw: It was carried out with the white rot fungus *P. chrysosporium* NCIM-1197. The medium supplemented with modified NREL salts (Keller *et al.*, 2003) was prepared with 0.22 g $NaNO_3$, 0.56 g KC1, 0.78 g $MgSO_4.7H_2O$, 8 mg $FeSO_4.7H_2O$, 2.22 g KH_2PO_4 and 1 mg thiamine per liter salt solution in acetate buffer (20 mM, pH 4.5). Further the modified NREL salts were supplemented with 1 g $MnSO_4.H_2O$ per liter salt solution to prepare the modified NREL salts with Mn^{2+} formulation (Brown *et al.*, 1990). Forty grams of solid substrate (rice straw powder) was moistened with 200 mL of salt solution to obtain 70%, w/w of moisture with pH 4.5, in 1 L Erlenmeyer conical flask were thoroughly mixed and autoclaved at 121°C for 30 min. The flask containing the substrate was inoculated with the fungal spore suspension of 2.5×10^6 spores g^{-1} dry matter. After thorough mixing, the flasks were incubated in incubator at 37±1°C for 40 days. The moisture content was maintained by spraying the above liquid fermentation medium on solid substrate. After the development of mycelia over the entire substrate, the contents of the Erlenmeyer conical flasks were collected, dried, sieved and used as substrate for the production of cellulases.

FTIR spectrum of biological pretreated rice straw: A Perkin Elmer Spectrum 1 FTIR was used. The samples were used in the form of KBR discs, which were prepared by grinding 1 mg sample/100 mg pre-dried KBR. The spectra were recorded in the range of 450-4000 cm K^{-1}.

Enzyme production: The submerged cultures were carried out in 500 mL Erlenmeyer flasks containing 100 mL of the production medium comprising of 10% (v/v) of salt medium (Mandels and Reese, 1957), 5% (w/v) of biologically pretreated rice straw, 0.2% wheat bran, 0.1% (w/v) Tween 80 and 89.7% of distillated water. In the control cultures, cellulose (CEL) and Untreated Rice Straw (URS) were used. The flasks were sterilized at 121°C for 15 min and inoculated with 1×10^5 conidia mL^{-1} from *A. niger* isolate and kept under shaking at 180 rpm at 28°C for 8 days. The samples were removed at different time intervals and filtered. The filtered broth was collected for determination of enzymatic activity and reducing sugars. The experiments were carried out in triplicate.

Enzyme assay: Determination of the Filter Paper Activity (FPA) was carried out according to Ghose (1987). The β-glucosidase activity was determined using salicine as the substrate, according to Chahal (1985). The endoglucanase assay was carried out according to Ghose (1987) using a 2% carboxymethyl cellulose solution in citrate buffer at pH 4.8. The xylanase activity was determined according to Bailey *et al.* (1992). The reducing sugars were estimated by the method of Miller (1959) with 3,5-dinitrosalicylic acid (DNS). One unit of enzymatic activity was defined as the quantity of enzyme required to liberate 1 µmoL of reducing sugars of the substrate, given per mL min^{-1} of enzymatic filtrate under the analysis conditions.

Glucosamine determination: For fungal chitin hydrolysis into N-acetyl glucosamine, 20 mg dried biomass was incubated with 2 mL of H_2SO_4 (72%) in a test tube. After continuous shaking (130 rpm) for 60 min at 25°C, it was diluted with 3 mL of distilled water and autoclaved at 121°C for 2 h. The hydrolyzate was neutralized to pH 7.0 with 10 M and then with 0.5 M NaOH and diluted to 100 mL. Finally, glucosamine was assayed by the colorimetric method described by Tsuji *et al.* (1969) and modified by Ride and Drysdale (1972). Hydrolysate (1 mL diluted) was mixed with 1 mL of $NaNO_2$ (5%) and 1 mL of $KHSO_4$ (5%) in a centrifuge tube. After shaking occasionally for 15 min, it was centrifuged at 3500 rpm for 5 min; 2 mL of supernatant was mixed with 0.67 mL of $NH_4SO_3NH_2$ (12.5%) and shaken for 3 min. To the mixture was added 0.67 mL of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH; 0.5%, prepared daily) and then the mixture was boiled for 3 min and immediately cooled to room temperature; 0.67 mL of $FeCl_3$ (0.5%, prepared within 3 days) was added. After standing for 30 min, the absorbance at 650 nm was measured spectrophotometrically. The glucosamine content was calculated as milligrams per gram (mg g^{-1}) of fungal biomass using a standard curve.

