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Research Article

CHARACTERISATION OF BIOLOGICALLY PRETREATED RAW MATERIALS FOR BIOPULPING PROCESS

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ABSTRACT: Biopulping, the treatment of wood chips by white rot fungi and subsequent chip refining is envisioned as a method for saving energy and making a stronger paper product. The present study aims to find suitability of two fungal isolates *Phellinus pectinatus* and *Daedaleopsis confragosa* for the process of biopulping and the characteristion of the biologically pretreated raw materials for biopulping. Two combinations of raw samples, Bamboo: wood shavings and Bamboo: wood shavings: *Sorghum halepense* culm were prepared and subjected to four different pretreatment. *Daedaleopsis confragosa* was found to be effective in biopulping with a supplement of Potato dextrose broth medium to the raw material.

Keywords: *Daedaleopsis confragosa , Phellinus pectinatus,* Weight loss, Biopulping Lignin loss, Cellulose loss

INTRODUCTION

Paper consumption is continuously increasing across the world. World paper consumption was about 300 million tonnes in 1996–1997 and is expected to rise above 400 million tonnes by the year 2010 (Hurter and Ricco 1998). Wood is the primary raw material for the pulp and paper industry because it is the main source of cellulose fibre. The available wood supply in many countries will not meet the growing demand. Much of this increase in fiber demand will be met by increased forestry production in tropical countries, but higher fiber recovery rates and the use of nonwood fibers will also play their part. Non-wood materials, for example cotton and cereal crops such as straw, can be used for papermaking but the advantage of a year-round wood supply, combined with the product diversity made possible by mixing wood fibres, has made it the most practical and economic option.

The using of agricultural residuals in the pulp and paper industry has increased substantially. According to Bajpai et al., (2004) agriculture based fibers are particularly relevant in countries where demand for pulp and paper is increasing but the wood resources are limited. Nieschlag et al., (1960) stated that promising non woody species for fiber production have been found in plant families Gramineae, Leguminosae, and Malvaceae, Of these most attention in recent years has been focused on grasses and other monocotyledons (Kordsachia et al. 1992, Olsson et al. 1994 , Pahkala 2001, Madakadze et al., 2010). In Gujarat grasslands cover a wide area. Large numbers of grass species grow in these grasslands wildly of which many of them are fast-growing, with very high biomass but highly unpalatable. These are not preferred by livestock and so are used as fuel. Many of the grass species have high fiber content and the property more or less equals that of the hardwood. Fibre markets for perennial grasses currently include their use for livestock bedding, "straw bale" housing, and as a compost substrate for mushroom production. Promising future markets include the pulp and paper and composite industries as well as energy industries. Pulping studies have found that the stem component of grasses, followed by the sheaths and then the leaves, has the highest pulp yield, fibre length, and brightness (Goel et al., 1998). Biodegradation of wood chips with ligninolytic microorganisms is being considered a suitable or complementary alternative to traditional method due to its contribution to reduce the environmental impact of paper mill industries and to save energy and chemical cost (Arias et al., 2010, Leathem et al., 1990). Unlike the weak chemically softened fiber, microbial treatment produced soft, whiter fibers having better tensile strength and elongation (44.6–44.8%) properties (Rajan et al., 2005). White rot fungi has shown to have a great potential to be used for the process of pulping (Akhtar et al.,1992,1993, Leathem et al., 1990, Singh et al., 2010). Biopulping has the potential to be an environmentally kind means of improving both the economics of pulp production and quality of the pulp produced.

In the present study two well known white rot basidiomycetes have been evaluated for its potentiality to be used in the modified process of biopulping. Wood blocks, bamboo culm, wood shavings and culm of the grass *Sorghum halepense* have been used in the mixture of raw materials. Before the pre-treatment of raw materials with fungi six different modified processing of raw materials have been attempted and evaluated for effective biopulping process.

MATERIALS AND METHODS

Selection of material

On a visit to the well known paper industry , J K paper industry (one of the major papers producing industry of the country) located at Songadh in Gujarat it was understood that the raw material used in industry for the paper making is 70% bamboo and 30% of other wood species. Wood shavings are also lignocellulosic waste material which could be used as an alternative source in mixture with wood chips. Wood shavings from the carpenters (residue after carpentry work) were collected and also used as raw material in the present experiment.

