International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol 3, Issue 4, 2012, pp 790 -796
http://www.bipublication.com

# SCREENING AND SELECTION OF WHITE ROT FUNGI FOR BIOLOGICAL DELIGNIFICATION OF AGRICULTURAL RESIDUES

**[1] Sivaramaiah Nallapeta, [2] Vinod Kumar Nigam, [2]Prasanth Survajahala and [2]Krishna Mohan**

[1]Bioclues organization, IKP Knowledge Park, Secunderabad-500009
[2]Birla Institute of Scientific Research, Statue Circle, Jaipur- 302001
Corresponding author: E-mail: nallapeta@bioclues.org

[Received - 10/10/2012, Accepted - 30/11/2012]

## ABSTRACT

Rapid industrialization and population explosion are some of the major causes which has depleted and polluted the natural resources like never before. The large availability of agricultural and agro industrial residues makes it possible to use these materials for energy applications and as chemical feedstock . The major steps in the conversion system are pre-treatment and enzymatic saccharification, to convert into fermentable sugars. Delignification is necessary since the presence of lignin makes the residue more resistant to enzymatic attack. Cellulose is available in great abundance in agricultural crop residues and forestry wastes, and offers a great scope for bioconversion.

Among the biological delignification methods white-rot fungi belonging to the class Basidiomycetes are mainly used. In the present study 20 known white rot fungi were used for screening using bagasse, rice straw and wheat straw. Out of which five cultures i.e. *Phanerochaetes chrysosporium, Ganoderma applanatum, Lenzites adusta , Lenzites acuta* and *Dadela flavida* were found significant in lignin removal. Preferential substrate for all selected white rot fungi are bagasse in comparison to rice straw and wheat straw. *Phanerochaetes chrysosporium* showed maximum delignification upto 40% among all the tested isolates.

**Keywords:** White rot fungi, Delignification, Agricultural biomass.

## INTRODUCTION

The lignocellulose bioconversion is naturally slow and limited to few microorganisms, due to its complex and heterogeneous structure. The white rot fungi stand out for being especially active as lignocellulose decomposers. They are the only fungi that can take the complete lignin mineralization [1, 2]. These organisms are also able of delignifying lingocellulosic substrate selectively, modifying or degrading the lignin and transforming the lignocellulose substrate of the decomposition to high quality feed for ruminants [3], or utilizing the polysaccharides liberated by hydrolysis and fermentation, in order to produce fuels and other chemicals [4] There are few white rot fungi which can efficiently decompose lignocellulosic substrate without the need of pre-treatment. Thus a great variety of agricultural residues can be used as substrates and simultaneously favor its recycling [5]

Lignin is a heterogeneous polymer consisting of phenylpropanoid structures connected through

various covalent linkages. White rot fungi are the organisms able to mineralise lignin efficiently to carbon dioxide and water. They produce lignin modifying enzymes such as lignin peroxiases (Li-P), manganese peroxidases (MnP), laccases and glyoxal oxidase (GlOX) during the process of lignin mineralization. However, all these enzymes are not produced in single organism. Therefore, it is important to screen the organisms that remove lignin efficiently and to study their enzymatic process. These lignolytic enzymes have many biotechnological applications ranging from the production of biofuels, chemicals, and proteins, improving textiles, wood pulping and animal feeds for domesticated herbivores. Hence, our current study is focused on screening of various white rot fungi that can remove lignin using various agricultural residues.

## MATERIALS AND METHODS

### Strains and growth conditions

A total of twenty white rot fungus were procured from Forest Research Institute (FRI), Dehradun and National Centre for Industrial Microorganisms (NCIM) Pune, India. All the cultures were maintained on 2% malt extract plates and Potato dextrose agar media.

### Culture conditions

5.0 g of milled agricultural residue (<2 mm size) in 250 ml flask is mixed with 50 ml distilled water and autoclaved for one hour at $121^0$C. Twenty blocks of 5 mm dia fungal discs were inoculated into each flask aseptically and incubated at $28^0$C for 28 days. Bagasse, wheat straw and rice straw were procured from the market and used as substrates.