Statistical analysis: All the experiments were carried out in triplicate and the mean value and standard deviation were presented. Student's t-test has been used to compare the mean values. The data were analyzed by one-way analysis of variance (ANOVA) using SPSS, version 12.0 unless otherwise stated.

RESULTS

FT-IR spectrum of pretreated rice straw: Spectral characteristics of pretreated rice straw were done by FT-IR spectroscopy. The rice straw sample was pretreated with *P. chrysosporium* after 40 days was used for this study. The Fig. 1 shows the IR spectrum of extracted cellulose using *P. chrysosporium* pretreatment method by FTIR. The IR spectrum is similar with standard library of cellulose (Kondo, 1997). The IR spectrum shows the typical adsorption of cellulose backbone at 1600 cm^{-1}. From the hydrogen bonded OH stretching at 4000-2995 cm^{-1}, is due to the H boned OH groups and the stretching frequency of the-OH group as well as intramolecular and intermolecular hydrogen bonds (Richard, 2002) and the C = C stretch has weak intensity is made at 2359.97 cm^{-1} frequency. The wide band between 3,500 and 3,000 cm^{-1} for all of the samples are due to OH stretching vibrations of alcohols and phenols (Singh *et al.*, 2005). The stretching frequency of 3435.33 cm^{-1} is observed due to-OH bond whose intensity is strong and broad. The absorption peak region at 1629.97 cm^{-1} is not as intense as that seen for C = O. It is variable and may be fairly small in symmetrical, or nearly symmetrical cases and indicates the occurrence of alkene C = C bond which is weak in intensity. Multiple sharp, medium peaks were observed at 1571.74 cm^{-1} with aromatic C = C stretch vibration. The pattern of peaks varies depending upon the substitution

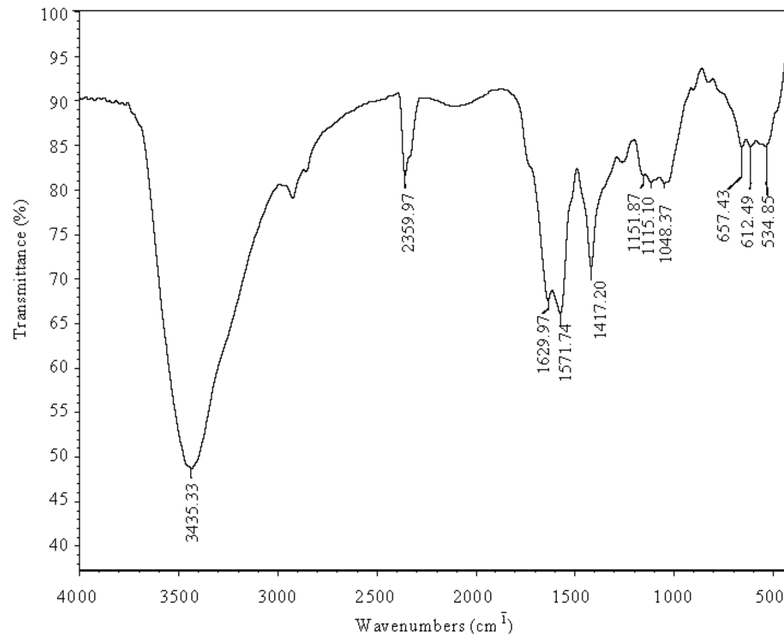

Fig. 1: FTIR spectrum of biologically pretreated rice straw

pattern. The peak at 1151.87 cm^{-1} confirmed the presence of tertiary alcoholic bond (C-O) which is medium. The absorption peak at 1115.10 cm^{-1} indicates the aromatic C-H inplane deformation and typical for syringyl units and indicates the occurrence of secondary alcohols and shows the C = C stretching. The frequency of 1048.37 cm^{-1} is seen for C-O stretch and the bond intensity is strong. The spectra of 1048 cm^{-1} for C-O stretch in cellulose and hemicellulose. The band at 657.43 cm^{-1} is due to C-S vibration of sulfonic group. The band at 655 cm^{-1}, characteristic of lignosulfonate (Nada *et al*., 1998), appears at 650 cm^{-1} for LS8 and at 618-650 cm^{-1} for LS9 and LS10. The presence of sulfur has been confirmed by elemental analysis.

