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Keywords: Lignin biodegradation, *Pleurotus eryngii*, Wheat straw, Ruminant feed

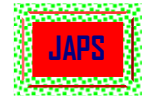
1 SUMMARY

In this study, Klason lignin (KL) biodegradation of wheat straw (WS) by *Pleurotus eryngii* (DC. ex Fr.) Quel. under solid state fermentation (SSF) was studied. Besides, in this study the effects of the addition of different rates (5, 10% w/w) of rice bran (RB) on studied parameters (lignin degradation, total protein level, Carbon/Nitrogen (C/N) ratio, Sulphur (S) level) were investigated. Klason lignin degradation was followed during different growth periods of cultivation, such as spawn running, primordia initiation and fruit body yield. crude protein levels were determined, C:N alteration, and C, N and S levels of spent substrate. While substrate initially contained 21.33% klason lignin the maximum lignin loss of $46.63 \pm 0.18\%$, occurred on WS without RB. Nonfermented wheat straw initially contained 2.43% crude protein and after incubation this level increased to $6.48 \pm 0.77\%$. It was also observed that addition of different concentration of rice bran revealed statistically ($P < 0.05$) different results in the studied parameters. Efficiency of this fungus under the solid-state fermentation on high value ruminant food production is discussed.

2 INTRODUCTION

White Rot Fungi (WRF) are a physiological group comprising of fungi that are capable of biodegrading lignin. The name white rot is derived from the white appearance of the wood attacked by WRF, where lignin removal gives a bleached appearance (Pointing, 2001). Taxonomically, WRF are mostly basidiomycetes, although few ascomycetes are also capable of white-rot decay (Eaton & Hale, 1993). White-rot fungi have powerful lignin degrading enzymes that enable them in nature to bridge the lignin barrier and, hence, overcome the rate-limiting step in the carbon

cycle (Elder & Kelly, 1994). The *Pleurotus eryngii* belongs to the family of oyster mushrooms (*Pleurotaceae*). The wild species of *Pleurotus eryngii* (*Pleurotus eryngii*/ De CANDOLLE ex FRIES) can be found in large areas of Europe. Its natural habitat is on the dead root of the weed *Eryngium campestre*. Its common name originated from it also. It can be collected from the wild between October and December but rarely in early spring (Gyôrfi & Hajdú, 2007). Lignin is a highly branched polyphenolic, amorphous polymer with wide range of functional groups consisting of phenyl



propanoid monomers of coniferyl, sinapyl, and p-coumaryl alcohols (Dence & Lin, 1992; Chang & Chang, 1995). Sadly, much of the lignocellulose waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon (Levine, 1996). Lignocellulosic crop residues represent a potential source of dietary energy for ruminants. These residues are characterised by high percentages of cellulose and hemicellulose, but are poor in protein content. This limits their utilisation as an ideal animal feed. In addition, they pose poor digestibility and poor palatability. In order to improve their utilisation, it is necessary to improve their nutritional quality. This could be achieved using several physical, chemical and microbial methods (Singh *et al.*, 1996). Since physical and chemical methods are energy intensive and are expensive, focus then has been made on developing microbial methods. SSF has been termed potential for this (Balagopalan, 1996; Bano *et al.*, 1996).

SSF is generally defined as the growth of microorganisms on solid materials in the absence or near 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. (Pérez-Guerra *et al.*, 2003).

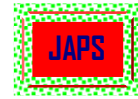
Lignin, which physically and chemically forms a complex with cellulose and hemicellulose, makes the polysaccharides less accessible to ruminal microbial digestion by blocking access to rumen bacteria and their enzymes (Karunanandaa *et al.*, 1995). Moreover, ruminal microbial populations do not possess ligninolytic activity (Zadrazil *et al.*, 1995). Hence, partial delignification of lignocellulosic feeds may promote feed intake and animal

productivity. Microbiological delignification is required to be a less energy consuming process where the amount of carbohydrate consumption by the organisms needs to be minimum in respect of delignification rate. This method has now become popular for improving the nutritional qualities of ruminant feed especially proteins and sugars as well as its digestibility, thereby upgrading the economic value of lignocellulosic waste (Zadrazil & Brunnert, 1982; Kamra *et al.*, 1993; Dhanda *et al.*, 1994; Reid, 1995).

