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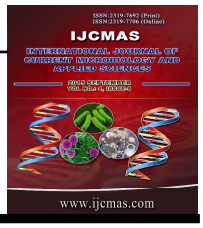

Original Research Article

Biological Pretreatment of Agroresidues with Lignolytic Fungi for Delignification and Recovery of Cellulose and Hemicellulose

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Cellulose, Hemicellulose, Lignin, Lignolytic fungi, Pretreatment

A B S T R A C T

Five agro-residues namely sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk and corn stover with particle sizes of 0.5 mm, 1.0 mm and 10.0 mm were subjected for pretreatments with different lignolytic fungi. The pretreatment with *Phanerochaete chrysosporium* resulted in maximum weight loss in 0.5 mm particle size at the end of 30 days incubation. This fungi produced the cellulose yield 0.427, 0.407, 0..400, 0.413 and 0.413 (g/g) in sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk and corn stover, respectively with particles size of 0.5 mm in 30 days incubation period however did not show much increase in hemicelluloses content. The fungi *Pleurotus florida* had resulted in a maximum reduction in lignin content between 28.57 and 30.07% in the particles of size 0.5 mm in the substrates.

Introduction

Biomass is a sustainable alternative to fossil energy sources which are used to produce fuels, electricity, chemicals, and other goods. At the moment, the main biobased products are obtained by the conversion of biomass to basic products like starch, oil and cellulose. Recovery of cellulose is a critical process from the biomass in order to be subjected for bioethanol production. The recalcitrant nature of the lignocellulose where the lignin surrounds cellulose and hemicellulose makes the abundantly available agricultural biomass, a difficult raw material for the recovery of cellulose and hemicelluloses (Mosier *et al.*, 2005).

Pre-treatment is required to alter the biomass of its macroscopic and microscopic size, structure as well as its submicroscopic chemical composition, so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields. Several pretreatment methods have been used for effective delignification and recovery of cellulose and hemicelluloses sugars from various substrates *viz.* physical,

chemical (Shankarappa and Geeta, 2013), combination of physical and chemical (Soderstrom *et al.,* 2003; Shankarappa and Geeta, 2013) and biological pretreatments (Valmaseda *et al.,* 1991). In recent years, biological pre-treatment has received emphasis because of its advantages such as its low cost and environmental compatibility to degrade plant cell materials. Several microorganisms are capable of degrading Lignin through their multiple enzymes systems and make the plant biomass accessible to saccarification of polysaccharides (Rodríguez *et al.,* 2003; Valaskova *et al.,* 2007). Therefore, the abundantly available agroresidues which are outside the human food *viz.* sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk and corn stover were subjected for biological pretreatment with lignin degrading fungi to achieve delignification and recovery of cellulose and hemicellulose sugars.

Materials and Methods

Five fresh substrates *viz.*, sugarcane bagasse (procured from Malaprabha sahakari sugar factory, M. K. Hubli, Belgaum, sugarcane trash (Co-8014) and sugarcane tops (Co-8014) from the fields of Mr. Basavaraj, Yettinagudda, Dharwad, Karnataka and corn stover (Arjun) and corn husk (Arjun) from Main Agricultural Research Station, University of Agricltural Sciences, Dharwad, Karnataka were brought to the laboratory, chopped into small pieces, dried at 60^0C in a hot air oven for 12 h and powdered by dry milling (Willey mill) to obtain different particle sizes of 0.5 mm, 1.0 mm and 10 mm (Karimi *et al.,* 2006) and were subjected for biological pretreatment with three efficient lignolytic white rot fungi cultures namely *Phanerochaete chrysosporium, Pleurotus florida* and UASD-LF1.