### Gravimetric analysis

### Determination of weight loss

Weight loss of the substrate was determined at regular intervals of time by directly weighing the substrate after drying in an oven at $60^0$C till constant weight. Percent weight loss of the substrate was determined gravimetrically. Delignification activity i.e percent lignin removal, cellulose and hemicellulose left in the residue were also determined at regular intervals by standard methods.

### Determination of different components of bagasse, wheat straw and rice straw

The contents of lignin, cellulose and hemicellulose in all the substrates were determined using the methods recommended in TAPPI 1993 [6].

### Preparation of Bagasse

Sugarcane bagasse was gathered from a local sugar mill and stored in a freezer until used. The bagasse was dried at 80°C in oven to a constant weight and then grinded to a fine powder in a wiley mill, which can pass through a 40-mesh sifter. The sieved bagasse was then stored in an air tight container after exposing to average atmospheric conditions and was used for all the experiments.

## RESULTS

Among 20 strains of fungi screened, five fungal strains viz. *Phanerochaete chrysosporium*, *Pycnoporous sangineus, Cyclomyces fabamus, Dadela flavida and Flavida flavus* were shown to be the most selective molds in delignification of rice straw, wheat straw and bagasse. However, these species showed varying delignification abilities depending upon the substrate used. Under certain culture conditions white-rot fungi use lignin preferentially, producing soluble monomers, breaking up the cellulose-hemicellulose matrix, and making the solid more susceptible to further enzymatic action.

These conditions, however, remain as yet specific for each fungus-substrate combination. All of them have enzymatic capacity to use the cellulose components as a source of carbon and energy. These make the solid-state fungal delignification process an attractive and alternative for converting lignocellulose into utilizable substances. Recent research reported on this subject has been briefly reported [7, 8] and most of the work has been done with cereal straw and very less information is available regarding utilization of rice straw. Hence in our

studies we report the results of solid-state growth of 20 basidiomycetes on rice, wheat straw and bagasse and its effect on chemical composition.

**Chemical composition of bagasse, rice straw and wheat straw**

The chemical composition and comparison of all the three substrates used in the present investigation are shown in the figures [2] and table [1]. Bagasse had 2.2 % ash which is much higher than that of wood (usually less than 1%) but, lower than that of rice and wheat straw (5.1 and 3.6% respectively). Compared with bagasse and wheat straw, rice straw had more lignin (Klason lignin) which constituted upto 18%. Cellulose, susceptible to enzymatic hydrolysis to simple sugars, represented 39% for bagasse, 34% and 37% for rice and wheat straw respectively. Maximum of 2.9% moisture content was noted in bagasse where as wheat and rice straw contained 2.1 and 1.8% respectively. It was noted that bagasse contained maximum of 1.1% nitrogen content. Rice straw contained 0.3% less than $1/4^{th}$ of that in bagasse where as wheat straw (0.6%) showed almost half the nitrogen content in comparison to bagasse. Since protein is a main component of nitrogenous constituents in plants [9] it can be deduced that rice straw contained very less protein in comparison to wheat straw and bagasse as shown below. This could explain an experimental observation that wheat straw was more easily delignified than rice straw because protein tends to condense with lignin and retard subsequent delignification [10] However, residues like rice straw and wood shavings with high lignin and low readily available nitrogen contents have slow decomposition rates [11]

**Solid state fermentation with basiodiomycetes**

The delignification process was studied using SSF and for that cultures of white rot fungi were prepared and allowed to grow on moistened baggase as solid substrate at optimal conditions [Fig 1] The gravimetric analysis (weight loss of the substrate) at regular time intervals of the investigation is shown in [Table 2]. It was observed that weight loss of the substrate (bagasse) varied markedly with fungal sps. All the tested fungi showed weight loss; an average of 25% after 28 days of incubation. Fungi showing significant weight loss i.e. more than 30 % are *Lenzites adusta, Flavida flavus, Phanerochaete chrysosporium, Sporotrichum, Ganoderma lucidium, Pleurotus ostreatus, Trametes hirsute* and *Dadela flavida.* Further five best cultures causing significant weight loss of the substrate were analyzed for their delignification ability.

