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Full Length Article

The Effectiveness of *Pleurotus eryngii* Strains in Biodelignification of Cotton Stalk, Causing Environmental Hazard and Disposable Problem

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ABSTRACT

Biodelignification of cotton stalk (CS) by *Pleurotus eryngii* collected from Tunceli and Elazig province of Turkey under solid state fermentation (SSF) was studied. The effect of the addition of varying concentration (5 & 10% w/w) of rice bran (RB) on lignin degradation and Carbon/Nitrogen (C/N) ratio were investigated. Klason lignin (KL) degradation was followed for 140 day. While substrate initially contained 28.66% KL, maximum lignin loss (69.68±1.05%), occurred on CS +5% RB, C/N ratio of non-fermented cotton stalk initially was 34.13±4.98% and after incubation this ratio decreased to 18.60±0.36% on CS without RB. It was concluded that *P. eryngii* strain collected from Tunceli province is very effective in delignifying cotton stalk.

Key Words: Lignin biodegradation; *Pleurotus eryngii*; Cotton stalk; Rice bran; C:N ratio

INTRODUCTION

Lignocellulosic wastes refer to discarded plant biomass composed of cellulose, hemicellulose and lignin. These wastes may be grouped into different categories such as wood residues, grasses, waste paper, agricultural residues (including straw, stalks, bagasse), domestic wastes (lignocellulosic garbage & sewage), municipal solid wastes and alike (Rodriguez *et al.,* 2008; Godliving, 2009). White rot fungi belong to the order Basidomycetes that participates in the biodegradation of lignin in nature, which is essential for global carbon recycling (Pointing, 2001; Trojanowski *et al.,* 1985). The *Pleurotus eryngii* belongs to the family of oyster mushrooms (*Pleurotaceae*). Its natural habitat is dead roots of the weed *Eryngium campestre*. It can be collected from the wild between October and December but rarely in early spring (Gyorfi & Hajdu, 2007).

Lignin is an aromatic and amorphous polymer present in all layers of woody cell walls. In fibers and tracheids, the thin middle lamella has the highest lignin content, whilst most of the lignin exists in the thick secondary wall layers embedded with cellulose microfibrils and hemicellulose (Chang & Chang, 1995). Lignocellulose consists of three major components: cellulose, hemicellulose and lignin. In addition, small amounts of other materials such as ash, proteins and pectin can be found in lignocellulosic residues in varying degrees based on the source (Sanchez, 2009). Microbiological delignification is required to be a less energy consuming process where the amount of carbohydrate consumption by the organisms needs to be minimum for delignification (Kamra *et al*., 1993; Dhanda *et al*., 1994; Reid, 1995).

SSF is generally defined as the growth of microorganisms on solid materials in the absence or near the absence of free water (Pandey, 1992). SSF has been usually exploited for the production of value-added products (antibiotics, alkaloids, plant growth factors, etc.), biofuel, enzymes, organic acids, aroma compounds and also for bioremediation of hazardous compounds, biological detoxification of agroindustrial residues, nutritional enrichment, biopulping, biopharmaceutical products, etc., (Perez-Guerra *et al*., 2003).

The problem of increasing the utility of lignocellulose wastes has been known for decades. In addition to the growing demand for traditional applications (paper manufacture, biomass fuels, composting, animal feed, etc.), novel markets for lignocellulosics have been identified in recent years. The intensity of research and the magnitude of capital investment in this field increased vastly once commercial viability seemed probable for many of these new applications (e.g., fuel ethanol, acetone & butanol) (Lee, 1997; Wheals *et al.,* 1999; Kaylen *et al.,* 2000).

Most fungal strains produce various lignolytic enzymes into the environment, thus contributing significantly to the decay of lignocellulosic residues in nature. The breakdown of lignocellulosic biomass involves

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the formation of long-chain polysaccharides, mainly cellulose and hemicellulose and the subsequent hydrolysis of these polysaccharides into their component 5-and 6-carbon chain sugars (Zhou & Ingram, 2000; Dashtban *et al.,* 2009).

The cotton stalks could be delignified in order to reduce environmental hazard. In this study, different *P. eryngii* strains were tested for their ability to biodelignify cotton stalks.

