Recent Advances in Biotechnological Applications in Pulp & Paper Industry

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ABSTRACT

Biotechnological applications have received considerable attention during the last one & half decades due to its commercial potential in number of areas. Pulp & Paper Industry is no longer an exception.
A brief look at the process technological trends world wide show a clear leaning towards use of enzymes for various applications in pulp & paper industry & most important among various applications are the use of enzyme as an aid to pulping & paper making for reduction in the use of cooking & bleaching chemicals, there by, reducing the energy consumption during pulping & refining, management of wood and industrial waste minimisation.
Biopulping is the term applied to delignification and might involve either lignin deploymerizing enzymes or growth of whole organisms on fibrous raw materials. Either strategy would be a pre-treatment that lowers the use of subsequent chemicals employed for pulping & energy required for refining is also reduced to a great extent with improved pulp properties.
The annual plants, predominant raw materials in Indian Paper Industry could combine some favourable characteristics for biological delignification (related to histoligical features, like lower lignin content & higher amount of phenolic unit in lignin etc.)
The present article highlight a review on the status of biological pulping of wood & nonwood based raw materials emphasing identified fungal strains and potential of its applications in Indian Paper Industry.

INTRODUCTION

The high energy & chemical requirement of conventional pulping proceses as well as the environmental concern are forcing the pulp & paper industry to turn to more cost effective & environmental friendly alternatives. Increased productivity, product quality as well as energy savings & improvement of the environmental impact are the main strategies of pulp & paper production. Biotechnological processes are consistent with these strategies and are getting access to the pulp & paper industry. Biotechnology

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implies the technical exploitation of biological processes. The technology has received increasing attention during past fifteen years because of its commercial potential in number of areas. The pulp & paper industry is no longer an exception. In view of increased pressure from the environment pollution control & the continuously increasing energy cost component in pulp & paper, the industry continuously tries to improve its processes & products. However, due to economical & technical constraints new approaches to pulp and paper manufacture are rare. Biotechnology can, however, give rise to new possibilities. Until recently, the use of enzymes in pulp & paper industry was not considered feasible because of non availability of suitable enzymes. A limited use of the starch degrading enzyme, amylase was the only industrial application in pulp & paper industry.

In order to improve the chemical & energy efficiency during pulping, biopulping processes show promising future, where in certain microorganisms are able to delignify the fibrous raw material under controlled conditions. The processes however, need to be developed suiting to the raw materials, process conditions employed in Indian Paper Industry.

Central Pulp & Paper Research Institute has initiated its activities in the area of biotechnological applicion in pulp & paper. The present paper highlights a review on biopulping emphasing the status and its potential in Indian Paper Industry.

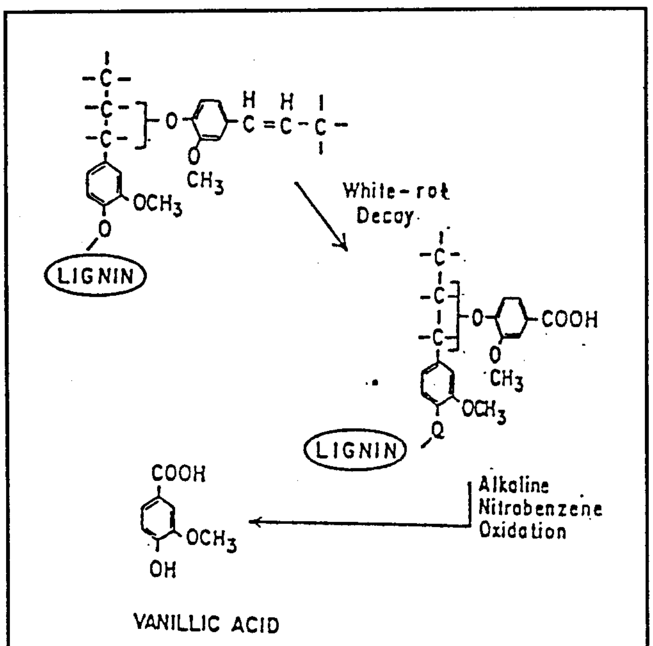


Fig-1 Degradation of Phenylpropanoid End Groups in Lignin by white- Rot Fungi.

MICROBIOLOGICAL DELIGNIFICATION OF LIGNOCELLULOSIC RAW MATERIALS BIOPULPING:

The use of fungi for the pretreatment of fibrous raw materials like bamboo & wood chips, agro residue prior to pulping is often referred to as biopulping. Biopulping is the bioconversion of these raw materials by a class of micro organisms. Certain micro organisms are able to partially delignify these fibrous material raising the possibilities of biopulping.

