Biodegradation (2011) 22:823–831
DOI 10.1007/s10532-010-9408-2

ORIGINAL PAPER

# White-rot fungal conversion of wheat straw to energy rich cattle feed

**Bhuvnesh Shrivastava · Shilpi Thakur · Yogender Pal Khasa · Akshaya Gupte · Anil Kumar Puniya · Ramesh Chander Kuhad**

Received: 4 May 2010 / Accepted: 13 August 2010 / Published online: 25 August 2010
© Springer Science+Business Media B.V. 2010

**Abstract** In order to improve the digestibility and nutrient availability in rumen, wheat straw was subjected to solid state fermentation (SSF) with white-rot fungi (i.e. *Pleurotus ostreatus* and *Trametes versicolor*) and the fermented biomass (called myco-straw) was evaluated for biochemical, enzymatic and nutritional parameters. The fungal treatment after 30 days led to significant decrease ($P < 0.05$) in cell wall constituents viz, acid detergent fiber (ADF), neutral detergent fiber (NDF), hemicellulose, lignin and cellulose to the extent of 35.00, 38.88, 45.00, 37.48 and 37.86%, respectively in *P. ostreatus* fermented straw, while 30.04, 33.85, 39.90, 31.29 and 34.00%, respectively in *T. versicolor* fermented straw. However, maximum efficiency of fermentation in terms of low carbohydrate consumption per unit of lignin degradation, favoring cattle feed production was observed for *P. ostreatus* on the 10th day (17.12%) as compared with *T. versicolor* on the 30th day (16.91%). The myco-straw was found to contain significantly high ($P < 0.05$) crude protein (CP; 4.77% *T. versicolor*, 5.08% *P. ostreatus*) as compared to control straw (3.37%). Metabolizable energy (ME, MJ/kg DM), percent organic matter digestibility (OMD) and short chain fatty acids (SCFAs; mmol) production also increased considerably from control straw (4.40, 29.91 and 0.292) to a maximum up to *P. ostreatus* fermented straw (4.92, 33.39 and 0.376 on 20th day) and *T. versicolor* fermented straw (4.66, 31.74 and 0.334 on 10th day), respectively. Moreover, the myco-straw had lower organic carbon and was rich in nitrogen with lower C/N ratio as compared to control wheat straw. Results suggest that the fungal fermentation of wheat straw effectively improved CP content, OM digestibility, SCFAs production, ME value and simultaneously lowered the C/N ratio, thus showing potential for bioconversion of lignin rich wheat straw into high energy cattle feed.

**Keywords** Bioconversion · Fermentation · White-rot fungi · Myco-straw · Animal feed · Metabolizable energy · Digestibility

B. Shrivastava · Y. P. Khasa · R. C. Kuhad (✉)
Lignocellulose Biotechnology Laboratory, Department of Microbiology, University of Delhi South Campus, New Delhi 110021, India
e-mail: kuhad85@gmail.com

S. Thakur · A. Gupte
Natubhai V Patel College of Pure and Applied Sciences, Vallabh Vidya Nagar, Gujarat 388120, India

A. K. Puniya
Dairy Microbiology Division, National Dairy Research Institute (Deemed University), Karnal 132001, India

## Introduction

Agricultural crop-residues constitute a major part of the ruminant's diet and among them wheat straw is one of the most abundant plant residue available throughout the world. However, rumen microbial

utilization of energy-rich cell walls of wheat straw is hindered by the presence of lignin, which limits its overall digestion process and can significantly influence the animal performance in livestock production systems (Basu et al. 2002). Thus, for the maximum utilization of wheat straw as cattle feed either complete or partial degradation of lignin from lignocellulosic complex is necessary. Among various pretreatment methods used to improve the digestibility of wheat straw, the biological delignification has several advantages over chemical and mechanical treatments, including mild reaction conditions, avoidance of toxic and corrosive chemicals, higher product yields, fewer side reactions, less energy demands and less reactor resistance (Teymouri et al. 2005; Kuhar et al. 2008; Okano et al. 2009).

White-rot fungi (WRF) have been studied for degradation of crop-residues under solid-state fermentation (SSF), as they are reported to degrade lignin more efficiently than any other group of microorganisms (Kuhad and Singh 2007; Kuhar et al. 2008; Shabtay et al. 2009). The white-rot fungi mainly degrade polysaccharide by hydrolytic enzymes (cellulases and xylanases) and lignin by oxidative ligninolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. A majority of WRF degrade polysaccharide and lignin simultaneously, while some degrade lignin selectively under SSF (Eriksson et al. 1990; Kuhad et al. 1997). SSF is a polyfactorial event, in which the fungus, its enzymes, physical structure of substrate, physiological factors of fermentation and culture and nutritional conditions play an important role in controlling lignin degradation and digestibility of fermented substrate (Zadrazil 1986).

White-rot fungi such as *Phanerochaete chrysosporium*, *Pleurotus* sp. *Lentinus edodes*, *Coriolus versicolor Phelbia* sp. and *Ceriporiopsis subvermispora* have largely been studied for their ability to ferment the different crop residues (wheat straw, olive mill solid waste, madake bamboo etc.) to produce improved animal feed (Zafar et al. 1989; Yadav and Tripathi 1991; Moyson and Verachtert 1991; Tripathi and Yadav 1992; Basu et al. 2002; Shabtay et al. 2009; Okano et al. 2009). Recently *Pleurotus ostreatus* has shown a higher rate of substrate decomposition and increase in digestibility of cowpea shells and olive mill solid wastes (Kinfemi et al. 2009; Shabtay et al. 2009). For large scale production of animal feed, it is necessary to obtain higher degradation of lignin with minimum cellulose utilization (Jung and Vogel 1986; Basu et al. 2002). According to Basu et al. (2002) inoculum type/seed culture has a major role to play in achieving high level of lignin degradation under SSF. They further prioritized that seed culture in the form of individual and separated pellets as opposed to mycelia, for uniform inoculation of moist wheat straw substrate. The fungal pellets as inoculum has been reported to be advantageous as compared to filamentous inoculum in obtaining decreased viscosity, desirable mixing, better mass and oxygen transfer into the biomass and lower energy consumption for aeration and agitation (Suijdam et al. 1980; Liao et al. 2007). Thus use of fungal pellet inoculum would improve the biodegradation of lignin and eventually improve the digestibility of the fermented feed in ruminants.