Enzyme production in submerged fermentation: Tests were carried out using biologically pretreated rice straw with the fungus *P. chrysosporium* NCIM-1197 to verify the secretion of cellulases by the strain *A. niger* isolate. Cultures grown with Microcrystalline Cellulose (CEL) and Untreated Rice Straw (URS) were kept as a control. The results of enzymatic analyses are given in Fig. 2a-d.

The enzymes were produced both during growth and stationary phases, indicating that the process of these enzymes production and secretion is a growth-associated one. It was found that the cellulose culture (0.68±0.04 U mL^{-1}) and PRS culture (1.03±0.06 U mL^{-1}) had greater FPase activity than the culture supplemented with untreated rice straw (0.48±0.08 U mL^{-1}). However, the FPA activity of untreated rice straw culture on 4th and 5th days was similar with the media supplemented with cellulose. This indicated that the milling of rice straw can reduce the crystallinity of lignocellulose and exposes the available cellulose surface area for enzyme activity (Fig. 2a). But, FPase activity of cellulose culture was higher at 6th day than the URS.

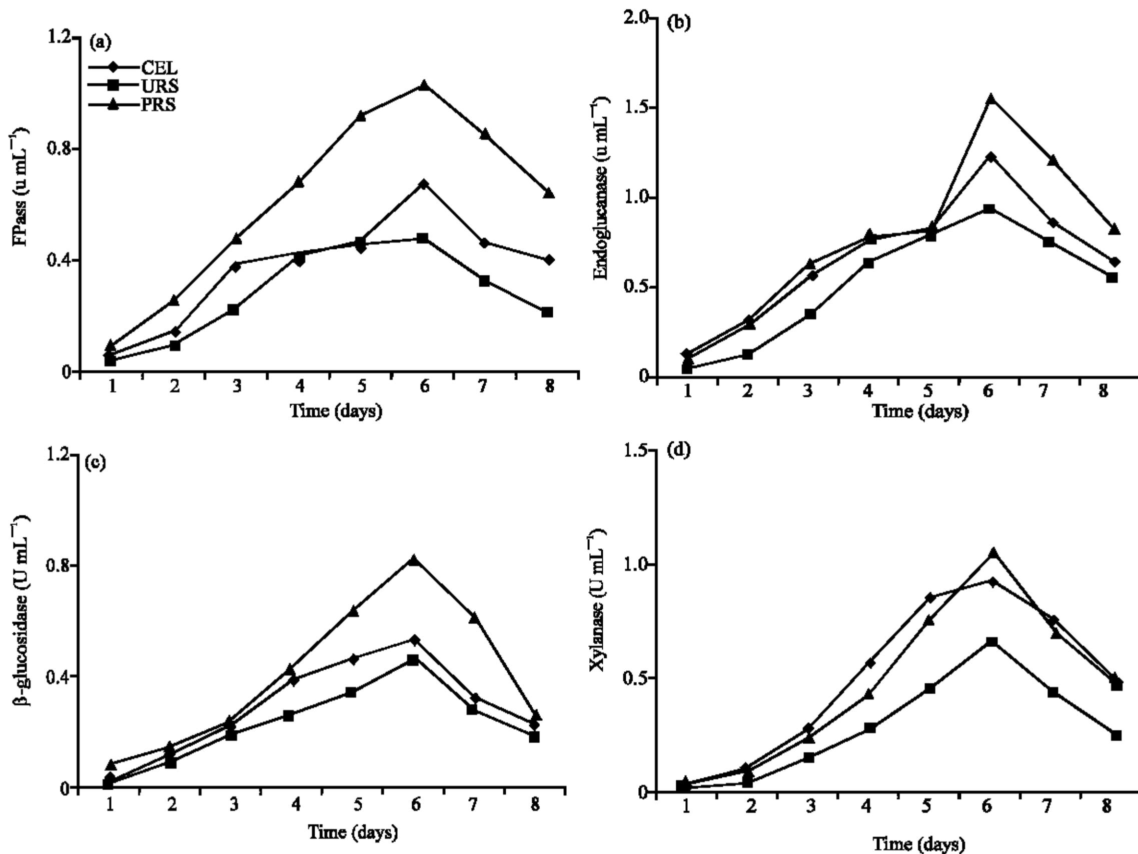

Fig. 2(a-d): Variation in filter paper activity (a), endoglucanase (b), β-glucosidases (c) and xylanases (d) of submerged cultures with 1% of substrate in flasks kept under reciprocal agitation, using the strain *Asperigillus niger* isolate. URS: untreated rice straw, CEL: cellulose, PRS: biological pretreated rice straw. Values (averages) with the same letters for the same day do not differ significantly in the student t-test