**Biochemical changes in lignocellulosic fraction of bagasse, wheat and rice straw**

With the analytical data of weight loss of substrate from each fungal treatment and the corresponding yields, a mass balance of lignocellulose fraction (estimated lignin, cellulose and hemicellulose loss) was made. The resulting data are shown in table 3 for bagasse, table 4 for wheat straw and table 5 for rice straw, expressed in percent change relative to original sample values. All the five tested fungi showed lignolytic activity with an average lignin loss of 35% for bagasse, 28% for wheat and 29.6% for rice straw respectively. *Phanerochaete chrysosporium, Ganoderma lucidium* and *Pleurotus ostreatus* showed highest lignin losses with bagasse, wheat straw and rice straw respectively after 28 days of incubation at optimal conditions. Practically all fungi showed preference to degrade hemicellulose over cellulose. The ratios of hemicellulose/lignin and hemicellulose/cellulose losses were observed to be on average 1.26 & 1.17 with bagasse as substrate, 1.67 & 1.26 with wheat straw and 1.41 & 1.38 with rice straw as substrate respectively.

In lignocellulosic material, lignin forms a part of lignin-carbohydrate complex which is stabilized by phenolic acids such as ferulic acid, p-coumaric acids and acetyl constituents of the cell walls [12, 13]. Hence, it is not unexpected to have the fungi degrade about the same amount of hemicellulose and lignin. These lignin-carbohydrate complexes are usually chemically

modified during microbial attack [14] and are not recovered in the residue. According to Zabell and Morrell, 1995 [15], hemicellulose is the cell wall component that is often degraded first by white rot fungi. This is probably due to fact that hemicellulose has shorter chain and lower molecular weight compared to cellulose, higher ability to dissolve and locate at open side around cellulose microfibril. Lignin can be degraded without loss in cellulose; however hemicellulose will be simultaneously degraded. This fact implies that white-rot fungi needs carbon source that is relatively easy to be metabolized. Hence, hemicellulose/lignin (ratio) loss can be considered as important parameter to evaluate biodelignification data.

For bagasse, hemicellulose/lignin loss ratio was greater than 1 ranging from 1.2 to 1.7, with the exception of *Phanerochaete chrysoporium* which degraded more lignin than hemicellulose. In case of wheat straw four strains showed ratio greater than 1 with exception *Ganedorma lucidium* degrading more lignin than hemicellulose. However, *Dadela flavida* caused maximum hemicellulose loss in wheat straw. Whereas, using rice straw as substrate all the tested strains showed a ratio of >1 ranging from 1.4-1.9. Though, all the tested fungi degraded more hemicellulose loss than the lignin, two of the molds *Lenzites adusta* and *Dadela fl*avida caused more hemicellulose loss.

## DISCUSSION

The ranges of experimental weight losses in our results observed to be 18-36% for bagasse, 21-34% for wheat straw and 29-39% for rice straw after 28 days of incubation using five different molds. Antai and Crawford [16] showed that in 10 weeks at $28^0$C, *C. versicolor* degraded more dry matter of 53.8%, in grass. Some what higher weight losses have been reported for cereal straws and with some specific white rot fungi. Studies conducted by Breccia et al., 2004 [17] revealed that white rot fungi when incubated on long fibre sugarcane baggase, ratio of residual lignin to residual cellulose reached lower than 0.4 after 60 days of incubation indicating mainly lignin was degraded. *Strophoria rugosoannulata* and *Pleurotus cornucopiae* degraded wheat straw 60 to 65 %; *P. florida* degraded 45% and *A. aegerita* degraded wheat straw up to 25% only in 17 weeks at $30^0$C incubation [18]. *P. chrysosporium* when tested individually caused 26.45% weight loss of the substrate and 28.95% lignin loss when grown on wheat straw for six weeks, where as caused lignin loss upto 36% when combination of *P. chrysosprium* and *D. flavida* were used.

Masayuki et al., 2005 [19] reported three white rot fungi *Pleurotus ostreatus*, *Phanerochaete chrysosporium* and *Trametes versicolor* that cause 41, 21 and 37% lignin loss when grown on rice straw for 60 days at $25^0$C. Sirlene et al., 2002 [20] reported white rot fungus *Ceriporiopsis subvermispora* caused higher loss of dry weight (32%) in bagasse when incubated for 30 days with 1% inoculum under solid state fermentation. Xin Li et al., 2002 [21] reported *Phlebia* sp. can cause more than 50% lignin and 10% holocellulose loss when incubated on sugarcane bagasse, which further can be employed as a raw material in the pulp and paper industry after biopulping with *Phlebia*.