In this study we examined the effect of fungal pellet inoculum of *Pleurotus ostreatus* (F6) (Jaquin ex Fr.) Kammer and *Trametes versicolor* (L. ex Fr.) Quelet (ATCC 11235) on bioconversion of wheat straw into improved animal feed in relation to digestibility, secretion of degradative enzymes, metabolizable energy and nutritive value.

## Materials and methods

### Substrate

Wheat straw collected locally was sieved to a uniform particle size (1.5–2.0 cm) and dried at 60°C before SSF. The unfermented and fermented straws were powdered by milling in the laboratory mill (Remi Motors, Delhi, India) and sieved (30 mesh) for analytical purposes.

### Organisms and inoculum preparation

Fungal cultures, *P. ostreatus* (F6) (Jaquin ex Fr.) Kammer and *T. versicolor* (L. ex Fr.) Quelet (ATCC 11235) procured from Institute of Forest Botanisches, Gottingen, Germany, were grown and maintained on malt extract agar (MEA) containing ($gl^{-1}$): malt extract, 20.0; $KH_2PO_4$, 0.5; $MgSO_4{\cdot}7H_2O$, 0.5; $Ca(NO_3)_2{\cdot}4H_2O$, 0.5; agar, 20.0 (pH 5.5) at 30°C (Dhawan and Kuhad 2003, Vasdev et al. 2005). The cultures were stored at 4°C and sub-cultured every fortnight.

Inoculum was prepared by growing each fungus in 250 ml Erlenmeyer flasks having 50 ml of sterile malt extract broth (MEB). Each flask was inoculated with 2 mycelial discs (8 mm dia. each) from the periphery of 7 days old fungal culture growing on MEA. The mycelial mat thus obtained was further homogenized and inoculated in 2 l Erlenmeyer flasks containing 500 ml MEB each. The flasks were incubated on a rotary shaker (Innova 43, New Brunswick Scientific, Germany) at 200 rpm and 30°C for 4 days and the pellets formed were used as inoculum. The fungal mass was separated from the culture broth by filtering through dried and preweighed whatman filter paper no. 1. The fungal pellet mass was thoroughly washed with deionized water and weighed after drying at 55°C till constant weight.

## Solid state fermentation

Enamel trays (25 × 20 $cm^2$) containing 50 g of wheat straw were moistened with mineral salt solution containing ($gl^{-1}$): $KH_2PO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $Ca(NO_3)_2 \cdot 4H_2O$, 0.5 (pH 5.5) and autoclaved at 121°C for 15 min. Each sterilized tray was inoculated with desired volume of inoculums to obtain 0.25 g ± 0.016 (0.5% w/w) of fungal dry mass. Sterile mineral salt solution was added to the trays to obtain a final substrate to moisture ratio of 1:3 and incubated at 30°C for 30 days. The uninoculated trays without fungal mycelia were used as control. The trays in duplicates were harvested at a regular interval of 5 days and a fixed amount of wet fermented straw was taken out for enzyme extraction. Weight loss of wheat straw was determined by deducting the oven dried myco-straw at 60°C to a constant weight from the weight of dried unfermented (control) straw.

## Enzyme assays

The myco-straw (wheat straw along with fungal mycelium; 5 g wet weight) removed aseptically from flasks after various intervals, was suspended in 20 ml acetate buffer (20 mM, pH 5.0) and shaken gently for 2 h. The extrudates were squeezed through muslin cloth for maximizing the enzyme extraction and centrifuged at 6708 × *g* for 20 min at 4°C. The enzyme solution thus obtained was assayed for various enzyme activities. Laccase (E.C. 1.10.3.2) activity was determined by monitoring the $A_{420}$ change related to the rate of oxidation of 1 mM 2,2′-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS, $\varepsilon$ = 36,000 $cm^{-1}$ $M^{-1}$) following the method described by Paavola et al. (1990). The assay was performed in 1 ml cuvette at 25°C with 100 µl of adequately diluted culture liquid, 100 µl of 1 mM ABTS and 800 µl of 50 mM sodium acetate buffer (pH 5.0). One unit of enzyme activity (U) was defined as the amount of enzyme which leads to the oxidation of 1 µM of ABTS/min. Laccase activity was calculated as follows:

$$\text{Enzyme (U/ml)} = \Delta D \times V \times 10^6 / \Delta E\ \varepsilon \times \Delta t$$

where ΔD = O.D difference for 1 min; V = volume of assay mixture (1 ml); ΔE = volume of enzyme (100 µl); $\varepsilon$ = Molar extinction coefficient (36,000 $cm^{-1}$ $M^{-1}$ for ABTS); Δt = time in minutes (1 min)

$$\begin{aligned}\text{Enzyme(units/g of substrate)} = \{&\text{Enzyme (units/ml)} \\ &\times \text{volume of total enzyme solution} \\ &\text{obtained after harvesting}\}/ \\ &\{\text{Dry weight of solid substrate (myco-straw)}\}\end{aligned}$$