There was induction of endoglucanase activity in the cultures with PRS, although the production of endoglucanases was greater in the control cellulose medium (Fig. 2b). On day 4 and 5th day the cellulose and PRS cultures showed similar endoglucanase enzymic activities, while the endoglucanase activity of URS culture was lower on 4th day and the enzymatic activity on 5th day reached nearer to both cellulose and PRS altered. On the 6th day the PRS culture had the greatest activity (1.54±0.08 U mL^{-1}) followed by the culture supplemented with cellulose (1.24 ±0.11 U mL^{-1}) and the URS culture had activities of 0.94±0.10 U mL^{-1}. The behavior of the PRS culture as well as the culture with cellulose also indicated a higher activity of this enzyme. Additionally, according to the results obtained regarding the production of endoglucanases, the optimum time for the culture to be interrupted is on the 4 or 5th day, since after this period, although small increases in the enzymatic activity on 6th day was observed, that there was a consequent decrease in productivity.

The data for the β-glucosidase activity are shown in Fig. 2c. On the 4th day in the control culture and PRS culture, the β-glucosidase activity was similar but with little bit of variation. The β-glucosidase activity in URS culture on 4 and 5th day were lower than the control culture and

PRS culture and attained the maximum activity of 0.46±0.12 U mL^{-1} on 6th day. The maximum enzyme activity in control culture and in PRS on 6th day was 0.54±0.16 U mL^{-1} and 0.82±0.14 U mL^{-1}, respectively. Further incubation resulted in decreased enzyme activity.

Relating to the xylanase activity in Fig. 2d, the untreated rice straw culture had lower enzyme activity than the control and PRS cultures. Interestingly, the xylanase activity on 4 and 5th day in cellulose cultures was higher than the PRS and URS cultures, but on 6th day the activity was decreased when compared to PRS sample. The maximum xylanase activity of PRS sample on 6th day was 1.02±0.08 U mL^{-1}, followed by culture supplemented with cellulose (0.90±0.06 U mL^{-1}) and culture supplemented with URS (0.64±0.14 U mL^{-1}). In the present study the xylanase activity was higher in PRS cultures and the pretreatment facilitated to degradation of hemicellulosic components and increased the reducing sugar concentration either in the form of glucose from cellulose or xylose from hemicellulose. The cellulose and xylose content in the PRS culture resulted in increased activity of xylanase as compared to control culture and URS (Fig. 2d).

The reducing sugar concentration in three fermented culture media samples (Fig. 3) showed higher reducing sugars concentration in URS and PRS than CEL. However, in relation to the quantities of reducing sugars, values were significantly lower in the PRS samples than in those of URS without pretreatment.

Determination of mycelial mass, glucosamine and protein: As the direct determination of fungal cell-mass in solid state fermentation is not possible, a specific component of fungal cell-mass namely the Chitin was used as an indicator for the determination of fungal cell-mass in SSF (Fang *et al.*, 2010). In the process of hydrolysis of the fermented samples, chitin is converted into glucosamine which can be determined using Spectrophotometer.

The quantity of mycelial mass was estimated indirectly by the quantity of N-acetyl glucosamine. It was found that the PRS culture showed the highest quantities of mycelial mass, followed by the cellulose culture, while the cellulose culture showed the lowest average for mycelial mass (Fig. 4). It was observed that there was higher N-acetylglucosamine content in the PRS culture than the URS culture (Fig. 5). A routine correlation was found between glucosamine levels and the fungal

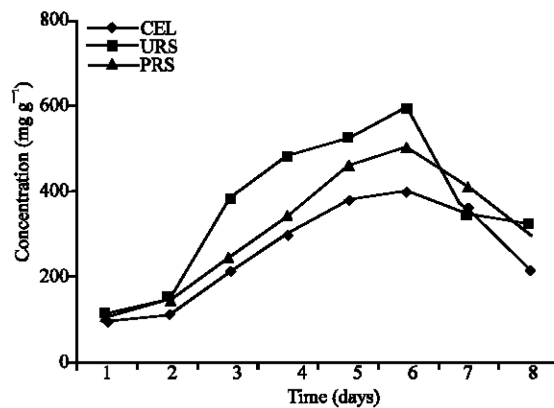

Fig. 3: Reducing sugars production by *Asperigillus niger* isolate cultured on CEL, URS and PRS (mg g^{-1})

growth by Aidoo *et al.* (1981) in koji fermentation with particular reference to spore concentrations of *A. oryzae*.