Differences in lignin and polysaccharide losses were also evident in all the tested substrates by different strains of white rot fungus. Five different isolates differed in the extent of weight loss, as well lignin and polysaccharide degradation. Kirk and Moore [22] first reported that lignin removal by white rot fungi from aspen and birch woods was always accompanied by removal of polysaccharide, although not necessarily correlated with removal of any particular fraction. In fact, lignin degradation by *Phanerochaete chrysosporium* was stimulated by addition of carbohydrates in the medium [23]. Agricultural residues contain polysaccharides which provide the energy required for lignin attack. So, during or previous to lignolysis, holocellulose degradation activity is shown. This

phenomenon has been studied by Martinez 1994 [24] using transmission electron microscopy (TEM) of wheat straw degraded by *P. chrysosporium* and *P. eryngii* and reported that in delignified areas the middle lamella was degraded, causing an extensive cell defiberization. Most of the literature on biodelignification of agricultural residues with white rot fungi till today supports that lignin loss is accompanied by polysaccharide loss, however varying loss percents depends upon the culture and incubation conditions. Our results are also no exception.

Inorganic and organic nitrogen available in the substrate or medium are quickly assimilated by the fungus and free intracellular glutamate accumulates, repressing lignolytic enzymes [25]. Reducing the nitrogen concentration in the medium greatly enhanced degradation of lignin from wheat lignocellulose by *P. chrysosporium* and *C. versicolor* [26,27]. Agricultural residues bagasse, wheat straw and rice straw known to contain less amount of nitrogen content than in wood hence our results showed some what higher delignification values than others.

## CONCLUSION

Finally comparing our data and literature data it is clear that the effects of white rot fungi on the lignocellulose matrix is a complex phenomenon controlled by many variables and their interactions. The results observed so far seem quite fungi-substrate specific, hence its very hard to define general trend and reasons. Delignification can not be predicted only by the chemical composition of the residue, the type of bonds hydrolysed, the type of new compounds synthesized by fungi but, other physical characteristics of the residue also play major roles in determining the extent and rate of degradation.

## ACKNOWLEDGEMENT

The author is thankful to Department of Science and Industrial Research, Govt of India (File no: DISR/TePP/1021/2011) for funding this research work

## REFERENCES

1. Augistine, B and Roy, G. K. [1981]. Recovery of chemical from agricultural residues: A critical review. J. Inst. Engineers. 62:1-6.
2. Moore-Landecker E [1996] Fundamentals of the fungi . Prentice Hall , New jersy, 574p.
3. Chaudhary, A.S [1998] hemical and biological procedures to upgrade cereal straws for ruminants. Nutr.Abs.Rev. 68:319-331.
4. Puniya, A. K and Singh, K. [998] Solid state fermentation of lignocellulosics, in fungi in biotechnology. CBS Publishers, NewDelhi. 177-186
5. Hadar Y., Kerem Z, Gorodecki B, Ardon O [1992] Utilisation of lignocellulose waste by edible mushroom *Pleurotus*. *Biodegradation*, **3:** 189-205.
6. Tappi Test Methods [1992-93] TAPPI, Atlanta, Georgia.
7. Ch Vijaya and Mallikarjuna Reddy [2012]. Bio-delignification ability of locally available edible mushrooms for the biological treatment of crop residues. Indian J. Biotechnol. 11:191-196
8. Jurado, M., Martinez, A.T. and Martinez M.J. [2011]. Application of white rot fungi in transformation, detoxification of agricultural wastes. Comprehensive Biotechnology. 6:595-603
9. Durzan DJ [1989] Nitrogen extractives. In: Rowe, J. W. (Ed.), Natural products of woody plants I. *Springer-Verlag*, Berlin, pp 33-61
10. Whitmore FW [1982] Lignin-protein complex in cell walls of *Pinus elliottii*: amino acid constituents. *Phytochemistry* 21: 315–318.
11. Janzen H, Kucey RM [1988] C N and S mineralization of crop residues as influenced by crop species and nutrient requirements. *Plant Soil* 106: 35-41.
12. Tanner G.R. and Morrison, I.M. [1983] Phenolic-carbohydrate complexes in the cell walls of Lolium perenne. *Phytochemistry* 22:1433-1439.
13. Chesson, A., Gordon, A.H and Lomax, J. [1983] Substituent groups linked by alkali-labile bonds to arabinose and xylose residues of legume, grass and cereal straw cell walls and their fate during digestion by rumen microorganisms. *J. Sci. Food Agric*. 34:1330-1340.
14. Gaillard B.D and Richards, G.N [1975] Presence of soluble lignin-carbohydrate complexes in the bovine rumen. *Carbohydr. Res*. 42:135-145.
15. Zabell RA and Morell J [1995] Decay and its Prevention, J. Wood Microbiology: *Academic Press Inc*., California.