Manganese dependent peroxidase (MnP) activity (E.C. 1.11.1.13) was measured by oxidation of DMP (2,6-dimethoxy phenol, $\varepsilon$ = 27,500 $cm^{-1}$ $M^{-1}$) in the presence of $H_2O_2$ and $MnSO_4$. Increase in absorbance was measured with spectrophotometer $A_{469}$ nm and one unit of enzyme activity (U) was defined as the amount of enzyme catalyzing the oxidation of 1 µM of DMP/min (Martinez et al. 1996). Presence of laccase activity was corrected as laccase assay was performed in 3 ml cuvette with 500 µl of adequately diluted culture liquid, 500 µl of 1 mM $MnSO_4$, 1.0 ml of 50 mM sodium acetate buffer (pH 5.0) and 1 ml of 1 mM DMP without addition of $H_2O_2$. The xylanase activity was determined by measuring the release of reducing sugars from Birchwood xylan (1.0% w/v) by dinitrosalicylic acid method (Miller, 1959). The assay mixture consisted of 490.0 µl of substrate in citrate–phosphate buffer (100.0 mM; pH 6.0) and 10.0 µl of enzyme solution. The reaction mixture was incubated at 60°C for 10.0 min. One unit of xylanase was defined as the amount of enzyme required to release 1 µM of xylose from Birchwood xylan in 1 min under the standard assay conditions described elsewhere (Kapoor et al. 2008; Kuhar et al. 2008).

### Proximate and chemical composition of wheat straw

The dried straw was analysed in triplicates for moisture content, dry matter, ether extract, crude fiber, total carbohydrate, CP and total ash (AOAC 1995). The contents of ADF, NDF, cellulose, hemicellulose and lignin were determined by methods described by Van Soest (Goering and Van Soest 1970; Van Soest et al. 1991). Carbon and Nitrogen in fermented samples were estimated using CHNS analyzer (Elementar—Vario EL III, Analysensysteme GmbH, Germany). Percent efficiency of SSF process is expressed as the loss of lignin compared to carbohydrate breakdown and calculated using the method described by Moyson and Verachtert (1991).

Losses of components of wheat straw after treatment were calculated as follows:

$$U_1 = [(G_0C_0 - G_1C_1)/G_0C_0] \times 100$$

Where, $G_0$ and $G_1$ are the substrates dry weight at the beginning and end of fermentation. $C_0$ and $C_1$ are the dry weights of the components at the beginning and end of fermentation.

### Estimation of in vitro gas production, ME, OMD and SCFAs production

Mineral buffer solution was prepared and placed in a water bath at 39°C under continuous flushing with $CO_2$. Rumen fluid was collected into pre-warmed insulated bottles and filtered through three layers of muslin cloth and purged with $CO_2$. Thereafter, this rumen fluid was added to the mineral buffer solution (1:2 v/v), and mixed. Samples (200 ± 10 mg) of predried fermented straw were accurately weighed into syringes fitted with plungers. Buffered rumen fluid (30 ml) was pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39°C. Three syringes with only buffered rumen fluid were incubated and considered as blanks. The syringes were gently shaken after every 2 h and the gas production was recorded after 24 and 48 h of incubation. Total gas values were corrected for the blank incubation and reported gas values are expressed in ml per 200 mg of dry matter (DM). The post incubation parameters such as ME and OMD were estimated at 24 h (GP24) post gas collection according to Menke et al. (1979) and Menke and Steingass (1988). While, the short chain fatty acids (SCFA) were predicted according to Getachew et al. (2002).

### Statistical analysis

Differences in various components through fungal treatments were tested by one way analysis of variance (ANOVA) followed by Bonferroni's posthoc test ($P < 0.05$). Statistical analyses were performed using SYSTATE (6.0.1) software. However, the level of significance was tested between control and fungal treatments of wheat straw on different incubation periods.

## Results and discussion

### Enzyme production and substrate transformation during fungal fermentation of wheat straw

Lignocellulosic residues have been shown to stimulate enzyme production by basidiomycetes (Eichlerova et al. 2000). In this study, *P. ostreatus* produced maximum laccase (3518 U/g) and MnP (213.4 U/g) on the 10th day, while *T. versicolor* exhibited maximum laccase (2711 U/g) and MnP (834 U/g) production on 5th day and the enzyme production declined thereafter in both the fungi (Figs. 1, 2). A second peak in laccase and MnP production was observed for *P. ostreatus* and *T. versicolor* on 25th and 20th day, respectively. Whereas maximum xylanase activity obtained for *P. ostreatus* and