Ten grams of oven dried substrates of three particle sizes were transferred separately in to 250ml capacity Erlenmeyer flasks. Each substrate was wetted with nutrient solution on the basis of its water holding capacity (approximately @ 5.0 ml g^{-1} dry substrate). The nutrient solution consisted of $(NH_4)_2 SO_4$ (10.0 g^{-1}L), KH_2PO_4 (3.0 g^{-1}L), $MgSO_4 7H_2O$ (0.50 g^{-1}L) and $CaCl_2 H_2O$ (0.50 g^{-1} L), (Singhania *et al.,* 2006; Valaskova *et al.,* 2007). The lignolytic cultures maintained on PDA were used as inoculums for the substrate pre-treatment. The respective fungal cultures were grown on liquid potato dextrose broth, 50 ml in 150 ml Erlenmeyer flasks for five days on rotary shaker (150 rpm) at 28 ± 1^0 C. The mycelia was homogenised and the homogenate having 20 × 10^8 CFU per ml was inoculated to the substrates aseptically @ 5.0% and incubated for 10, 20 and 30 days at 28 ± 1^0 C (Valmaseda *et al.,* 1991). One un-inoculated control also was maintained for all the substrates and all the particle sizes. After an interval of 10, 20 and 30 days solid state fermentation, the growth of fungi was arrested by autoclaving at 121°C for 15 min. The residues obtained after the treatments were dried in a hot air oven at 60°C to constant weight and the cellulose, hemicelluloses and lignin contents were estimated by determining Neutral detergent fibre, Acid detergent fibre and Acid detergent lignin as per method described by Goerging and Van Soest (1975). The cellulose, hemicellulose and lignin contents were expressed in grams. The treatments were replicated three times and the data was statistically analyzed using factorial, completely randomized design.

Results and Discussion

The five agro-residues selected for bioethanol production studies differed in their composition of cellulose,

hemicelluloses and lignin contents (Table 1). Sugarcane bagasse contained the highest cellulose (0.353 g g^{-1}) and hemi cellulose (0.293 g g^{-1}), whereas corn stover was found to contain the lowest cellulose content of 0.327 g per g. Sugarcane tops had the lowest hemi cellulose content of 0.227 g per g. The highest lignin content of 0.160 g per g was observed in sugarcane trash and the lowest lignin content of 0.140 g per g both in sugarcane bagasse and corn husk (Shankarappa and Geeta, 2013). The difference in chemical composition of the agro-residues is due to variation in the composition of cell wall as it varies with plant species, tissue type and, region within the cell wall and development stages of the cell wall (Carpita and Mc Cann, 2000).

Loss in weight

With regards to weight loss, the inoculation of lignolytic fungi showed significant differences at different incubation periods (Table 4). The mean maximum loss of weight was observed with *P. chrysosporium* at 30 days of incubation period with a value of 0.125 g per g. It was found to be on par with *P. florida* (0.124 g g^{-1}) and significantly superior over UASD-LF1 (0.119 g g^{-1}) at 30 days incubation period. The weight loss observed at 30 days incubation was found to be significantly superior over 20 days and 10 days incubation in all the lignolytic fungi. Among the different substrates pre-treated with lignolytic fungi, corn husk showed the significantly highest mean maximum weight loss, 0.093 g per g. The sugarcane bagasse with mean weight loss of 0.054 g per g resulted in lowest weight loss. All the particle sizes of the substrates exhibited significant differences with each other in mean weight loss. The particle size 0.50 mm recorded significantly maximum mean weight loss of 0.095 g per g as compared to 1.0 mm (0.085 g g^{-1}) and 10.0 mm (0.066 g g^{-1}) particle size. The loss in weight of the substrates is attributed to non selective and rapid degradation of the organic components by the lignolytic fungi (Kerem *et al.*, 1992).

Cellulose content

The inoculation of lignolytic fungi influenced the cellulose content (Table 3). The mean maximum cellulose content (0.396 g g^{-1}) was observed with inoculation of *P. chrysosporium*, followed by both *P. florida* and UASD-LF1 (0.393 g g^{-1}) at 30 days incubation. All the three lignolytic fungi were found to be on par with each other at 30 days incubation and significantly superior over 20 days and 10 days incubation with regards to cellulose content.