16. Antai SP, and LD Crawford [1982] Degradation of extractive-free lignocellulose by Coriolus versicolor and Poria placenta. *Eur. J. Appl. Microbiol. Biotechnol*. 14:165-168.
17. Breccia JD, Siñeriz. F, Bettucci L and Piaggio M . [2004] Degradation of sugarcane by several white rot fungi. *Acta Biotechnologica*. 17: 177-184
18. Zadrazil F, and Brunnert H. [1980] The influence of ammonium nitrate supplementation on degradation and in vitro digestibility of strain colonized by higher fungi. *Eur. J. Appl. Microbiol. Biotechnol*. 9:37-44.
19. Mayasuki Taniguchi, Suzuki H, Watanabe D, Sakai K, and Tanaka T. [2005] Evaluation of Pretreatment with *Pleurotus ostreatus* for Enzymatic Hydrolysis of Rice Straw. *Journal of Bioscience and Bioengineering* 100: 637-642.
20. Sirlene M Costa, Gonçalves A and Elisa Esposito [2004] Symposium on Biotechnology for *Fuels and Chemicals*, Chattanooga, US.
21. Xin Li, Kundo R and Sakkai K. [2002] Biodegradation of sugarcane with marine fungus *Phlebia* sp. MG-60. *Journal of Wood Science* 48: 159-162.
22. Kirk T. K and Moore W. E. [1972] Removing lignin from wood with white rot fungi and digestibility of resulting wood. *Wood Fiber* 4: 72-79
23. Maijala P. [2000] Enzymology of cellulose, hemicellulose and lignin degradation. *PhD dissertation*, University of Helsniki.
24. Martinez, T. A. Susana, C, Guillen, F., Varela, E., Martinez, J., Jose, M. B. and Katia, R. [1994]. Progress in Biopulping on non-woody materials: Chemical, enzymatic and ultrastructural aspects of wheat straw delignification with lignolytic fungi from the genus *Pleurotus FEMS Microbiology Reviews* **13**:265-274.
25. Leisola MS, Ulmer DC and Fletcher A. [1994] Factors affecting lignin degradation in lignocellulose by *Phanerochaete chrysosporium. Arch. Microbiol*. 137: 171-175
26. Ander P, Eriksson and Yu HS. [1983] Physiological requirements for degradation of lignin related substances by *Sporotrichum pulveruletum*. *Eur. J. Appl. Microbiol. Biotechnol.* 18: 374-380.
**27.** Kenealy WR, Diane M and Dietrich [2004] Growth response of *Phanerochaete chrysosporium* to oxygen limitation. *Enyzme and Microbial Technology*. 34: 490-498.

**Table 1:** Comparative chemical composition of various agriculture residues

| Constituent (%) | Bagasse | Rice straw | Wheat straw |
|---|---|---|---|
| Ash | 2.2 | 5.1 | 3.6 |
| Cellulose | 39 | 34 | 37 |
| Hemicellulose | 29 | 25 | 31 |
| Lignin | 16 | 18 | 14 |
| Moisture content | 2.9 | 1.8 | 2.1 |
| Nitrogen content | 1.1 | 0.3 | 0.6 |
| Crude protein (nitrogen x 6.25) | 6.87 | 1.875 | 3.75 |