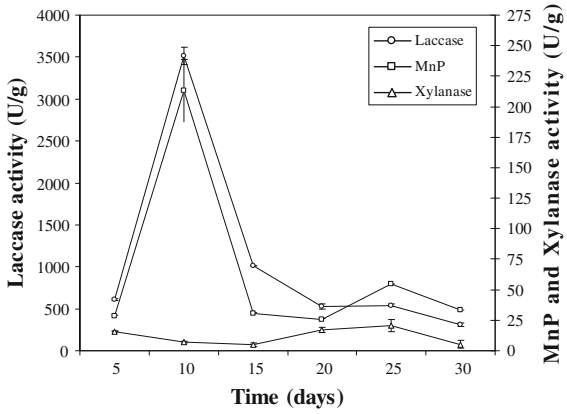


**Fig. 1** Ligninolytic and Xylanolytic activities of *P. ostreatus* during the cultivation on wheat straw

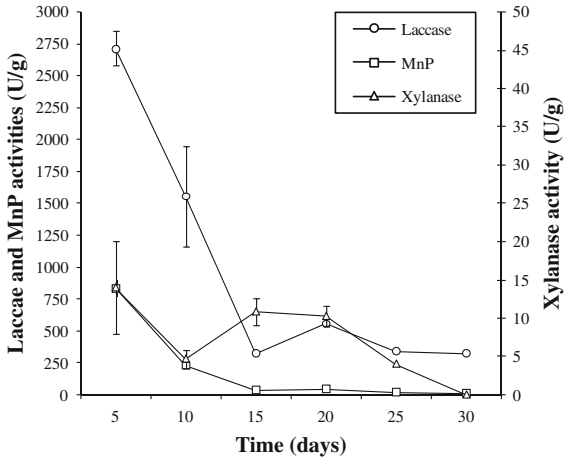


**Fig. 2** Ligninolytic and Xylanolytic activities of *T. versicolor* during the cultivation on wheat straw

*T. versicolor* were 16 and 14 U/g, respectively on 5th day and xylanase production declined thereafter. However it peaked again after 20–25 days for *P. ostreatus* (Fig. 1) and 15 days for *T. versicolor* (Fig. 2). The twin peak in enzyme production during studied process could be attributed to mycelial autolysis and release of mycelial bound enzymes or it might be due to nitrogen starvation in the second phase of cultivation (Arora et al. 2002; Dhawan et al. 2005; Gupte et al. 2007).

Both fungi colonized and degraded wheat straw rapidly and brought about variable dry matter loss (DML). *P. ostreatus* resulted in a higher DML in wheat straw (30.9%) as compared to *T. versicolor* (24.38%) after 30 days of incubation. Interestingly, *P. ostreatus* exhibited a higher DML at the initial

**Table 1** Chemical composition (g/100 g DM) of wheat straw subjected to treatment with *P. ostreatus* for various incubation periods

| Days of incubation | Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Weight | ADF | NDF | HC | Lignin | Cellulose | CP | ESSF (%) |
| 0 | 100 | 48.97 ± 0.29 | 79.78 ± 0.41 | 30.81 ± 0.69 | 9.77 ± 0.08 | 34.10 ± 0.21 | 3.37 ± 0.35 | – |
| 5 | 94.88 ± 1.17 | 47.95 ± 1.24 | 74.60 ± 1.71 | 26.65 ± 2.91 | 9.22 ± 0.98 | 33.28 ± 1.50$^{c}$ | 3.67 ± 0.30 | 11.12 |
| 10 | 84.68 ± 0.57 | 41.98 ± 0.58$^{c}$ | 66.41 ± 3.28$^{c}$ | 24.44 ± 3.77 | 7.77 ± 0.28$^{b}$ | 28.77 ± 0.37$^{c}$ | 3.72 ± 0.36 | 17.12 |
| 15 | 73.58 ± 3.07 | 35.27 ± 1.15$^{c}$ | 55.78 ± 2.37$^{c}$ | 20.51 ± 2.83$^{a}$ | 6.73 ± 0.21$^{c}$ | 24.2 ± 0.98$^{c}$ | 4.08 ± 0.10 | 15.03 |
| 20 | 71.15 ± 0.50 | 33.70 ± 1.29$^{c}$ | 51.99 ± 2.44$^{c}$ | 18.29 ± 3.63$^{b}$ | 6.36 ± 0.44$^{c}$ | 22.92 ± 1.40$^{c}$ | 4.17 ± 0.22 | 14.40 |
| 25 | 70.46 ± 2.19 | 33.28 ± 1.26$^{c}$ | 50.76 ± 2.31$^{c}$ | 17.49 ± 2.62$^{b}$ | 6.26 ± 0.78$^{c}$ | 22.37 ± 0.73$^{c}$ | 4.64 ± 0.47$^{b}$ | 14.72 |
| 30 | 69.10 ± 1.34 | 31.83 ± 1.16$^{c}$ | 48.76 ± 1.94$^{c}$ | 16.93 ± 3.10$^{b}$ | 6.11 ± 0.19$^{c}$ | 21.19 ± 1.31$^{c}$ | 5.08 ± 0.14$^{c}$ | 10.98 |

*ADF* Acid detergent fiber, *NDF* Neutral detergent fiber, *HC* Hemicellulose, *CP* Crude protein, *ESSF* Efficiency of SSF

Data are presented as mean ± SD of 3 replicates: Significance ($^{a}$ $p < 0.05$; $^{b}$ $p < 0.01$; $^{c}$ $p < 0.001$)

**Table 2** Chemical composition (g/100 g DM) of wheat straw subjected to treatment with *T. versicolor* for various incubation periods