The cellulose content was found to vary differently with different substrates. The mean maximum cellulose content of 0.377 g per g was noticed in sugarcane bagasse. It was observed to be significantly superior over other substrates. The rest of the substrates with a range of maximum (0.367 g g^{-1}) and minimum (0.363 g g^{-1}) cellulose content found to be on par with each other. The particle size of substrates indicated significant variation in cellulose content. Mean maximum cellulose content, 0.376 g per g was observed with 0.50 Mm particle size and it was significantly superior over particle size of 1.0 mm (0.368 g g^{-1}) and 10.00 mm (0.356 g g^{-1}). The interaction between lignolytic fungi and particle size of substrates showed significant variations in cellulose content. The significantly highest cellulose content of 0.412 g per g was recorded with inoculation of *P. chrysosporium* in 0.50 mm particle size substrates. The trend of increase in cellulose content was observed with decrease in particle size, where particle size of 0.5 mm was found to contain more cellulose than particles size of 1.0 mm and 10.0 mm.

The inoculation of *Phanerochaete chrysosporium* fungi produced the cellulose yield of 0.427, 0.407, 0..400, 0.413 and 0.413 in sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk and corn stover respectively with particles size of 0.50 mm in 30 days incubation period.

The observed per cent increase in cellulose content were 20.96, 22.22, 16.62 24.02 and 26.69% respectively with sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk and corn stover. The increase in cellulose content with inoculation of lignolytic fungi was because of the degradation of lignin to carbon dioxide and water (Kirk and Farrel, 1987), as the white rot fungi possess many kinds of lignin degrading enzymes and release cellulose and hemicellulose during delignification process (Buswell *et al.,* 1995; Viswanath *et al.,* 2008). Although part of the cellulose is used up by the fungi for the synthesis of cellular material.

Hemicellulose content

The pre-treatment of substrates with different lignolytic fungi indicated significant variations in hemicellulose content (Table 4). The mean maximum hemicellulose content (0.274 g g^{-1}) was observed with *P. florida* inoculated substrates at the end of 30 days incubation. All the lignolytic fungi cultures were found to be on par in hemicellulose content at all incubation periods except in case of *P. chrysosporium*, which showed significantly less hemicellulose content (0.264 g g^{-1}) at 10 days incubation when compared to *P. florida* (0.272 g g^{-1}) at 30 days incubation.

The different substrates differed significantly due to microbial pre-treatment in hemicellulose content. The significantly highest mean hemicellulose content (0.300 g g^{-1}) was observed in sugarcane bagasse. It was highly superior over corn stover (0.264 g g^{-1}), corn husk (0.263 g g^{-1}), sugarcane trash (0.254 g g^{-1}) and sugarcane tops (0.244 g g^{-1}). The particle size of the substrates also indicated significant variations in hemicellulose content. The mean maximum hemicellulose content, 0.269 g per g was observed with 0.50 mm particle size. It was found to be significantly superior over particle sizes of 1.0 mm (0.264 g g^{-1}) and 10.0 mm (0.261 g g^{-1}). The different pretreated substrates showed zero to 11.60 per cent increase in hemicellulose for lignolytic fungi inoculation where lignin degrading enzymes release hemicellulose during delignification process (Leontievsky *et al.,* 1997).

Lignin content

The lignin content in the substrates was found to decrease due to fungal cultures (Table 5). The mean minimum 0.115 g per g lignin content was seen with *P. florida* and it was found to be on par with *P. chrysosporium* (0.116 g g^{-1}) and UASD-LF1 (0.117 g g^{-1}) at 30 days incubation period and it was significantly lower when compared to 20 days incubation with all the fungal cultures.