MATERIALS AND METHODS

Fungal strain: *Pleurotus eryngii* was collected from Tunceli and Elazig Province, Turkey (Table I). *P. eryngii* is a known wood-degrading fungus. Cultures were grown on malt extract agar (MEA; Merck) at 25ºC in the dark for 8 days before being transferred for specific assays.

Spawn preparation: One kg wheat grain was used for spawn production. The grain was cooked for 40 min and washed in tap-water. The grain was drained and supplemented with 2 g lime and 8 g gypsum and mixed manually. Then, 120 g of grain, cooked and supplemented, was placed in erlenmayer flask (250 mL), closed and sterilized in autoclave at 121ºC, for 15 min. After cooling, each Erlenmayer flask was inoculated with two agar disks of 6 mm diameter containing mycelium (actively growing mycelial growth on MEA plates) and incubated at 25ºC in full darkness for two weeks.

Experimental design: In this study cotton stalks that are usually burned or left in the field to rot in Diyarbakir, Turkey was used as a main material for fermentation of *P. eryngii*. Cotton stalk cut into long pieces (5–10 cm) was soaked overnight in tap water (control). The stalks were then mixed with rice bran (RB) at a ratio of 5 and 10% (w/w), and then in order to obtain the desired pH values (5.5-6.5), for one kg material, 35 g of lime and 35 g of gypsum was added to compost. For each ratio of RB three replicates were prepared. Polypropylene bags (height 18 cm, diameter 15 cm) were filled with 200 g of substrate, sterilized for 45 min at 121°C and allowed to cool to 23°C. All substrates were then inoculated with 3% (w/w) spawn under aseptic conditions.

Cultivation conditions: The closed bags were incubated in a cultivation room maintained at 25±1°C temperature and 85±5% relative humidity. The bags were neither aerated nor illuminated during the mycelial growth phase. After the substrate was fully colonized the bags were opened and then incubated at 20±2°C with a light intensity of 600 lux m^{-2} for 12 h day^{-1} by fluorescent lamps.

Lignin degradation: The samples, taken from the bags periodically (spawn running, primordia initiation & 140 d) consisted of 3 g of substrate colonized with mycelium. The samples were oven dried at 60°C for 24 h. For preparation of chemical analyses, the dried samples were ground in a polymix laboratory mill (Kinematica, Germany). For lignin analyses, test method of TAPPI (1988) were used. In this method lignin is obtained by treating the sample with 13.5 M sulphuric acid. The polysaccharides were hydrolyzed, and lignin, as Klason lignin (KL), was recovered as an insoluble residue.

Table I: *Pleurotus eryngii* strains and sources used in lignin degradation study

Strains	Source
Pleurotus eryngii - P.e (E)	Elazig Province, Turkey
Pleurotus eryngii - P.e (T)	Tunceli-Mazgirt Province, Turkey

Lignin Degradation (%): $100 \times (L_x\text{-}L_y)/L_x$

Where, L_x: Lignin content of unfermented substrate, L_y: Lignin content of spent substrate.

C/N ratio determination: At the end of 140 days, amounts of C and N of spent substrate were determined in an elemental analyzer (Leco CHNS-932).

Statistical analyses: The experimental design was completely randomized (CRD) with three replications. Data was statistically analyzed for means and standard error.

RESULTS AND DISCUSSION

The effect of RB on lignin degradation: Results on lignin degradation of CS by *P. eryngii* fermentation are given in Fig. 1. When total lignin degradation by P.e (E) in CS medium was 55.11%, at CS+5% RB and CS+10% RB medium were determined as 56.40 and 55.41%, respectively. When total lignin degradation by P.e (T) at CS, CS+5% RB and CS+10% RB medium was 68.11, 69.68 and 64.50%, respectively. From this study, it was observed that the addition of RB as a nitrogen source to fermentation medium inhibit lignin degradation, especially at high concentration (10% RB). Similarly, Reid (1989) and Yildirim and Yildiz (2010) reported that addition of N generally leads to repression of lignin degradation.