| Days of incubation | Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Weight | ADF | NDF | HC | Lignin | Cellulose | CP | % ESSF |
| 0 | 100 | 48.97 ± 0.29 | 79.78 ± 0.41$^{a}$ | 30.81 ± 0.69 | 9.77 ± 0.08 | 34.10 ± 0.21 | 3.37 ± 0.35 | – |
| 5 | 96.73 ± 0.40 | 46.30 ± 1.37 | 74.32 ± 2.23$^{c}$ | 28.03 ± 2.72 | 9.52 ± 0.35 | 31.38 ± 1.05 | 3.85 ± 0.24 | 4.61 |
| 10 | 93.70 ± 0.34 | 45.05 ± 1.44$^{a}$ | 69.91 ± 1.71$^{c}$ | 24.86 ± 1.16$^{b}$ | 9.04 ± 0.53 | 29.95 ± 1.49$^{a}$ | 3.86 ± 0.20 | 7.20 |
| 15 | 92.87 ± 0.56 | 43.97 ± 0.68$^{b}$ | 68.21 ± 1.38$^{c}$ | 24.24 ± 0.78$^{b}$ | 8.9 ± 0.24 | 29.69 ± 0.45$^{b}$ | 3.81 ± 0.39 | 7.94 |
| 20 | 86.07 ± 1.10 | 39.62 ± 1.19$^{c}$ | 61.06 ± 2.54$^{c}$ | 21.44 ± 1.37$^{c}$ | 7.82 ± 0.61$^{c}$ | 26.52 ± 0.93$^{c}$ | 4.59 ± 0.69$^{a}$ | 11.50 |
| 25 | 77.90 ± 1.34 | 34.69 ± 1.67$^{c}$ | 53.93 ± 2.08$^{c}$ | 19.23 ± 0.52$^{c}$ | 7.00 ± 0.06$^{c}$ | 22.15 ± 1.65$^{c}$ | 4.77 ± 1.16$^{b}$ | 14.00 |
| 30 | 69.61 ± 2.72 | 34.26 ± 1.44$^{c}$ | 52.78 ± 0.45$^{c}$ | 18.52 ± 1.81$^{c}$ | 6.70 ± 0.16$^{c}$ | 22.50 ± 1.33$^{c}$ | 4.69 ± 0.22$^{a}$ | 16.91 |

*ADF* Acid detergent fiber, *NDF* Neutral detergent fiber, *HC* Hemicellulose, *CP* Crude protein, *ESSF* Efficiency of SSF

Data are presented as mean ± SD of 3 replicates: Significance ($^{a}$ $p < 0.05$, $^{b}$ $p < 0.01$, $^{c}$ $p < 0.001$)

stage of SSF and comparatively caused lesser DML after the 15th to 30th day of SSF (Tables 1, 2). While, *T. versicolor* colonized straw poorly in the initial stage of SSF and caused a comparatively less DML, which increased after 15th day of SSF. The degradation of lignin was higher in *P. ostreatus* treated straw (37.48%) compared with *T. versicolor* (31.29%) with a decrease in cellulose content up to 37.86 and 34.00%, respectively till the 30th day. However, when statistically analyzed the difference in lignin degradation between *P. ostreatus* and *T. versicolor* was not found significant ($P = 0.070$). The high loss in cellulose in the present study is well in accordance with the findings of Okano et al. (2006), where despite being a selective lignin degrader *Ceriporiopsis subvermispora* caused higher cellulose loss than *Lentinus edodes,* which is not a selective lignin degrader. This could be because of difference in cell wall assembly and chemical structure of lignin and lignin–carbohydrate complex. *P. ostreatus* and *T. versicolor* both degraded ADF, NDF and hemicellulose up to 35.00, 38.88, 45.00 and 30.04, 33.85, 39.89%, respectively after 30th day of incubation (Table 3). The decrease in ADF, NDF and hemicellulose was more pronounced during preliminary stage of SSF with *P. ostreatus,* but in contrast, degradation of ADF, lignin and cellulose with *T. versicolor* was more apparent after 20 days of incubation. Moreover, *T. versicolor* exhibited initially greater consumption of hemicellulose and lesser but consistent cellulose consumption throughout the SSF of straw, indicating its simultaneous degrading nature (Table 3). Arora and Sharma (2009) also reported an increase in DML along with hemicellulose and cellulose degradation and *P. ostreatus* was found to dominantly degrade the lignin over hemicellulose and cellulose. Adamovic et al. (1998) while studying the biodegradation of wheat straw by *P. ostreatus* for cattle feed production also reported the similar pattern of degradation and the fungus consumed more hemicellulose (27%) than cellulose and lignin (3.75% and 20%, respectively) till the 30th day of incubation. Various workers have reported similar degradative capabilities of both the fungi and showed DM, hemicellulose, cellulose and lignin losses ranging from 5–50, 2–20, 5–30 and 2–40%, respectively (Yadav and Tripathi 1991; Tripathi and Yadav 1992; Shabtay et al. 2009).

The degradation of wheat straw (i.e. in terms of substrate weight loss, lignin, cellulose and hemicellulose degradation) continued throughout the studied period and substrate degradation was not found to be directly correlated with the production of laccase, manganese peroxidase and xylanase by *P. ostreatus* and *T. versicolor* (Figs. 1 and 2, Table 3), as has been reported by Li et al. (2008) and Mendonca et al. (2008). Recently, Sharma and Arora (2010) have also observed that the enzyme production profile could not be correlated well with the degradation of specific polymer, which showed that fiber degradation not only depends upon the production of the enzymes but also regulated by a variety of physiological factors. The progressive degradation of the substrate by *Pleurotus ostreatus* and *Trametes versicolor* throughout the studied process could be as a consequence of several enzyme complexes working in consortia and are active at different stages of incubation. It is well documented in the literature that several additional

