

Bio-softening of mature coconut husk for facile coir recovery

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Abstract Bio-softening of the mature coconut husk using Basidiomyceteous fungi was attempted to recover the soft and whiter fibers. The process was faster and more efficient in degrading lignin and toxic phenolics. *Phanerochaete chrysosporium*, *Pleurotus eryngii* and *Ceriporiopsis subvermispota* were found to degrade lignin efficiently without any appreciable loss of cellulose, yielding good quality fiber ideal for dyeing.

Keywords Biosoftening · Coconut husk · Basidiomycetes · Lignin degradation

Introduction

Coir is a coarse fiber obtained from the mesocarp of coconut. The fiber cell walls are thick and made up of cellulose and lignin. Lignin is a complex chemical responsible for its stiffness and dark colour. Retting is a traditional fiber recovery process carried out in the backwater lagoons. This is an environmentally hazardous process due to the release of the toxic compounds such as polyphenols¹. Bio-softening is an ecofriendly method to extract fiber by selective removal of lignin using specific microorganisms^{2,3}. This also results in the reduction of retting time and is safe for the environment⁴.

We used the white rot fungi for depolymerization of lignin and remove resins and parenchyma cells (pith) to obtain bleached coconut fibre.

Materials and Methods

Lignin degrading white rot fungi namely *Phanerochaete chrysosporium*⁵, *Ceriporiopsis subvermispota*⁶, *Pleurotus eryngii*⁷ and *Ganoderma lucidum*⁸ were used in this study. *P. chrysosporium* (BKM-F-1767) and *C. subvermispota* cultures (FP-105752-Sp) were obtained from the United States Department of Agriculture, Wisconsin. *G. lucidum* culture was obtained from the Tamil Nadu Agricultural University, Coimbatore. *P. eryngii* (NBIMCC 2199) was obtained from the culture collection of Bulgaria, Sofia. *P. chrysosporium* and *C. subvermispota* were maintained on Potato dextrose broth⁹ whereas *P. eryngii* and *G. lucidum* were grown in glucose peptone broth⁹ and malt extract broth⁹ respectively.

Homogenized fungal cultures (100 ml of 10 d old) were added separately to 10g husk samples in conical flasks, each with three replications and incubated at 30°C. Lignin and

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cellulose contents in fiber, and extracellular enzymes and tannin in the spent medium were analyzed after 30, 60 and 90d of incubation. Husk and medium without any fungus served as the control.

Cellulose and lignin contents were estimated by the standard procedure⁵. Lignin peroxidase (Lip) was measured by monitoring the oxidation of 2 mM veratryl alcohol to veratraldehyde in 0.1M sodium tartrate (pH 3) supplemented with 0.4 mM H₂O₂⁸. Laccase (Lac) activity was assayed by the rate of oxidation of guaiacol in 0.1 M sodium phosphate buffer (pH 6)⁸. Manganese peroxidase (Mnp) activity was assayed by monitoring the formation of the Mn³⁺-tartrate complex from 0.1 mM MnSO₄ in 0.1 M sodium tartrate (pH 5) supplemented with 0.1 mM H₂O₂⁸. Cellulase content was assayed by the standard procedure⁸. One unit of enzyme activity is defined as the amount of enzyme required for the transformation of 1μmol of substrate per minute. Tannin content was estimated by Lowenthal permanganate titration method¹⁰. Dye uptake percentage of the coir samples was calculated by determining the reduction in absorbance of malachite green solution at 660 nm before and after the treatment of the fibre.

Results

Lignin and cellulose contents of the coconut husk before biosoftening were 45.84 and 43.44% respectively. Reduction in the lignin content of the treated coir (Table 1) clearly indicated that lignin was degraded by the fungi. *P. chrysosporium* enhanced the reduction in lignin content from 24.3 % after 30d to 44.74% after 90d accompanied by 8.4–13.9% reduction in cellulose content. Reduction in the lignin content by *G. lucidum* was only 26.24% after 90d and was adjudged as a poor degrader of lignin.

P. eryngii reduced the lignin content from 16.7% after 30d to 36.36% after 90d accompanied by 7.7–14.8% reduction in cellulose content. *C. subvermispora* brought about a reduction in the lignin content from 19.87% after 30d to 34.99% after 90d accompanied by 5.7–9.9% reduction in the cellulose content. The observations are supported by earlier reports^{2,11} on lignin degradation by the white rot fungi causing fiber individualization and decolourization in paper and pulp industry. Gradual increase in the Lip, Mnp and Lac activities in the spent medium implied more lignin degradation in coir (Table 2). The observations suggested that Lip, Mnp and Lac activities could contribute more for lignin deg-

Table 1 Effect of white rot fungi on lignin and cellulose contents^a of coir.