White rot fungi are able to degrade lignins as well as the other wood components. The normal pattern of wood breakdown involves a simultaneous attack on both the polysaccharides and the lignin. A totally specific attack on the lignin component probably does not occur. The white rot fungi need polysaccharides and/or low molecular weight sugars in order to degrade lignin, the sugar being necessary partly to provide the energy for growth and metabolism and partly to produce hydrogen peroxide, which is an important requirement for lignin degradation (1-3). Thus, if the capacity of white rot fungi to attack lignin was made more specific it should be possible to obtain energy savings not only during mechanical pulping but also in the production of chemical pulp. In addition, a specific removal of lignin from agro-residue such as straws & bagasse would yield a more easily digestable & most likely a more energy/rich fodder for ruminants.

REACTION MECHANISM DURING ENZYMATIC DELIGNIFICATION

Basically, lignin degrading enzymes (Ligninase or lignin peroxdases) have been found to be involved during enzymatic delignification and/ or lignin degradation. The key reactions involved are-

- Ligninase- catalysed one electron oxidation of aromatic rings in lignin to form cation radicals. These redicals degrade via many spontaneous reactions, some of which cleave inter molecular linkages in lignin & some of which cleave aromatic nuclei. The ligninase-catalysed oxidation requires extracellular H_2O_2 which means that the enzyme system supplying cofactor is also a key component of the in vivo lignin degradation system.

- A second peroxidase (termed mangnese peroxidase) also is involved, but its role is not yet clear. Fig.1 - shows degradation of phenyl propanoid

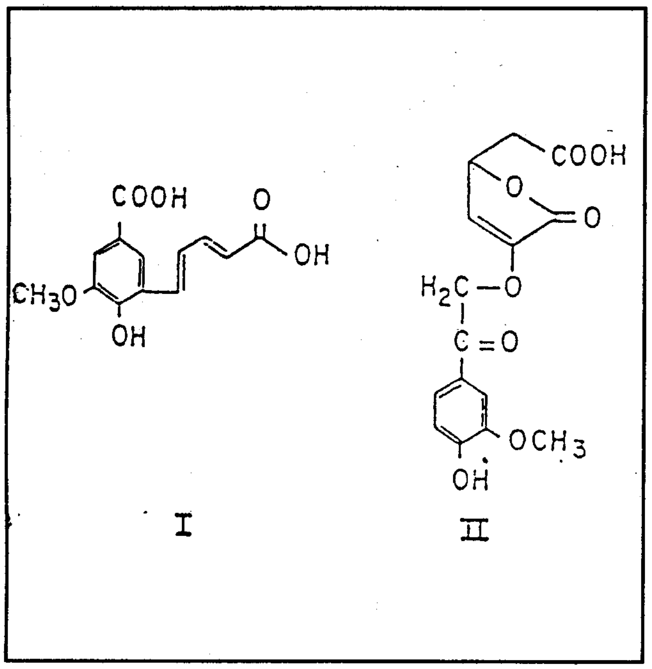


Fig-2 Two of Thirty Lignin Fragments Released From Spruce Wood During Decay Phanerochaete Chrysospolium.

end groups in lignin by white - rot fungi and Fig.2 - shows two of thirty lignin, decay fragments released from spruce wood during decay by Phane rochaete chrysosporium. Compound 1 was probably derived from a biphenyl lignin structure by cleavage of one ring while it was still attached to the polymer. Like wise compound II may be derived from an arylglycerol B-aryl ether structure by ring cleavage of the intact polymer.

STATUS OF BIOPULPING:

BIOPULPING OF FOREST BASED RAW MATERIALS:

Research on the development of cellulase less mutant of white-rot fungi & their use as microbial delignification was pioneered at STFI in Stockholm.

One of the first attempts to exploit this opportunity was in pretreating wood for mechanical pulping. In 1971 an unpublished cooperate study between the US Forest Products Laboratory & North Carolina State University, showed that aspen wood pretreated with Fomes ulmarius gave refiner mechanical pulp, with better strength properties than untreated aspen wood refined with the same amount of energy(5). In Sweden Ander & Eriksson (6) found in the Laboratory that birch & pine woods slightly delignified by cellulase negative mutant of Phanerochaete chrysosporium required 25-30% less energy for refining & gave pulps with better strength properties than controls. However, subsequent approach for an industrial evaluation met with only in different success(7,8).