Biomass, glucosamine and fungal protein were determined from *A. niger* isolate strain grown in liquid media separately on substrates CEL, URS and PRS for 8 days (Fig. 4-6). The fungal growth almost stopped after 5 days of cultivation and the biomass maintained at a relatively stable value. The glucosamine content of mycelium grown in liquid media containing CEL, URS and PRS was 116.4±2.20, 108.2±4.06 and 120.6±2.48 mg g^{-1}, respectively and remained almost constant for the rest of the monitored time. However, gluocosamine content of PRS was higher than URS and control cellulose cultures.

The fungal protein content of mycelium grown in liquid media containing CEL, URS and PRS individually was 385.4±2.73, 325.4±4.12 and 374.2±4.12 mg g^{-1}, respectively and maintained at a nearly constant or little variation during the monitored time. The higher fungal protein content was observed in PRS culture (394.6±1.08 mg g^{-1}) after 5 day than the other control CEL and URS cultures. The data show that even with different carbon sources the glucosamine, fungal protein contents could be considered as good indicators of the biomass growth. There was good correlation between glucosamine content and fungal protein in the data obtained. As shown in Fig. 5 and 6, the glucosamine and fungal protein contents varied in CEL, URS and PRS samples.

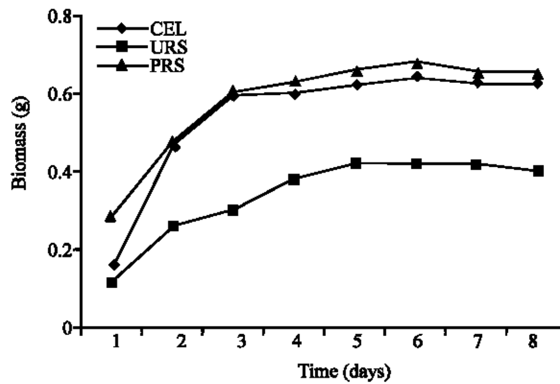

Fig. 4: Biomass content of fungi on cellulose (CEL), untreated rice straw (URS) and pretreated rice straw (PRS) during cultivation

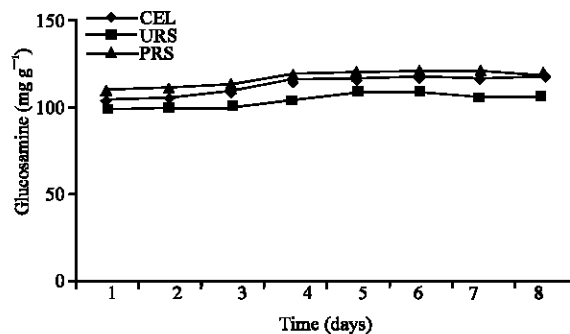

Fig. 5: Glucosamine content (mg of glucosamine g^{-1} of biomass) of fungi on cellulose (CEL), untreated rice straw (URS) and pretreated rice straw (PRS) during cultivation

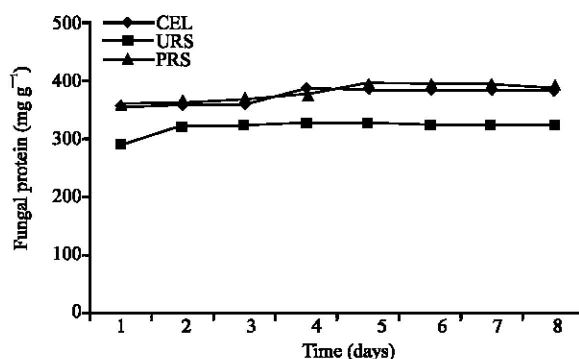

Fig. 6: Fungal protein content (mg of protein g^{-1} of biomass) on cellulose (CEL), untreated rice straw (URS) and pretreated rice straw (PRS) during cultivation

DISCUSSION

In the plant cell wall the lignin forms a matrix that surrounds cellulose and hemicellulose. Degradation of lignin is a prerequisite for hydrolysis of cellulose and hemicelluloses and thus for the usage of the main carbon and energy sources by the fungi. However, due to hydrophobicity and the complex random structure lacking regular hydrolysable bonds, lignin is poorly degraded by many microorganisms capable of cellulose degradation. However, the basidiomycetous whiterot fungi are capable of efficient depolymerization and mineralization of lignin. The enzymes degrading lignin are oxidative, non-specific and act via non-protein mediators in contrast to hydrolytic cellulases and hemicellulases. The main fungal ligninolytic enzymes are manganese peroxidases (MnP; EC 1.11.1.13), lignin peroxidases (LiP; EC 1.11.1.14) that catalyse a variety of oxidative reactions that are dependent on H_2O_2 and laccases (EC 1.10.3.1) that oxidize phenolic compounds and reduce molecular oxygen to water. In addition, extracellular hydrogen peroxide-generating enzymes, glyoxal oxidase and glucose-2-oxidase (EC 1.1.3.10), that generate peroxides essential for peroxidase function, play a role in the delignification process (Aro *et al.*, 2005).