As an alternative source of raw material, in the present study *Sorghum halepense* an unpalatable Graminaceous member with high fibre content and property (Albert et al.2011), has been selected and tried in mixture with wood shavings and bamboo culm. Bamboo culms were procured from a timber merchant in Dandia Bazaar, Vadodara, Gujarat, wood shavings from Laxmi saw mill, Chhani road, Vadodara, Gujarat and *Sorghum halepense* culms collected from Rampura grass lands, Dahod, Gujarat.

Isolation of fungi

Fruiting bodies of fungi were collected, surface sterilized with 0.1 % $HgCl_2$ and inoculated in the petridish containing PDA(Potato-Dextrose-Agar) medium under aseptic condition and incubated for 7 days. After development of colony for sub culturing these were transferred into slants. The fruiting bodies were sent to FRI Dehradun and authentically identified by Dr.N.S.K. Harsh, Forest Research Institute, Dehradun.

Method for screening of enzymatic activity (Bavendamm test)

Screening of enzymatic activity was done by substituting the malt extract agar medium (3%) with enzyme substrate tannic acid. The PH of medium is adjusted to 5.8 autoclaved and then poured to sterile petriplates aseptically. On this medium, fungal culture was inoculated in the center and incubated at 28 ±1ºC for 1 week. Ligninolytic activity was assessed by observing the dark brown colored zone around the respective fungal colonies (Bains et al., 2006).

Preparation of raw material mixture for experiment

In the present study a mixture of the grass *Sorghum halepense*, wood shavings and Bamboo Culm has been tried as raw materials for the pulping experiments.

Bamboo and *Sorghum* culms were separately chopped into pieces of 4 to 5 cms. Two sample mixtures were prepared for the study.

Sample mixture 1:- Bamboo (B) wood shavings (W) in the ratio of 70:30- (WB)

Sample mixture 2:- Bamboo (B): wood shavings (W) : *Sorghum halepense* culm(S) in the ratio of 35: 35: 30 – (WBS)

Before the fungal pretreatment both the raw material sample mixtures were processed in four different ways,

Mixture of raw materials soaked in 10 ml water for 24 hrs and then autoclaved for 45 min.

($WB-H_2O$, $WBS-H_2O$)

10 ml Potato dextrose broth added to the raw material mixture and then autoclaved for 45 min. (WB-PDA,WBS-PDA)

Most of the Graminaceous members have high silica content. Silica is the main quality barrier preventing perennial grasses and straw from being more widely utilized in both the pulp and paper industry and the energy sector. Silica, which is the single largest component of ash in perennial grasses, varies greatly in quantity between species. In the pulp and paper industry, silica-rich feedstocks complicate the recycling of chemicals from recovery boilers, and increase maintenance costs while shortening the lifespan of machinery. Hence in the present study the raw material has been pretreated by hydrofluoric acid (HF) to remove the silica content.

The following methods were followed:-

Raw material was soaked in 5 ml 3% HF for 24 hrs, Then washed in running water for 24 hrs and then autoclaved for 45 min.

(WB-HF, WBS-HF)

Raw material was soaked in 5 ml 3% HF for 24 hrs, washed in running water for 24 hrs, oven dried, 10 ml potato dextrose broth was added to it and autoclaved for 45 min.

(WB-PDA-HF, WBS-PDA-HF).

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Weight loss experiment

Weight loss experiments were conducted in autoclavable polypropylene plastic bags. The raw materials in different combinations after the different pretreatments were inoculated with fungi *Phellinus pectinatus* and *Daedaleopsis confragosa* and incubated for 10, 20, and 30 days. After completion of the incubation period raw materials were taken out from the polypropylene bags, oven dried for 48 hrs at 80^{o}C and dry weight was measured. These samples were then used for analysis of lignin and cellulose.