**Table 3** Disappearance (%) of cell wall components in wheat straw subjected to treatment with *P. ostreatus* (PO) and *T. versicolor* (TV) for various periods

| Days of incubation | Disappearance (%) of cell wall components | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DML | | ADF | | NDF | | HC | | Lignin | | Cellulose | |
| | PO | TV | PO | TV | PO | TV | PO | TV | PO | TV | PO | TV |
| 5th day | 5.12 | 3.26 | 2.09 | 5.46 | 6.50 | 6.84 | 13.50 | 9.04 | 5.67 | 2.60 | 2.41 | 7.97 |
| 10th day | 15.32 | 6.30 | 14.28 | 8.00 | 16.75 | 12.38 | 20.69 | 19.33 | 20.52 | 7.45 | 15.64 | 12.18 |
| 15th day | 26.42 | 7.12 | 27.98 | 10.21 | 30.08 | 14.50 | 33.43 | 21.34 | 31.09 | 8.93 | 29.03 | 12.92 |
| 20th day | 28.85 | 13.92 | 31.18 | 19.10 | 34.83 | 23.47 | 40.64 | 30.41 | 34.92 | 19.94 | 32.79 | 22.22 |
| 25th day | 29.85 | 22.10 | 32.05 | 29.15 | 36.37 | 32.40 | 43.25 | 37.57 | 35.91 | 28.40 | 34.39 | 35.03 |
| 30th day | 30.9 | 24.38 | 35.00 | 30.04 | 38.88 | 33.85 | 45.05 | 39.89 | 37.48 | 31.29 | 37.86 | 34.00 |

*DML* dry matter loss, *ADF* acid detergent fiber, *NDF* neutral detergent fiber, *HC* hemicellulose

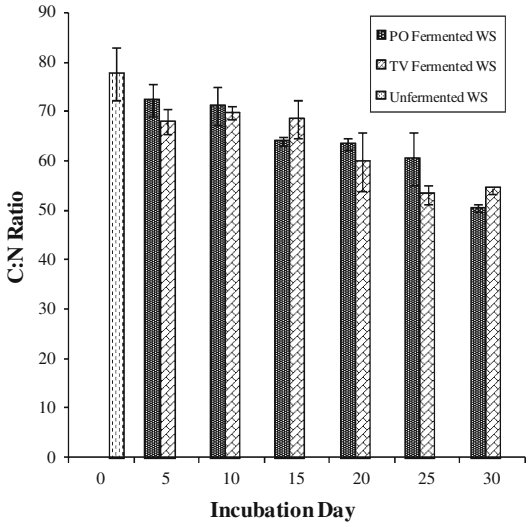


**Fig. 3** Carbon Nitrogen ratio (C/N) of unfermented and fermented wheat straw with *P. ostreatus* and *T. versicolor*

enzymes such as glyoxal oxidases, Cellobiose oxidase, Cellobiose oxidase: quinone oxidoreductases (CBQase) are reported to play an important role in substrate degradation (Cullen and Kersten 1996).

*Pleurotus ostreatus* and *Trametes versicolor* exhibited maximum SSF efficiency (~17%) on 10th and 30th day of incubation, respectively. Higher SSF efficiency resulted in better assimilation of nutrients for fungi and thereby causing a consistent increase in CP content of myco-straw (41–50%) (Tables 1, 2). Similarly Basu et al. (2002) also reported the maximum desirability coefficient on the 6th day (achieving 19.5% lignin and 17.8% cellulose degradation) although the fungus kept continued to degrade the substrate thereafter as well. In addition to that, our results showed lower C/N ratio in fermented substrate, however *P. ostreatus* consistently brought it down to a minimum of 40.45 (30th day) compared with *T. versicolor* (54.62 on 30th day) and control (77.6) (Fig. 3). This observation is consistent with the earlier report of Bisaria et al. (1997).

## In vitro gas production (IVGP) and estimated parameters (ME, OMD and SCFAs)

*Pleurotus ostreatus* and *Trametes versicolor* fermented straw when tested, produced more gas (IVGP) (18.25 and 16.5 ml/200 mg DM, on 20th and 10th day, respectively) as compared to the unfermented straw (14.75 ml/200 mg DM). The elevation in gas production evidenced the higher availability of ME (MJ/kg DM) and increase in percent OMD in straw fermented with *P. ostreatus* (4.92, 33.39) and *T. versicolor* (4.66, 31.74) on the 20th and 10th day, respectively than in unfermented straw (ME: 4.4, OMD: 29.91). Similar higher gas production (11–13 ml/100 mg unfermented and 13.5–14 ml/100 mg in fermented) is reported by Okano et al. (2005) in wheat straw degraded by *Pleurotus* sp. Moreover, in the present study, ME and OMD in *P. ostreatus* and *T. versicolor* fermented straw were observed to be declined after 20th and 10th day, respectively which could be attributed to the addition of degradable nitrogen compound to fiber rich feeds and due to that improved capturing of nutrients and higher production of microbial protein and the carbon source may possibly be diverted from gas to microbial protein (Menke and Steingass 1988).

The maximum increase in ME, OMD and SCFAs were 11.81, 11.63, 28.76% and 6.00, 6.11, 14.3% for *P. ostreatus* and *T. versicolor,* respectively on the 20th and the 10th day of incubation (Table 4). Zadrazil and Puniya (1995) also reported that culturing bagasse with *P. eryngii* raised OMD (3–16%). Similarly, Kinfemi et al. (2009) reported an increase in OMD (45.65–62.14%) and ME (6.97–9.41 MJ/kg DM), while degrading cowpea husk using *P. ostreatus* for ruminant feed production. They correlated an increase in CP with OMD and described that fungal degradation of cellulose and hemicelluloses into monomers could serve as a carbon source for the fungus to produce biomass and high gas production. Although the decrease in hemicellulose and cellulose causes a loss of nutrients as feedstuff, this undesirable change in fungal treatment could be compensated by increase in digestible substrate, consequently providing higher gas production.