The lignin content between the substrates also varied significantly. The mean maximum of 0.142 g per g lignin content was observed with sugarcane trash and it was on par with sugarcane tops (0.139 g g^{-1}) and significantly superior over rest of the substrates. The lowest mean lignin content, 0.123 g per g was recorded in both sugarcane bagasse and corn husk. The mean lignin content in particle size of 0.50 mm was found to be lowest (0.126 g g^{-1}) when compared to 1.0 mm (0.131 g g^{-1}) and 10.0 mm (0.138 g g^{-1}).

Table.1 Initial chemical composition of agro-residues used for bioethanol production studies

Sl. No	Substrate	Cellulose (g g^{-1})	Hemicellulose (g g^{-1})	Lignin (g g^{-1})
1	Sugarcane bagasse	0.353	0.293	0.140
2	Sugarcane tops	0.333	0.227	0.157
3	Sugarcane trash	0.343	0.247	0.160
4	Corn husk	0.333	0.253	0.140
5	Corn stover	0.327	0.260	0.153

Table.2 Effect of lignolytic fungi on weight loss of substrates

Lignolytic fungi	Particle size (mm)	Substrates (g g^{-1})					Mean
		Sugarcane bagasse	Sugarcane tops	Sugarcane trash	Corn husk	Corn stover	
Phanerochaete chrysosporium (10 days)	0.5	0.052	0.130	0.123	0.135	0.125	**0.099**
	1.0	0.049	0.107	0.115	0.115	0.124	
	10.0	0.032	0.085	0.088	0.109	0.102	
Pleurotus florida (10 days)	0.5	0.061	0.126	0.103	0.131	0.121	**0.092**
	1.0	0.054	0.099	0.091	0.117	0.107	
	10.0	0.035	0.080	0.070	0.099	0.085	
UASD-LF1 (10 days)	0.5	0.053	0.121	0.095	0.109	0.100	**0.081**
	1.0	0.045	0.097	0.086	0.093	0.092	
	10.0	0.029	0.077	0.061	0.083	0.079	
Control (10 days)	0.5	0.010	0.007	0.010	0.010	0.010	**0.008**
	1.0	0.010	0.010	0.010	0.010	0.010	
	10.0	0.002	0.007	0.003	0.003	0.001	
Phanerochaete chrysosporium (20 days)	0.5	0.064	0.117	0.142	0.139	0.136	**0.109**
	1.0	0.063	0.109	0.133	0.128	0.128	
	10.0	0.039	0.098	0.108	0.117	0.111	
Pleurotus florida (20 days)	0.5	0.101	0.139	0.139	0.132	0.126	**0.108**
	1.0	0.081	0.117	0.128	0.117	0.112	
	10.0	0.058	0.087	0.096	0.101	0.087	
UASD-LF1 (20 days)	0.5	0.104	0.137	0.140	0.127	0.125	**0.108**
	1.0	0.084	0.123	0.127	0.113	0.113	
	10.0	0.061	0.082	0.097	0.099	0.081	
Control (20 days)	0.5	0.010	0.007	0.010	0.010	0.010	**0.008**
	1.0	0.010	0.010	0.010	0.010	0.010	
	10.0	0.002	0.007	0.003	0.003	0.001	
Phanerochaete chrysosporium (30 days)	0.5	0.095	0.151	0.159	0.159	0.144	**0.125**
	1.0	0.079	0.137	0.150	0.143	0.139	
	10.0	0.059	0.113	0.095	0.127	0.126	
Pleurotus florida (30 days)	0.5	0.129	0.142	0.156	0.150	0.141	**0.124**
	1.0	0.100	0.134	0.146	0.137	0.129	
	10.0	0.074	0.099	0.101	0.118	0.111	
UASD-LF1 (30 days)	0.5	0.117	0.143	0.152	0.147	0.137	**0.119**
	1.0	0.093	0.128	0.139	0.133	0.120	
	10.0	0.063	0.103	0.097	0.116	0.096	
Control (30 days)	0.5	0.010	0.007	0.010	0.010	0.010	**0.008**
	1.0	0.010	0.010	0.010	0.010	0.010	
	10.0	0.002	0.007	0.003	0.003	0.001	
Mean		**0.054**	**0.088**	**0.089**	**0.093**	**0.088**	
Mean	0.5			0.095			
	1.0			0.085			
	10.0			0.066			