One of the goals of biological delignification of agricultural wastes using white-rot fungi is to make as much possible of the digestible substrate carbohydrate and reduce environmental hazard (Adenipekun & Fasidi, 2005). The KL content decreased during the fermentation, showing that lignin was degraded (Fig. 1). Lignin degradation over the 140 d was determined as 69.68%. This value is higher than previously reported values of 46% lignin degradation of horticultural plant residues by *Phanerochaete flavido-alba* (Lopez *et al.,* 2006) and 32% degradation of the insoluble lignin component by *Streptomyces badius* (Borgmeyer & Crawford, 1985). Pallat *et al.* (1984) reported that *Pleurotus* strains degraded 10-56% of lignin present in cotton straws. In this study, lignin degradation by P.e (T) was higher than this previously reported range. Decrease in the lignin fractions could be due to the production of various enzymes during the vegetative and reproductive phases with lignocellulose degrading properties. This agrees with the result obtained in this study (Belewu & Balewu, 2005).

Fig. 1: The effect of RB on lignin degradation at different growth periods

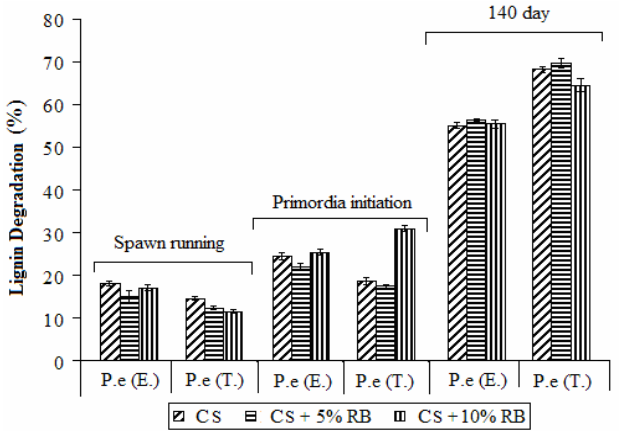


Fig. 2: The effect of rice bran on C, N levels and C/N ratio of spent substrate fermented by P.e (E)

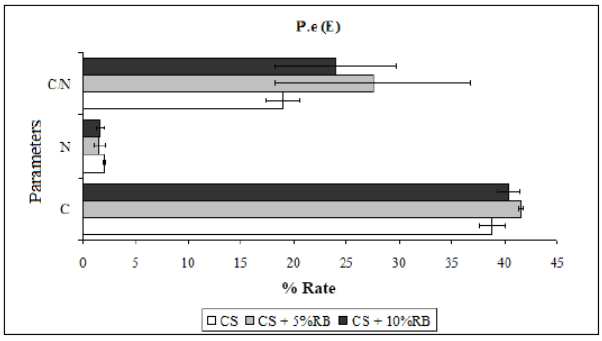
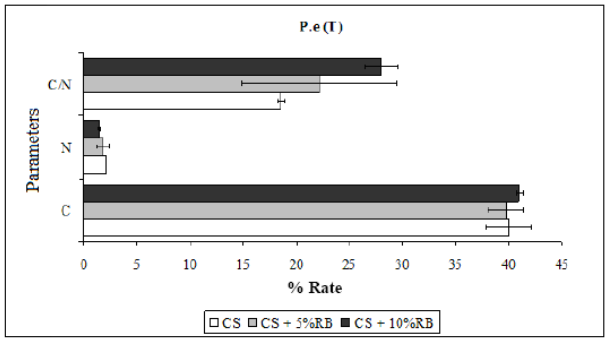


Fig. 3: The effect of RB on C, N levels and C/N ratio of spent substrate fermented by P.e (T)



Changes in C/N ratio: The initial C/N ratio of the substrate was 34.13 (Table II) and decreased to 19.05, 27.48 and 23.99% at CS, CS+5% RB and CS+10% RB groups after incubation by P.e (E), respectively (Fig. 2). C/N ratio decreased to 18.6, 22.15 and 28.01% at CS, CS+5% RB and CS+10% RB groups after incubation by P.e (T), respectively (Fig. 3). Carbon (C) content of substrate was decreased, while nitrogen (N) content increased by fungal fermentation. Similarly, Dilly *et al.* (2001) and Ballaminut and Matheus (2007) reported that C/N ratio of substrate was decreased by fungal fermentation. Ballaminut and Matheus (2007) observed that initial C/N ratios of the substrates used for inoculation of *Lentinus crinitus* and *Psilocybe castanella* were 80 and 58, respectively and decreased to 60 and 43 after 30 days of incubation, respectively. This agrees with the result obtained in this study.

CONCLUSION

It is concluded that *P. eryngii* collected Tunceli province was very effective in delignifying cotton stalk. However, the effect of RB was dependent on fungal strain and nature of the cotton stalk.

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