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 new applications. (e.g. fuel ethanol, acetone and butanol) (Kaylen *et al.*, 2000; Lee, 1997; Wheals *et al.*, 1999).

Cellulose is the most important source of carbon and energy in a ruminant's diet, although the animal itself does not produce cellulose-hydrolyzing enzymes (Czerkowski, 1986). Rumen microorganisms utilize cellulose and other plant carbohydrates as their source of carbon and energy. Thus, the microorganisms convert the carbohydrates in large amounts of acetic, propionic and butyric acids, which the higher animal can use as its energy and carbon sources (Colberg, 1988). The concept of preferential delignification of lignocellulose materials by white-rot fungi has been applied to increase the nutritional value of forages (Akin, *et al.*, 1995; Zadrazil & Isikhuehmen, 1997). This increased digestibility provides organic carbon that can be fermented to organic acids in an anaerobic environment, such as the rumen.

These wastes could be put into appropriate use in order to reduce environmental hazard and



pollution. In this study, *P. eryngii* was studied for its ability to bioconvert oat straw into

high-value ruminant food additives.

3 MATERIALS AND METHODS

3.1 Fungal strain: *Pleurotus eryngii* (DC.ex Fr.) Quel. Was obtained from the culture collection of Hacettepe University, Department of Biotechnology, Ankara, Turkey. *Pleurotus eryngii* is a known wood-degrading fungus. Cultures were grown on malt extract agar (MEA; Merck) at 25°C in the dark for 8 days before being transferred for specific assays.

3.2 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 erlenmeyer flask (250 ml), closed and sterilized in an autoclave at 121 °C, for 15 min. After cooling, each erlenmeyer flask was inoculated with two agar disks of 6 mm diam., containing mycelium (actively growing mycelial growth on MEA plates), and incubated at 25 °C in full darkness for two weeks.

3.3 Experimental design: In this study wheat straw that are usually burned or left in the field to rot in Diyarbakir, Turkey was used as a main material for cultivation of *P. eryngii* and it was obtained from Dicle University campus area. Wheat straw, cut into pieces (5–10) cm long was soaked overnight in tap water (control). The straw was 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. For each ratio of RB three replicates were prepared. Polypropylene bags (height 18 cm, diameter 15 cm) were filled with 1.2 kg of substrate. They were sterilized once for 45 min at 121°C and allowed to cool down to 23 °C in a dark room. All substrates were then under aseptic conditions inoculated vertically with 3% (w/w) spawn using a sterile metal pipe.

3.4 Cultivation conditions : The tightly closed bags were then incubated in a cultivation

room maintained at 25 ± 1°C and relative humidity 85 ± 5%. During the mycelial growth phase the bags were neither aerated nor illuminated. After the substrate was fully colonised at about 23 days (spawn running) the bags were opened and then incubated at 20 ± 2°C with a light intensity of 600 lux/m² for 12 h/day by fluorescent lamps. After the primordium formation, the CO₂ level was maintained around 1000 ppm by aeration.

3.5 Lignin Degradation : The samples, taken from the bags periodically (spawn running, primordia initiation, and fruit body yield phases) consisted of 3 g of substrate colonized with mycelium. They 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 methods of the Technical Association of the Pulp and Paper Industry TAPPI, (1998) were utilized. Lignin content was determined according to TAPPI standard T222 om-88. In this method lignin is obtained by treating the sample with 13.5M sulphuric acid. The polysaccharides are hydrolyzed, and lignin, as Klason lignin, is recovered as an insoluble residue.

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

L_x: Lignin content of unfermented substrate

L_y: Lignin content of spent substrate

3.6 Crude Protein, C/N ratio

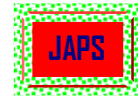
Determination : Amounts of C, N, S and crude protein of spent substrate were determined in an elemental analyzer Leco CHNS-932. Crude protein was calculated as N X 6.25 (Diez & Alvarez, 2001).

3.7 Statistical analyses : The experimental design was completely randomized with 3 replications. Data was statistically analyzed for standard error. Means were calculated and Duncan's new multiple range test was used to compare the groups (WS, WS+5%RB, and WS+10%RB).