	SE±	CD 1 %
Lignolytic fungi (A)	0.001	0.004
Substrates (B)	0.001	0.002
Particle size (C)	0.000	0.002
A x B	0.002	0.008
A x C	0.002	0.006
B x C	0.001	0.004
A x B x C	0.004	0.014

Table.3 Effect of lignolytic fungi on cellulose content of substrates

Lignolytic fungi	Particle size (mm)	Substrates (g g^{-1})					Mean
		Sugarcane bagasse	Sugarcane tops	Sugarcane trash	Corn husk	Corn stover	
Phanerochaete chrysosporium (10 days)	0.5	0.380	0.360	0.367	0.373	0.360	**0.358**
	1.0	0.367	0.360	0.360	0.360	0.347	
	10.0	0.360	0.340	0.347	0.347	0.340	
Pleurotus florida (10 days)	0.5	0.380	0.367	0.367	0.367	0.360	**0.357**
	1.0	0.367	0.360	0.360	0.360	0.347	
	10.0	0.353	0.340	0.340	0.347	0.340	
UASD-LF1 (10 days)	0.5	0.380	0.357	0.360	0.367	0.360	**0.355**
	1.0	0.367	0.360	0.347	0.360	0.353	
	10.0	0.360	0.337	0.340	0.347	0.337	
Control (10 days)	0.5	0.353	0.333	0.340	0.330	0.323	**0.336**
	1.0	0.353	0.333	0.337	0.330	0.333	
	10.0	0.350	0.333	0.340	0.330	0.327	
Phanerochaete chrysosporium (20 days)	0.5	0.400	0.387	0.387	0.403	0.387	**0.381**
	1.0	0.393	0.377	0.380	0.380	0.380	
	10.0	0.377	0.373	0.363	0.370	0.363	
Pleurotus florida (20 days)	0.5	0.403	0.397	0.390	0.403	0.400	**0.384**
	1.0	0.397	0.390	0.377	0.387	0.387	
	10.0	0.377	0.357	0.357	0.370	0.363	
UASD-LF1 (20 days)	0.5	0.397	0.387	0.387	0.397	0.403	**0.383**
	1.0	0.390	0.383	0.377	0.390	0.387	
	10.0	0.377	0.357	0.363	0.377	0.373	
Control (20 days)	0.5	0.353	0.333	0.340	0.330	0.323	**0.336**
	1.0	0.353	0.333	0.337	0.330	0.333	
	10.0	0.350	0.333	0.340	0.330	0.327	
Phanerochaete chrysosporium (30 days)	0.5	0.427	0.407	0.400	0.413	0.413	**0.396**
	1.0	0.413	0.393	0.387	0.400	0.393	
	10.0	0.387	0.377	0.373	0.383	0.367	
Pleurotus florida (30 days)	0.5	0.413	0.413	0.410	0.413	0.407	**0.393**
	1.0	0.400	0.400	0.387	0.400	0.393	
	10.0	0.390	0.367	0.367	0.367	0.367	
UASD-LF1 (30 days)	0.5	0.413	0.400	0.400	0.407	0.410	**0.393**
	1.0	0.400	0.387	0.393	0.397	0.397	
	10.0	0.380	0.367	0.377	0.377	0.390	
Control (30 days)	0.5	0.343	0.323	0.330	0.323	0.317	**0.329**
	1.0	0.343	0.323	0.327	0.317	0.323	
	10.0	0.340	0.330	0.340	0.327	0.327	
Mean		**0.377**	**0.363**	**0.364**	**0.367**	**0.363**	
Mean	0.5			0.376			
	1.0			0.368			
	10.0			0.356			