Estimation of cellulose (Anthrone method)

To a known amount of sample in a test tube 3 ml acetic –nitric reagent was added and mixed well. Test tube was then placed in a water bath at 100ºC for 30 min, cooled and centrifuged for 15-20 min at 5000 rpm. Supernatant was discarded. Residue was washed with distilled water and 10 ml of 67 % sulfuric acid was added and allowed to stand for 1 hr. 1 ml of the solution was diluted to 100 ml. To 1 ml of this solution 10 ml of anthrone reagent was added, mixed well and kept in boiling water bath for 10 min, cooled and the colour measured at 630 nm. A blank was set with anthrone reagent and distilled water. For the standard graph the D- glucose was taken in 1 mg/ml and different aliquots were treated with anthrone reagent, and the colour measured at 630 nm (Yem and Willis, 1954).

Estimation of lignin

Known amount of sample was taken and extracted with alcohol. From that the 0.5 gm of sample was taken and 4 ml 72% H_2SO_4 added to it , kept for 24 hours , diluted with 4% H_2SO_4, and autoclaved for 1 hour. Then it was filtered and dry weigt was measured to detect the lignin content (Dill and Kraepelin, 1986).

RESULT AND DISCUSSION

Mycologists have tried and successfully have differentiated white rot and brown rot fungi by the oxidase reaction termed as Bavendamn test which depends on the oxidative browning of tannic acid. In the present study, both *Daedaleopsis confragosa* and *Phellinus pectinatus* showed positive reaction to tannic acid used in Bavendamm test.

After seven days of incubation the medium which is substituted with Tannic acid showed the culture with brown coloration when viewed from the lower side of the petridish indicating presence of ligninolytic enzymes in *D. confragosa* and *P. pectinatus* respectively Fig. 1 (A), Fig. 1 (B). Petridish substituted with CMC (carboxy methyl cellulose) after one week when flooded with Congo red did not show red coloration confirming the presence of ligninolytic enzymes and absence of cellulolytic enzymes in *D. confragosa* and *P. pectinatus* respectively. Fig. 1 (C), Fig. 1 (D).

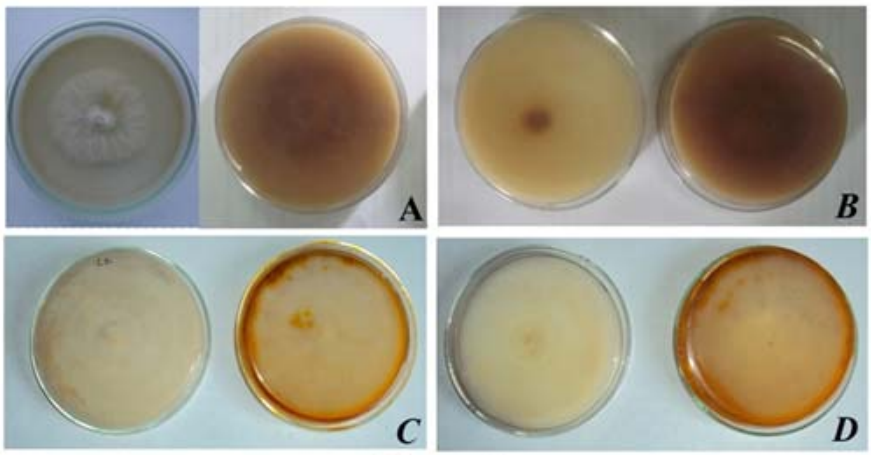

Figure 1

A: Brown coloured zone showing ligninolytic activity of *D. confragosa*.
B: Brown coloured zone showing ligninolytic activity of *P. pectinatus*.
C: Absence of red coloured zone.showing non-cellulolytic activity of *D. confragosa.*
D: Absence of red coloured zone.showing non-cellulolytic activity of *P. pectinatus*.

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Weight loss analysis

The untreated raw material and the pretreated raw materials after the incubation period were removed carefully from the polypropylene bags and analyzed for the weight loss. Differences in weight losses were evident in decay of raw material by inoculating two fungus. Result of the eight different combinations & pretreatments are presented in Table- I.