Higher digestibility also resulted in elevated production of SCFAs which was found to be 0.376 and 0.334 mmol for *P. ostreatus* and *T. versicolor*, respectively on the 20th and the 10th day. The elevation in SCFAs concentration might have resulted in part from more active digestion of fiber in straw, which may support the hypothesis that increased level of rumen N may result in an increased available amount of energy from low quality straw (Kinfemi et al. 2009). Blummel and Orskov (1993) have found that gas production from feed incubated in vitro in rumen fluid is closely related to the

**Table 4** Metabolizable energy, organic matter digestibility and short chain fatty acids using in vitro gas production in *P. ostreatus* (PO) and *T. versicolor* (TV) fermented wheat straw at different incubation periods

| Days of incubation | Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GP 24 (ml) | | ME (MJ/kg DM) | | OMD (%) | | SCFAs (mM) | |
| | PO | TV | PO | TV | PO | TV | PO | TV |
| 0 Day | 14.75 ± 1.06 | 14.75 ± 1.06 | 4.40 | 4.40 | 29.91 | 29.91 | 0.292 | 0.292 |
| 5th Day | 17.25 ± 0.35 | 12.75 ± 0.35 | 4.76 | 4.15 | 32.26 | 28.37 | 0.352 | 0.245 |
| 10th Day | 17.75 ± 2.47 | 16.50 ± 2.82 | 4.83 | 4.66 | 32.75 | 31.74 | 0.364 | 0.334 |
| 15th Day | 18.25 ± 1.77 | 15.00 ± 1.41 | 4.91 | 4.46 | 33.36 | 30.36 | 0.376 | 0.298 |
| 20th Day | 18.25 ± 1.76 | 10.00 ± 0.35 | 4.92 | 3.92 | 33.39 | 26.95 | 0.376 | 0.197 |
| 25th Day | 14.50 | 12.25 ± 1.06 | 4.44 | 4.14 | 30.29 | 28.39 | 0.286 | 0.233 |
| 30th Day | 13.50 | 15.00 ± 2.82 | 4.33 | 4.51 | 29.62 | 30.82 | 0.263 | 0.298 |

Data are presented as mean ± SD of 3 replicates

*Gp24* Gas production in 24 h, *ME* Metabolizable energy, *OMD* Organic matter digestibility, *SCFAs* Short chain fatty acids

production of SCFAs, which is based on carbohydrate fermentation and a high gas production is expected to yield higher value of SCFAs. High SCFAs production in the present study suggests that more energy was available to the ruminant in the myco-straw than unfermented wheat straw.

## Conclusion

The present findings suggest that *P. ostreatus,* an edible fungus can potentially be used for the bioconversion of wheat straw into enriched animal feed. Furthermore, the optimization of fungal inoculum in the form of pellets and minimizing the period of SSF is highly recommended for improving the animal feed.

**Acknowledgments** The authors are thankful to Dr. V.R.B. Sastry, former Senior Scientist, IVRI, Izatnagar, Prof. T. Satyanarayana, Department of Microbiology, University of Delhi South Campus, New Delhi and Ms. Urvashi Kuhad, Department of English, University of Delhi for their help during the preparation of manuscript. The financial support received from the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi, India, is highly acknowledged.

## References

Adamovic M, Grubic G, Milenkovic I, Jovanovic R, Protic R, Sretenovic L, Stoicevic L (1998) The biodegradation of wheat straw by *Pleurotus ostreatus* mushrooms and its use in cattle feeding. Anim Feed Sci Technol 71:357–362

AOAC (1995) Official methods of analysis, 16th edn. Association of Official Analytical Chemist, Airlington, VA

Arora DS, Sharma RK (2009) Enhancement in in vitro digestibility of wheat straw obtained from different geographical regions during solid state fermentation by white rot fungi. Bioresources 4:909–920

Arora DS, Chander M, Gill PK (2002) Involvement of lignin peroxidase, manganese peroxidase and laccase in degradation and selective ligninolysis of wheat straw. Int Biodet Biodeg 50:115–120

Basu S, Gaur R, Gomes J, Sreekrishnan TR, Bisaria VS (2002) Effect of seed culture on solid state bioconversion of wheat straw by *Phanerochaete chrysosporium* for animal feed production. J Biosci Bioeng 1:25–30

Bisaria R, Madan M, Vasudevan P (1997) Utilization of agroresidues as animal feed through bioconversion. Bioresour Technol 59:5–8

Blummel M, Orskov ER (1993) Comparison of in vitro gas production and nylon bag technique degradability of roughages in predicting feed intake in cattle. Anim Feed Sci Technol 40:109–119

Cullen D, Kersten PJ (1996) Enzymology and molecular biology of lignin degradation. In: Bramble R, Marzluf G (eds) The Mycota III. Springer-Verlag, Berlin, pp 297–314

Dhawan S, Kuhad RC (2003) Ethidium bromide stimulated hyper laccase production from bird's nest fungus *Cyuathus bulleri*. Lett Appl Microbiol 36:1–13

Dhawan S, Lal R, Hanspal M, Kuhad RC (2005) Effect of antibiotics on growth and laccase production from *Cyathus bulleri* and *Pycnoporus cinnabarinus*. Bioresour Technol 96:415–1418

Eichlerova I, Homolka L, Nerud F, Zadrazil F, Baldrian P, Gabriel J (2000) Screening of *Pleurotus ostreatus* isolates for their ligninolytic properties during cultivation on natural substrates. Biodegradation 11:279–287

Eriksson K-EL, Blanchette RA, Ander P (1990) Microbial and enzymatic degradation of wood and wood components. Springer, Berlin Heidelberg Newyork

Getachew G, Makkar HPS, Becker K (2002) Tropical browses: content of phenolic compounds, in vitro gas production

and stoichiometric relationship between short chain fatty acids and in vitro gas production. J Agric Sci 139:341

Goering HK, Van Soest PJ (1970) Forage fiber analysis agricultural handbook. 379. USDA, Washington D.C