	SE±	CD 1 %
Lignolytic fungi (A)	0.003	0.009
Substrates (B)	0.002	0.006
Particle size (C)	0.001	0.005
A x B	0.006	NS
A x C	0.004	0.016
B x C	0.003	NS
A x B x C	0.010	NS

Table.4 Effect of lignolytic fungi on hemicellulose content of substrates

Lignolytic fungi	Particle size (mm)	Substrates (g g^{-1})					Mean
		Sugarcane bagasse	Sugarcane tops	Sugarcane trash	Corn husk	Corn stover	
Phanerochaete chrysosporium (10 days)	0.5	0.303	0.243	0.257	0.267	0.273	**0.264**
	1.0	0.303	0.230	0.250	0.260	0.260	
	10.0	0.303	0.230	0.253	0.260	0.260	
Pleurotus florida (10 days)	0.5	0.300	0.253	0.260	0.267	0.273	**0.266**
	1.0	0.300	0.250	0.253	0.260	0.260	
	10.0	0.303	0.243	0.253	0.253	0.263	
UASD-LF1 (10 days)	0.5	0.307	0.260	0.273	0.273	0.273	**0.269**
	1.0	0.303	0.253	0.260	0.267	0.260	
	10.0	0.297	0.240	0.247	0.253	0.263	
Control (10 days)	0.5	0.293	0.223	0.240	0.260	0.250	**0.252**
	1.0	0.290	0.223	0.240	0.250	0.253	
	10.0	0.293	0.223	0.237	0.253	0.257	
Phanerochaete chrysosporium (20 days)	0.5	0.307	0.260	0.267	0.267	0.273	**0.269**
	1.0	0.303	0.253	0.260	0.260	0.267	
	10.0	0.303	0.237	0.253	0.263	0.267	
Pleurotus florida (20 days)	0.5	0.313	0.263	0.267	0.273	0.280	**0.270**
	1.0	0.300	0.247	0.253	0.267	0.267	
	10.0	0.297	0.250	0.247	0.260	0.260	
UASD-LF1 (20 days)	0.5	0.307	0.260	0.267	0.267	0.273	**0.270**
	1.0	0.300	0.263	0.260	0.270	0.267	
	10.0	0.303	0.247	0.253	0.257	0.260	
Control (20 days)	0.5	0.293	0.223	0.240	0.260	0.250	**0.252**
	1.0	0.290	0.223	0.240	0.250	0.253	
	10.0	0.293	0.223	0.237	0.253	0.257	
Phanerochaete chrysosporium (30 days)	0.5	0.307	0.260	0.267	0.267	0.283	**0.270**
	1.0	0.307	0.253	0.257	0.260	0.267	
	10.0	0.300	0.247	0.250	0.260	0.270	
Pleurotus florida (30 days)	0.5	0.310	0.260	0.273	0.267	0.283	**0.272**
	1.0	0.303	0.253	0.267	0.270	0.267	
	10.0	0.297	0.247	0.260	0.263	0.260	
UASD-LF1 (30 days)	0.5	0.310	0.260	0.273	0.280	0.280	**0.274**
	1.0	0.303	0.260	0.267	0.273	0.273	
	10.0	0.297	0.247	0.253	0.267	0.267	
Control (30 days)	0.5	0.283	0.217	0.230	0.253	0.240	**0.250**
	1.0	0.280	0.223	0.240	0.260	0.253	
	10.0	0.293	0.223	0.237	0.260	0.257	
Mean		**0.300**	**0.244**	**0.254**	**0.263**	**0.264**	
Mean	0.5			0.269			
	1.0			0.264			
	10.0			0.261			

	SE±	CD 1 %
Lignolytic fungi (A)	0.002	0.007
Substrates (B)	0.001	0.005
Particle size (C)	0.001	0.004
A x B	0.004	NS
A x C	0.003	NS
B x C	0.002	NS
A x B x C	0.008	NS