From the result it is clearly evident that among the two sample mixtures weight loss in 30 days is more in sample II compared to sample I. The maximum percentage weight loss in *Daedaleopsis confragosa* treated combinations was found to be in WBS – PDA + HF treated raw materials i.e. wood shavings : Bamboo : sorghum with Potato Dextrose broth inoculated after treating Bamboo and sorghum with Hydrofluoric Acid with an incubation period of 30 days (10.2%), While the maximum percentage weight loss of *Phellinus pectinatus* treated combination was found to be in WBS – HF i.e. wood shavings: Bamboo: sorghum (9.2%). pretreated with hydrofluoric Acid. (Table-1). In the initial 10 days incubation period percentage weight loss was found to be maximum in WBS – HF treated with both the fungi, but with increase in the time period percentage in WBS – PDA + HF combination treated with *Daedaleopsis confragosa* compared to WBS – HF *Phellinus pectinatus* treated combination wherein the percentage weight loss continued to increase to a maximum 9.2% in 30 days incubation period. This was higher compared to 8.4% loss in WBS – PDA + HF treated combination. In *Daedaleopsis confragosa* and *Phellinus pectinatus* treated sample mixture I the weight loss is maximum when the raw material is not treated with hydrofluoric acid but is supplemented with potato dextrose broth medium(8 and 8.8 respectively).

The weight loss experiment indicates *Daedaleopsis confragosa* to be more efficient fungi than *Phellinus pectinatus.* The experiments result also indicate that presence of silica dose not hinder the growth of the fungus as weight loss in the Hydrofluoric Acid (HF) treated and untreated samples is more or less the same.

Table 1. Percentage weight loss of pretreated raw materials infected with *Daedaleopsis confragosa* and *Phellinus pectinatus*

Sample mixtures	Pretreatments	*Daedaleopsis confragosa*			*Phellinus pectinatus*		
		10 days	20 days	30 days	10 days	20 days	30 days
I) Wood shavings : Bamboo culm	WB - H_2O	1	1.8	2.6	0.6	2.2	2.6
	WB – PDA	0.8	3	8	1	2.2	8.8
	WB – HF	3	5	6.8	3.2	3.8	7.2
	WB - PDA+HF	2.2	5.6	7.2	2.6	3.8	5
II) Wood shavings: Bamboo culm: *Sorghum halepense* culm	WBS - H_2O	2.2	4.2	8.6	1.6	2.4	5.6
	WBS – PDA	0.6	2.6	7.8	0.8	2.2	8.4
	WBS – HF	3.2	6	7.8	4.4	6.8	9.2
	WBS-PDA+HF	2	5.4	10.2	0.8	2.2	8.4

Table-2. Percentage loss of Klason Lignin (KL) and Chlorite Holocellulose (CHC) in control & decayed pretreated combinations of raw materials

		Daedaleopsis confragosa			*Phellinus pectinatus*		
		10 days	20 days	30 days	10 days	20 days	30 days
WB - H_2O	KL	8.33	25	25	16.67	25	25
	CHC	2.70	29.72	43.24	10.81	37.83	45.94
WB – PDA	KL	16.67	16.67	33.33	16.67	25	33.33
	CHC	10.81	24.32	24.32	21.62	27.02	62.16
WB – HF	KL	8.33	25	25	8.33	16.67	33.33
	CHC	13.51	35.13	35.13	21.62	51.35	72.97
WB- PDA+ HF	KL	16.67	33.33	41.66	16.67	25	33.33
	CHC	37.83	40.54	45.94	18.92	45.94	56.75
WBS- H_2O	KL	8.33	33.33	33.33	16.67	25	33.33
	CHC	0	24.32	45.94	5.40	18.92	64.86
WBS-PDA	KL	16.67	33.33	33.33	8.33	16.67	33.33
	CHC	10.81	21.62	37.83	5.40	24.32	40.54
WBS – HF	KL	8.33	25	25	16. 21	33.33	33.33
	CHC	0	0	24.32	16. 67	45.94	70.27
WBS-PDA+HF	KL	8.33	25	33.33	16.67	25	33.33
	CHC	0	5.40	16.21	18.92	32.43	75.67

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Chemical analysis of raw materials