**Table 2:** Gravimetric analysis of bagasse after treatment with various white rot fungi

| Name of White rot fungi | % weight loss of bagasse | | | |
|---|---|---|---|---|
| | 7d | 14d | 21d | 28d |
| *Daedalea radus* | 16 | 20 | 23 | 25 |
| *Hexagonia leuas* | 16 | 17 | 20 | 27 |
| *Trametes cingulata* | 16 | 20 | 25 | 28 |
| *Lenzites adusta* | 12 | 16 | 26 | 30 |
| *Pleurotus ostreatus* | 12 | 22 | 24 | 32 |
| *Trametes bisogenum* | 8 | 14 | 16 | 18 |
| *Pleurotus ascutaria* | 9 | 16 | 21 | 24 |
| *Flavida flavus* | 7 | 17 | 22 | 30 |
| *Polyporous xanthopus* | 6 | 13 | 18 | 21 |
| *Polyporus falipirae* | 8 | 12 | 23 | 27 |
| *Ganoderma applanatum* | 14 | 16 | 17 | 30 |
| *Polystictus paragamus* | 8 | 13 | 18 | 24 |
| *Trametes biforme* | 10 | 12 | 22 | 30 |
| *Cyclomyces fabamus* | 5 | 8 | 12 | 20 |
| *Phanerochaete chrysosporium* | 13 | 20 | 26 | 36 |
| *Ganoderma lucidium* | 15 | 22 | 25 | 29 |
| *Myrothecium veruccaria* | 12 | 15 | 20 | 22 |
| *Sporotricum sp.* | 13 | 19 | 28 | 35 |
| *Trametes hirsute* | 16 | 28 | 30 | 34 |
| *Daedela flavida* | 10 | 20 | 26 | 31 |
| *Phoma exigua* | 6 | 10 | 12 | 18 |

*Control values are for uninoculated material received the same sterilization proess and incubation

**Table 3:** Biochemical changes in the lignocellulose fraction of bagasse after 28d.

| White rot fungi | Cellulose loss | Lignin loss | Hemicellulose loss | H/L ratio | H/C ratio |
|---|---|---|---|---|---|
| *P. chrysosporium* | 30.1 | 40.5 | 35.8 | 0.88 | 1.18 |
| *L. adusta* | 48 | 34.5 | 50.4 | 1.46 | 1.05 |
| *P. ostreatus* | 54.1 | 32 | 40 | 1.25 | 0.75 |
| *G. applanatum* | 35.1 | 33.1 | 56 | 1.69 | 1.59 |
| *D. flavida* | 32 | 36 | 41 | 1.32 | 1.28 |
| Average | 39.86 | 35.22 | 44.64 | 1.26 | 1.17 |

**Table 4:** Biochemical changes in the lignocellulose fraction of wheat straw after 28d.

| White rot fungi | Cellulose loss | Lignin loss | Hemicellulose loss | H/L | H/C |
|---|---|---|---|---|---|
| *P. chrysosporium* | 38 | 32 | 34 | 1.06 | 0.89 |
| *L. adusta* | 50 | 24 | 43 | 1.79 | 0.86 |
| *P. ostreatus* | 29 | 28 | 51 | 1.82 | 1.75 |
| *G. applanatum* | 41 | 36 | 33 | 0.91 | 0.8 |
| *D. flavida* | 28 | 20 | 56 | 2.8 | 2.0 |
| Average | 37.2 | 28 | 43.4 | 1.67 | 1.26 |

**Table: 5** Biochemical changes in the lignocellulose fraction o straw after 28d.

| White rot fungi | Cellulose loss | Lignin loss | Hemicellulose loss | H/L | H/C |
|---|---|---|---|---|---|
| *P. chrysosporium* | 26.2 | 29 | 33.1 | 1.14 | 1.26 |
| *L. adusta* | 36 | 26 | 50 | 1.92 | 1.38 |
| *P. ostreatus* | 48 | 36 | 43 | 1.2 | 0.89 |
| *G. applanatum* | 51 | 31 | 44 | 1.41 | 0.86 |
| *D. flavida* | 20 | 26 | 51 | 1.9 | 2.55 |
| Average | 36.2 | 29.6 | 44.22 | 1.41 | 1.38 |

Loss of cellulose, lignin and hemicellulose is the % loss
H/L: Hemicellulose/Lignin ratio
H/C: Hemicellulose/Cellulose ratio

**Fig 1:** Solid-state delignification of agricultural residues


(A: Control, B: Inoculated with *P.chrysosporium*).

**Fig: 2** Chemical Composition of various agricultural residues used in the study