WS+10%RB medium were determined as 46.02% and 39.95% respectively. From this study, it was observed that the addition of RB as a nitrogen source to fermentation medium inhibited lignin

4 RESULTS AND DISCUSSION

4.1 The effect of RB on Lignin degradation: Lignin degradation of WS by *P. eryngii* fermentation are given in Table 2. When total lignin degradation at WS medium was 46.63%, at WS+5%RB and



degradation, especially at high concentration (10%RB). Similarly, Reid (1989) was reported that N addition generally leads to repression of lignin degradation.

The KL content decreased in the course of the experiment, showing that lignin was degraded (Table 2). Lignin degradation over the entire growth periods reached 46.63%. These values are in the range of previously reported values of 46% lignin degradation of horticultural plant residues by pure cultures of *Phanerochaete flavido-alba* (Lopez *et al.*, 2006) and 32% degradation of the insoluble lignin component by *Streptomyces badius* (Borgmeyer & Crawford, 1985).

Lignocellulosic materials possess lignin as barrier to rumen microorganisms, this complex molecule is unable to serve them as the sole carbon and energy source (Kirk *et al.*, 1976). One of the goals of biological delignification using white-rot fungi is to make as much possible of the digestible substrate carbohydrate (Adenipekun & Fasidi, 2005).

The changes in pH values of the *P. eryngii* fermentation wheat straw as the period of fermentation increased may be linked to the increase in metabolic products within the substrates. Fungal growth has been known to cause changes in pH of the straw mycelium (Zadrazil, 1977).

4.2 Changes in crude protein, C/N ratio, and ingredient elements: The crude protein content of the fermented spent substrate showed an increase at WS+5%RB and WS+10%RB groups reaching maximum values (5.94 ± 0.37) (Figure 1). However, it decreased at WS group, probably from proteolysis and also due to a higher increase of biological efficiency during cultivation. Similar change in crude protein content in paddy straw during cultivation of *P. florida* was reported by Dhanda *et al.*, (1996).

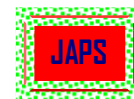
The initial C/N ratio of the substrates were 98.04 (Table 1), and increased to 135.73 at WS group, and decreased to 37.98 and 42.41 at WS+5%RB and WS+10%RB groups after incubation, respectively (Table 3). C (Carbon) rate of substrate was decreased, N (Nitrogen) and S (Sulphur) rates of substrate were increased by fungal fermentation (Table 3). Similarly, Dilly *et al.*, (2001) and Ballaminut & Matheus, (2007) were reported that

C/N ratio of substrate was decreased by fungal fermentation.

Zadrazil *et al.*, (1996) reviewed bioconversion of lignocellulose into protein enriched ruminant feed with white-rot fungi. In conclusion, the literature data and the results obtained in this work show that the effect of RB depends on fungal strain and nature of the compound tested.

4.3 Effect of RB on Growth Phases : Periods for various phases of *P. eryngii* fermentation are given in Table 4. White cottony mycelial mat on the surface of substrate was observed in all sets of the different rates of RB tested. The periods for complete mycelial covering recorded were variable with respect to the concentration of RB used. The most rapid spawn running took place within 20 days in the mixture of WS followed by 24 days in the combination of WS+ 5% RB while, WS + 10%RB had taken maximum 27 days to complete spawn running. The primordial formation in terms of pin heads was observed in all experimental sets. The duration of primordial formation was found to be variable for different groups. The results are presented in Table 4. The formation of primordia was observed within a lesser period of 67.63 days with the WS, whereas the combination WS+5%RB or 10%RB took more time of 77, 84.27 days respectively. The fruit body yield was observed within a lesser period of 105.34 days with the WS, whereas the combination WS+5%RB or 10%RB took more time of 110.24, 126.17 days respectively (Table 4). The higher doses of nitrogen rich supplements resulted in temperature increase (thermogenesis) sufficient to kill the mycelia (Lalley & JanBen 1993). The slower spawn running at higher concentrations of additives in this study may be due to the excess nitrogen, which is known to inhibit mushroom growth (Demirci, 1998; Baysal *et al.*, 2003). Gupta & Vijay (1991) also reported that supplementation above 2% resulted in undue heating of compost.

The growth of *Pleurotus eryngii* on wheat straw changes its chemical composition by increasing the organic matter content and modifying cell wall components, which may improve the nutritional quality of wheat straw. This process may allow using straw treated with *P. eryngii* for ruminant feeding.



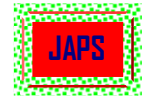
5 ACKNOWLEDGEMENTS

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authors wish to thank the reviewers for their valuable comments.