Chemical analysis of the treated combinations of raw materials indicated removal of lignin and cellulose. (Table - II)
The maximum percentage of lignin loss by *Daedaleopsis confragosa* & *Phellinus pectinatus* was found to be in WB – PDA + HF (41.66% & 33.33%respectively), (Table -2).
In the initial period of 10 days all the samples treated with *Daedaleopsis confragosa* showed loss of lignin to be more than cellulose. The percentage of lignin loss was also found to be the same (8.33%) in all samples except the samples WB-PDA, WB-PDA-HF and WBS-PDA (16.67%). In sample mixture II all the samples showed no loss of cellulose except WBS supplemented with potato dextrose broth. In 20 days period the loss of cellulose increased in sample mixture I, but in sample mixture II loss of lignin continued to be more than that of cellulose. But in 30days period the loss of cellulose was found to be more than lignin loss in all samples except WBS-PDA-HF and WB-PDA.
Unlike *Daedaleopsis confragosa* , in the initial period of 10 days all the samples treated with *Phellinus pectinatus* showed loss of cellulose to be more than lignin except in WB - H_2O, WBS- H_2O and WBS- PDA. Further in 20days period the loss of cellulose continued to be more except inWBS- H_2O and in 30days period the loss of cellulose was very high (Table 2).The percentage of lignin loss after an incubation period of 30 days remained same for all sample mixtures i.e. 33.33% except in WB –H_2O i.e. 25%. Compared to *Phellinus pectinatus* treated samples percentage loss of cellulose was less in *Daedaleopsis confragosa* treated samples after 30 days of incubation period.
In all experiments with *Phellinus pectinatus* the ratio of Klason Lignin (% KL) to Chlorite Holocellulose (%CHC) obtained indicated a moderate amount of delignification. The amount of loss of cellulose appeared to be more while in *Daedaleopsis confragosa* decayed raw materials a high amount of delignification was observed. Experiment in which silica was removed using HF and when supplemented with PDA it was observed that cellulose loss was more than lignin, while the raw materials when not supplemented with PDA it showed high rate of delignification.

CONCLUSIONS

That *Daedaleopsis confragosa* is efficient fungi compared to *Phellinus pectinatus* for biopulping.
The appropriate time for the delignification process for *Daedaleopsis confragosa* is 20 days
Presence of silica in the grasses does not hinder degradation process of the fungi and so can be applied for biopulping.
Rate of degradation is highest when the raw materials are untreated and inoculated with fungi and so is cost effective.
Attributes and abilities to degrade lignin and various plant cell types by lignin degrading white rot fungi differ considerably (Otjen et al., 1987,Messner and Srebotnik, 1994). According to Jennings (1995) the ability of a fungus to bring about lignocelluloses breakdown is governed by a range of factors. There is a tremendous variability among white tor fungi in how selectively they delignify wood. If the selectivity of delignification is poor, a large amount of carbohydrates (cellulose and hemicelluloses) will also be degraded during biotreatment which will decrease the yields of the pulp (Scott et al., 1998). Some species cause selective removal of lignin at one location and simultaneous removal of both lignin and cellulose at another location in the same wood sample. Even different strains of a single species can show considerable variation (Blanchette et al., 1992). Degradation pattern is also known to depend on environmental conditions. Moreover some white rot fungi seem to switch from selective to simultaneous degradation over time (Otjen et al., 1985).
In biopulping the selected fungi should have preferred action against hemicelluloses and lignin united with low activity on cellulose (Singh et al., 2010). Both the fungi *Phellinus pectinatus* and *Daedaleopsis confragosa* are selective lignin degraders .In the present study both the sample mixture mainly comprises of Bamboo and *Sorghum* culm both belonging to the grass family. The chemistry of grass lignocelluloses varies considerably from that of wood. As determined by various extractive procedures there is less lignin in grasses than in woody plants (Terashima et al., 1993, Van Soest and Wine 1968). Cellular composition of grass culm is mainly occupied by parenchymatous cortex in which the vascular bundles ensheathed with the lignified fibers are embedded. Though the two selected fungi are selectively ligninolytic, as the substrate (i.e., Bamboo and *Sorghum* culm) composition is mainly cellulose the two white rot fungi seem to have switched from selective to simultaneous degradation over time.

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