Table.5 Effect of lignolytic fungi on lignin content of substrates

Lignolytic fungi	Particle size (mm)	Substrates (g g^{-1})					Mean
		Sugarcane bagasse	Sugarcane tops	Sugarcane trash	Corn husk	Corn stover	
Phanerochaete chrysosporium (10 days)	0.5	0.117	0.137	0.133	0.120	0.133	**0.134**
	1.0	0.120	0.143	0.137	0.127	0.140	
	10.0	0.133	0.150	0.147	0.133	0.147	
Pleurotus florida (10 days)	0.5	0.120	0.133	0.133	0.120	0.133	**0.134**
	1.0	0.120	0.143	0.140	0.133	0.133	
	10.0	0.127	0.153	0.150	0.130	0.140	
UASD-LF1 (10 days)	0.5	0.120	0.140	0.140	0.120	0.133	**0.136**
	1.0	0.127	0.143	0.147	0.127	0.140	
	10.0	0.133	0.150	0.153	0.133	0.140	
Control (10 days)	0.5	0.140	0.157	0.160	0.140	0.153	**0.150**
	1.0	0.140	0.157	0.160	0.140	0.153	
	10.0	0.140	0.157	0.160	0.140	0.153	
Phanerochaete chrysosporium (20 days)	0.5	0.107	0.133	0.140	0.113	0.127	**0.130**
	1.0	0.120	0.140	0.140	0.120	0.133	
	10.0	0.120	0.140	0.147	0.127	0.140	
Pleurotus florida (20 days)	0.5	0.107	0.123	0.120	0.107	0.107	**0.123**
	1.0	0.113	0.127	0.133	0.113	0.120	
	10.0	0.127	0.140	0.147	0.127	0.133	
UASD-LF1 (20 days)	0.5	0.113	0.127	0.127	0.107	0.120	**0.127**
	1.0	0.120	0.133	0.140	0.113	0.120	
	10.0	0.127	0.140	0.147	0.133	0.140	
Control (20 days)	0.5	0.140	0.157	0.160	0.140	0.153	**0.150**
	1.0	0.140	0.157	0.160	0.140	0.153	
	10.0	0.140	0.157	0.160	0.140	0.153	
Phanerochaete chrysosporium (30 days)	0.5	0.100	0.113	0.120	0.100	0.107	**0.116**
	1.0	0.107	0.120	0.120	0.103	0.113	
	10.0	0.120	0.133	0.140	0.113	0.127	
Pleurotus florida (30 days)	0.5	0.100	0.113	0.113	0.100	0.100	**0.115**
	1.0	0.107	0.120	0.120	0.107	0.107	
	10.0	0.120	0.133	0.140	0.120	0.120	
UASD-LF1 (30 days)	0.5	0.100	0.113	0.123	0.100	0.107	**0.117**
	1.0	0.107	0.120	0.127	0.113	0.113	
	10.0	0.120	0.133	0.140	0.113	0.127	
Control (30 days)	0.5	0.140	0.157	0.160	0.140	0.153	**0.150**
	1.0	0.140	0.157	0.160	0.140	0.153	
	10.0	0.140	0.157	0.160	0.140	0.153	
Mean		**0.123**	**0.139**	**0.142**	**0.123**	**0.133**	
Mean	0.5			0.126			
	1.0			0.131			
	10.0			0.138			

	SE±	CD 1 %
Lignolytic fungi (A)	0.002	0.008
Substrates (B)	0.001	0.005
Particle size (C)	0.001	0.004
A x B	0.005	NS
A x C	0.004	NS
B x C	0.002	NS
A x B x C	0.008	NS

The inoculation of lignolytic fungi showed the highest reduction between 28.57 and 30.07 for the particle size 0.50 mm, 23.57 and 30.07 for 1.00 mm and 12.50 and 19.29% for 10.0 mm with *P. florida* in 30 days incubation. All the lignolytic fungi showed similar range of lignin reduction in 30 days incubation. The reduction in lignin content was accomplished by different lignin degrading enzymes secreted by the

fungi (Rodríguez *et al.*, 2003). The variations in lignin reduction observed with different substrates could be due to differential lignin content in the raw substrates.