According to Mosier *et al.* (2005), the pretreatments are efficient if their action releases the lignin, hemicellulose and cellulose, the polymers which constitute the lignocelluloses (Weil *et al.*, 1994). The higher N-acetylglucosamine content in the PRS samples for this culture in comparison to the URS samples is probably due to the presence of *P. chrysosporium* mycelia in the substrate. The white rot fungi, which preferentially attack the lignin, could be employed in the pretreatments due to their capacity to liberate oxidases and hydrolases (Perez *et al.*, 2002), or even to reduce the quantity of energy of pretreatment by the alternate steam explosion technique (Keller *et al.*, 2003).

Brown rots mainly attack cellulose, whereas white and soft rots attack both cellulose and lignin. Degradation of lignin by white-rot fungi occurs through the action of lignin-degrading enzymes such as peroxidases and laccase (Lee *et al.*, 2007). The white-rot fungus *P. chrysosporium* produces lignin degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism, in response to carbon or nitrogen limitation (Boominathan and Reddy, 1992). Although encouraging results have been reported in relation to biological pretreatments, from analyzing the enzymatic activities of the FPA, endoglucanases, β-glucosidases and xylanases, it can be observed that the enzymatic activities obtained with the PRS were higher than the control treatments carried out with URS and CEL.

Further study is needed to find out the effect of different days of treatment of rice straw with *P. chrysosporium* in cellulase production by *A. niger*. It is likely that with less days of treatment,

more cellulose is available for growth of *A. niger* for cellulase production. Haltrich *et al.* (1996) suggested that low molecular mass degradation products of xylan and cellulose hydrolysis penetrate into the cells and induce the production of hydrolytic enzymes. Ghosh *et al.* (1993) reported that xylose, the ultimate breakdown product of xylan, serves as a good inducer of this enzyme. However, the presence of xylane is not necessary for the occurrence of xylanases, given that the culture with cellulose also showed xylanase activity. It has been well documented that the carbon source is an important variable for the production of xylanases, with lignocellulose materials perhaps being better substrates than xylan for the production of xylanolytic enzymes (Damaso *et al.*, 2000; Papinutti and Forchiassin, 2007). Also, the fungus *Trichoderma reesei*, grown on cellulose, has shown high levels of endoxylanase (Olsson *et al.*, 2003). This effect may be attributed to the fact that the cellulose ACEII regulator also affects the regulation of xylanases. Thus, the presence of cellulose not only induces cellulases, but also induces the production of xylanases (Aro *et al.*, 2001).

Whiterot fungi were also cultivated on wheat straw and the residue was hydrolyzed with *Trichoderma reesei* cellulase. Of nineteen fungi examined, *Pleurotus ostreatus, Pleurotu* ssp. 535, *Pycnoporus cinnabarinus* 115, *Ischnoderma benzoinum*108, *Phanerochaete sordida* 37, *Phlebia radiata* 79 and two unidentified fungi were found suitable for pretreatment of the straw. The yields of reducing sugars and glucose based on original straw were markedly better compared with uninoculated straw after hydrolysis with cellulase. And these fungi also gave better results than *Polyporus versicolor*, a nonselective reference fungus (Cowling, 1961).

CONCLUSION

Whiterot basidiomycetes are suitable for biological pretreatment of rice straw, which represents a safe and environment-friendly carbon source. Moreover, the use of these fungi also reduces the requirement of conventional acid pretreatments of biomass. The biological pretreatment appears to be a promising method and has very evident advantages, including no chemical requirement, low energy input, mild and environmental friendly. However, disadvantage is that the biological pretreatment is slow and requires careful control of growth conditions and large amount of space to perform the treatment. In addition, most of the lignolytic microorganisms solubilize or consume not only lignin but also hemicellulose and cellulose substrates for their growth. Further studies to reduce the delignification time-interval using the white rot fungi and to improve the enzymatic activities by *A. niger* isolate through mutations, are under progress.

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