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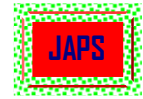


Table 1. Properties of wheat straw used as substrate

C	N	S	C/N	Klason lignin	Crude protein
38.13	0.39	1.25	98.04	21.33	2.43

Table 2. The effect of RB on total lignin degradation (%) at different growth phases

Growth phases	Groups					
	WS		WS + 5% RB		WS + 10% RB	
	Lignin degradadion (%)	pH	Lignin degradatio n (%)	pH	Lignin degradati on (%)	pH
Spawn running	22.71±1.16 ^{a*}	7,65	20.71±1.43 ^b	7,21	16.89±2.40 ^c	7,20
Primordia initiation	34.19±1.01 ^a	8,22	31.54±0.60 ^b	8,02	35.45±0.75 ^a	7,62
Fruit body yield	46.63±0.18 ^a	7,83	46.02±1.07 ^a	7,72	39.95±1.25 ^b	7,49

*Mean of three replicate ± standart error.

Values followed by the same letter along each row are not significantly different by Duncan's multiple range test ($p < 0.05$). Each value is an average of three replicates.

Table 3. The effect of RB on C, H, N and, S content and C/N ratio of nonfermented and spent substrate

Groups	C (%)	N (%)	S (%)	C/N (%)
SW (control)	35.52±2.58	0.29±0.10	1.31±0.81	135.73±29.56
SW + 5% RB	34.88±2.72	0.95±0.21	1.26±0.80	37.98±11.34
SW + 10% RB	34.72±0.50	0.82±0.04	1.48±0.11	42.41±2.81

*Mean of three replicate ± standart error.

Table 4. The effect of RB on growth periods

Growth period (day)			
Groups	Spawn running	Primordia initiation	Fruit body yield
WS	20.36±1.55 ^{c*}	67.63±2.14 ^c	105.34±.2.1 ^c
WS + 5% RB	24.00±0.47 ^b	77.00±1.22 ^b	110.24±3.6 ^b
WS + 10% RB	27.00±1.45 ^a	84.27±1.16 ^a	126.17±2.8 ^a

*Mean of three replicate ± standart error.

Values followed by the same letter along each column are not significantly different by Duncan's multiple range test ($p < 0.05$). Each value is an average of three replicates.

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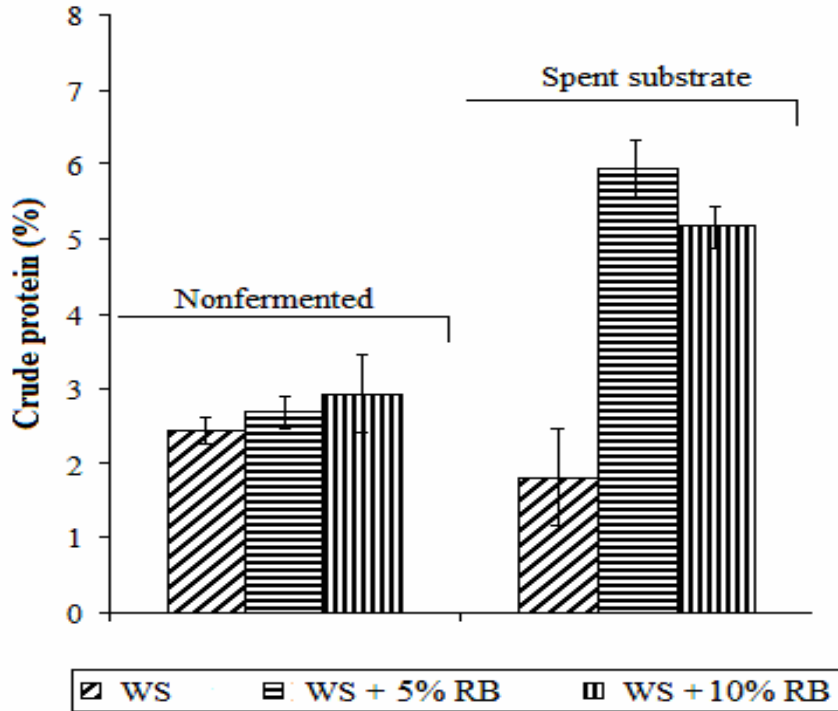


Figure 1. The effect of RB on crude protein content of spent substrate