Gupte A, Gupte S, Patel H (2007) Ligninolytic enzyme production under solid state fermentation by white-rot fungi. J Sci Ind Res 66:611–614

Jung HG, Vogel KP (1986) Influence of lignin on the digestibility of forage cell wall material. J Anim Sci 62: 1703–1707

Kapoor M, Nair LM, Kuhad RC (2008) Cost-effective xylanase production from free and immobilized *Bacillus pumilus* strain MK001 and its application in saccharification of *Prosopis juliflora*. Biochem Eng J 38:88–97

Kinfemi AA, Mohamed MI, Ayoade JA (2009) Biodegradation of cowpea shells by Pleurotus species for it use as ruminant feed. World J Agric Sci 5:639–645

Kuhad RC, Singh A (2007) Lignocellulose biotechnology future prospects. I. K. International Publishing House Pvt. Ltd., New Delhi

Kuhad RC, Singh A, Eriksson K-El (1997) Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol 57:45–125

Kuhar S, Nair LM, Kuhad RC (2008) Pretreatment of lignocellulosic material with fungi capable of higher lignin degradation and lower carbohydrate degradation improves substrate acid hydrolysis and the eventual conversion to ethanol. Can J Microbiol 54:305–313

Li L, Li XZ, Tang WZ, Zhao J, Qu YB (2008) Screening of a fungus capable of powerful and selective delignification of wheat straw. Lett Appl Microbiol 47:415–420

Liao W, Liu Y, Frear C, Chen S (2007) A new approach of pellet formation of a filamentous fungus—*Rhizopus oryzae*. Biores Technol 98:3415–3423

Martinez MJ, Ruiz-Duenas FJ, Guillen FA, Martinez AT (1996) Purification and catalytic properties of two manganese-peroxidase isoenzymes from *Pleurotus eryngii*. Eur J Biochem 237:424–432

Mendonca RT, Jara JF, Gonzalez V, Elissetche JP, Freer J (2008) Evaluation of the white rot fungi *Ganoderma australe* and *Ceriporiopsis subvermispora* in biotechnological applications. J Ind Microbiol Biotechnol 35:1323–1330

Menke KH, Steingass H (1988) Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen liquid. Anim Res Dev 28:7–55

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (1979) The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor. J Agric Sci 93:217–222

Miller GL (1959) Use of Dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Moyson E, Verachtert H (1991) Growth of higher fungi on wheat straw and their impact on the digestibility of the substrate. Appl Microbiol Biotechnol 36:42–424

Okano K, Boonlue S, Suzuki Y (2005) Effect of ammonium hydroxide treatment on the *in vitro* dry matter digestibility and gas production of wheat straw, sugarcane bagasse medium and konara oak rotted by edible basidiomycetes. Anim Sci J 76:147–152

Okano K, Iida Y, Samsuri M, Prasetya B, Usagava T, Watanabe T (2006) Comparision of in vitro digestibility and chemical composition among sugarcane bagasses treated by four white rot fungi. Anim Sci J 77:308–313

Okano K, Ohkoshi N, Nishiyama A, Usagawa T, Kitagawa M (2009) Improving the nutritive value of madake bamboo, *Phyllostachys bambusoides*, for ruminants by culturing with the white-rot fungus *Ceriporiopsis subvermispora*. Anim Feed Sci Technol 152(3):278–285

Paavola N, Raaska ML, Itavaara M (1990) Detection of white-rot fungi by fungi by a non-toxic strain. Mycol Res 94:27–31

Shabtay A, Hadar Y, Eitam H, Brosh A, Orlov A, Tadmor Y, Izhaki I, Kerem Z (2009) The potential of *Pleurotus* treated olive mill solid waste as cattle feed. Bioresour Technol 100:6457–6464

Sharma RK, Arora DS (2010) Changes in biochemical constituents of paddy straw during degradation by white rot fungi and its impact on *in vitro* digestibility. J Appl Microbiol 109:679–686

Suijdam JCV, Kossen NWF, Paul PG (1980) An inoculum technique for the production of fungal pellets. Eur J Appl Microbiol 10:211–221

Teymouri F, Laureano-Perez L, Alizadeh H, Dale BE (2005) Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. Bioresour Technol 96:2014–2018

Tripathi JP, Yadav JS (1992) Optimization of SSF of wheat straw into animal feed by *P. ostreatus*: A pilot effort. Anim Feed Sci Technol 37:59–72

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597

Vasdev K, Dhawan S, Kapoor RK, Kuhad RC (2005) Biochemical characterization and molecular evidences of a laccase from birds nest fungus *Cythus bulleri*. Fungal Gen Biol 42:684–693

Yadav JS, Tripathi JP (1991) Optimization of cultivation and nutrition conditions and substrate pretreatment for solid substrate fermentation of wheat straw by *Coriolus versicolor*. Folia Microbiol 36:294–301

Zadrazil F (1986) White-rot fungi and mushrooms grown on cereal straw: aim of the process, final products, scope for the future. In: Vandermeer JM, Rukens RA, Berranti MP (eds) Degradation of lignocellulosics in ruminants and in industrial processes. Institute for Livestock Feeding and Nutrition Research (IWO), Lelystad (Netherlands), pp 55–62

Zadrazil F, Puniya AK (1995) Studies on effect of particle size during solid state fermentation of sugarcane bagasse into animal feed using white-rot fungi. Bioresour Technol 54:85–87

Zafar SI, Sheeraz Q, Abdullah N (1989) Degradation of the lignocellulosic component on wheat straw—*Coriolus versicolor* solid state fermentation under nitrogen-starved conditions. Biol Waste 27:67–70