References

Buswell, J.A., Cai, Y., Chang, S. 1995. Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by *Lentinula* (Lentinus) *edodes*. *FEMS Microbiol. Lett.*, 128(1): 81–88.

Carpita, N., McCann, M. 2000. The cell wall. In: Biochemistry and molecular biology of plants. Buchanan, B.B., Gruissem, W., Jones, R.L. (Eds), American Society of Plant Physiologists, Rockville, Maryland. Pp. 52–108.

Goerging, H.D., Van Soest, J.P. 1975. Forage fibre analysis, US Department of Agriculture, Agric. Res. Ser., Washington, DC.

Karimi, K., Kheradmandinia, S., Taherzadeh, M.J. 2006. Conversion of rice straw to sugars by dilute acid hydrolysis. *Biomass Bioenergy*, 30: 247–253.

Kerem, Z., Friesem, D., Hadar, Y. 1992. Lignocellulose degradation during solid-state fermentation: *Pleurotus ostreatus* versus *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.*, 58: 1121–1127.

Kirk, T.K., Farell, R.L. 1987. Enzymatic "combustion": The microbial degradation of lignin. *Ann. Rev. Microbiol.*, 41(3): 465–505.

Leontievsky, A., Myasoedova, N., Pozdnyakova, N., Golovleva, L. 1997. 'Yellow' laccase of *Panus tigrinus* oxidises non-phenolic substrates without electron-transfer mediators. *FEBS Lett.*, 413(3): 446–448.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pre-treatment of lignocellulosic biomass. *Biores. Technol.*, 96(6): 673–686.

Rodríguez, C.S., Rodríguez, R., Gallego, P.P., Sanromán, A. 2003. Biodegradation of grape cluster stems and ligninolytic enzyme production by *Phanerochaete chrysosporium* during Semi-Solid-State cultivation. *Acta Biotechnologica*, 23(1): 65–74.

Shankarappa, T.H., Geeta, G.S. 2013. Alkali and autohydrolysis pretreatments for effective delignification and recovery of cellulose and hemicellulose in selected agro residues. *Karnataka J. Agric. Sci.*, 26(1): 67–75.

Singhania, R.R., Sukumaran, R.K., Pillai, A., Prema, P., Szakaks, G., Pandey, A. 2006. Solid state fermentation of lignocellulosic substrates for cellulase production by *Trichoderma reesei* NRRL 11460. *Indian J. Biotechnol.*, 5: 332–336.

Soderstrom, J., Pilcher, L., Galbe, M., Zacchi, G. 2003. Two-step steam pre-treatment of soft wood by dilute H_2SO_4 impregnation for ethanol production. *Biomass Bioenergy*, 24: 475–486.

Valaskova, V., Snajdr, J., Bittner, B., Cajthaml, T., Merhautova, V., Hofrichter, M., Baldrian, P. 2007. Production of lignocellulose-degrading enzymes and degradation of leaf litter by saprotrophic basidiomycetes isolated from a Quercus petraea forest. *Soil Biol. Biochem.*, 39: 2651–2660.

Valmaseda, M., Almendros, G., Martinez, A.T. 1991. Chemical transformation of wheat straw constituents after solid state fermentation with selected lignocellulose degrading fungi. *Biomass Bioenergy*, 1(5): 261–266.

Viswanath, B., Chandra, M.S., Pallavi, H., Reddy, B.R. 2008. Screening and assessment of laccase producing fungi isolated from different environmental samples. *Afr. J. Biotechnol.*, 7(8): 1129–1133.