Treatments	Lignin Incubation (d)			Cellulose Incubation (d)		
	30	60	90	30	60	90
Control ¹	45.51 ± 0.16	42.43 ± 0.13	41.42 ± 0.31	43.03 ± 0.13	42.00 ± 0.08	39.23 ± 0.21
<i>P. chrysosporium</i>	34.67 ± 1.70	30.37 ± 0.82	25.33 ± 0.50	39.78 ± 0.26	38.80 ± 0.22	37.38 ± 0.49
<i>C. subvermispora</i>	36.73 ± 0.79	34.51 ± 0.59	29.80 ± 0.40	40.93 ± 0.68	39.67 ± 1.25	39.13 ± 1.18
Control ²	43.65 ± 0.19	39.52 ± 0.23	35.25 ± 0.55	41.10 ± 0.08	40.09 ± 0.07	39.97 ± 0.05
<i>P. eryngii</i>	38.17 ± 0.79	37.25 ± 0.18	29.17 ± 0.67	40.07 ± 0.05	39.26 ± 0.57	37.00 ± 0.82
Control ³	42.64 ± 0.09	38.56 ± 0.05	35.21 ± 0.03	42.17 ± 0.05	41.30 ± 0.16	39.80 ± 0.25
<i>G. lucidum</i>	39.58 ± 0.10	37.43 ± 0.26	33.81 ± 0.20	42.03 ± 0.47	40.99 ± 0.08	39.20 ± 0.63
CD (P < 0.01)						
¹ Treatments x incubation period	2.16			1.99		
² Treatments x incubation period	1.52			1.25		
³ Treatments x incubation period	0.45			1.05		

^aMean of 3 replicated values in % w/w.

Table 2 Extracellular enzyme activity^a in *P. chrysosporium*, *P. eryngii* and *C. subvermispora*.

Sampling days	<i>P. chrysosporium</i>			<i>P. eryngii</i>			<i>C. subvermispora</i>		
	Lip	Mnp	Lac	Lip	Mnp	Lac	Lip	Mnp	Lac
30	0.052	0.030	0.057	0.050	0.025	0.020	0.037	0.014	0.050
60	0.059	0.051	0.090	0.052	0.030	0.021	0.041	0.018	0.114
90	0.067	0.062	0.100	0.064	0.041	0.024	0.050	0.021	0.131

^a Values in Unit/mg of protein.

Table 3 Tannin reduction and dye uptake of bio-softened (90 d duration) coir^a.

Treatments	Tannin (%)	Dye uptake (%)
Control	0.073	88.20
<i>P. chrysosporium</i>	0.030	97.44
<i>P. eryngii</i>	0.030	97.38
<i>C. subvermispora</i>	0.032	97.84

^a10 g husk + 100 ml water as retting mix.

radation in softening and bleaching of the husk. Role of Lip in lignin degradation has been reported earlier^{7,12}.

The tannin, a biofouling compound¹, was reduced to 57.14% in treated sample (Table 3), suggesting that biosoftening can also reduce biofouling to a extent greater than the conventional retting process. Further, dye uptake in the bio-softened coir increased to 97% over 88% in the control. Our study showed that *P. chrysosporium*, *C. subvermispora* and *P. eryngii* are suitable for biosoftening and biobleaching of coconut husk in an ecofriendly manner.

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References

- Nelliyat P (2003) Coconut husk retting and backwater pollution in coastal Kerala. 3rd biennial conference of the Indian Society for Ecological Economics, Kolkata.
- Eriksson KEL, Blanchette RA & Ander P (1990) Microbial and enzymatic degradation of wood and wood components. Springer Verlag, Germany: 60
- Camareo S, Galletti GC & Martinez AT (1994) Preferential degradation of phenolic lignin units by two white rot fungi. *Appl Environ Microbiol* 60:4509–4516
- Hammel KE (1989) Organopollutant degradation by ligninolytic fungi. *Enzyme Microb Technol* 11:776–777
- Kerem Z, Friesem D & Hadar Y (1992) Lignocellulose degradation during solid-state fermentation *Pleurotus ostreatus* versus *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 58:1121–1127
- Akin DE, Rigsby LL, Sethuraman A, Morrison WH, Gamble GR & Eriksson KE (1995) Biodegradability of grass lignocellulose treated with the white rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus*. *Appl Environ Microbiol* 61:1591–1598
- Guillén F, Martínez AT & Martínez M J (1992) Substrate specificity and properties of the aryl-alcohol oxidase from the ligninolytic fungus *Pleurotus eryngii*. *Eur J Biochem* 209: 603–611
- Trevor M D'Souza, Carlos S Merritt & Adinarayana Reddy C (1999) Lignin-modifying enzymes of the white rot Basidiomycete *Ganoderma lucidum*. *Appl Environ Microbiol* 65: 307–5313
- Subba Rao NS (1977) Soil microorganisms and plant growth. Oxford & IBH Publishing Co Pvt Ltd, NewDelhi: 287
- Burroughs LF & Whiting GC (1960) Annual Report, Long Ashton Research Station: 140–143
- Blanchette RA (1995) Degradation of the lignocellulose complex in wood. *Can J Bot* 73:999–1010
- Kirk TK & Chang HM (1981) Potential application of bioligninolytic systems. *Enzyme Microb Technol* 3: 189–196