One problem with fungal pretreatment is that the time required is a matter of weeks. This delay has led to suggestions that treatment might be carried out in connection with chip transport or storage or that treatment time could be reduced to days or even hours by optimising the fungal strains & treatment conditions(9). Work towards the goal of optimizing treatment has continued in a number of laboratories. In Japan Akamatsu et al (10) compared TMPS made from aspen wood pretreated with any of 10 different white-rot fungi to TMP made from untreated aspen wood. None of the 10 fungi were ligno-specific, yet each of them reduced refining energy by about 25% & three of them gave pulp with some what improved paper strength properties. At the University of Minnesota & the U.S. Forest Products Laboratory, Blanchette etal (11) have found that even with lignin specific organism, the strain of white rot fungus employed & the type of wood substrate treated may significantly influence the rate of lignin degradation & the selectivity of lignin removal. Therefore it should be possible to select & optimise strains for lignolytic activity on particular wood substrate.

Meyers et al (12) recently described solid substrate bioreactor used to treat aspen wood chips prior to refiner mechanical pulping. As in the earlier studies, the strength properties of biopulps prepared from chips treated in this bioreactor have been better than controls when compared at equal freeness, but treatment times are still in the order of weeks. Advances in genetic engineering and bioreactor concepts have already yielded biological compounds that attack lignin. For example test pulp made from spruce wood chips was treated for 14 days in Sweden by Ericksson & his Coworeis(4) with a cellulase less mutant at Sporotichum pulverulentem. Their experiments showed an average energy savings of 23% over the energy required to refine the untreated reference pulp. The results are shown in Table-I.

Some of the work carried out at Biotechnology Research Laboratory, Japan (13) on Biomechanical Pulping using white-rot fungi IZU-154 has been shown in Table -II & III, where-in the coarse beech mechanical pulp (MP) & wood meal were incubated with only the mycelia of three fungi IZU-154, Coriolus

TABLE 1

Energy damand for refining spruce and wood chips (5)		
Tensile strength, Nm/g	**Biologically treated Pulp², kWh/Ton**	**Reference kWh/Ton**
15	375	500
25	830	1000
35	1360	1860

Energy demand is for beating in a PFI mill following defibration. Pulp treated with cellulase less mutant of Sporotrichum pulverulentem

TABLE 2

Refining energy and strength properties of softwood MP (1 kg) before and after treatment with fungus IZU-154.						
Species	**Incubation time days**	**Refining kWh/ton**	**energy* %**	**Tensile Index*N.mg**	**Burst Index*kPa. m/g**	**Tear Index* mN.m/g**
Spruce						
Untreated	....	2627	100.0	33.3	2.77	8.54
Fungus treated	10	1700	64.7	38.8	2.76	8.22
Pine						
Untreated	....	2073	100.0	17.3	1.26	7.71
Fungus treated	14	1367	65.9	23.5	1.55	7.32

** Fungal treatment was carried out by inoculating the mycelia without supplemental nutrients.*
** To pulp freeness of 200 mL CSF.*

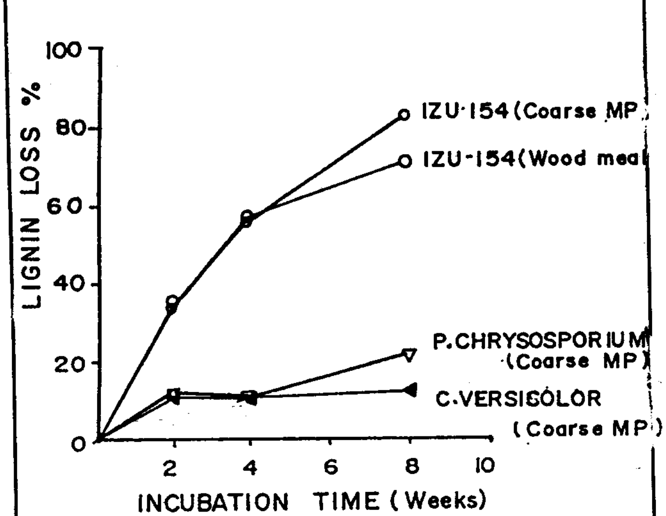


Fig-3 Rate of Lignin Loss During incubation of Hardwood Pulps (2g) With Three white-Rot Fungi.

versicolor & Phane-rochaete chrysosporium) for 2-8 weeks at 30°C. The results shown in Table- II clearly indicated that with the pretreatment of spruce and pine with fungal strain IZU-154, there is remarkable decrease in the requirement of refining energy to approximately 35% and the pulp properties also improved in respect of tensile, tear strength and burst index. Further, the rates of delignification (Klasson Lignin Losses) for these fungi are shown in Fig.3. The results clearly showed that the lignolytic activity of fungus IZU-154 was much greater than that of the other two fungi with the IZU-154 fungus showing about 35%, 60% & 70-80% lignin losses after respective incubation times of 2 weeks, 4 weeks & 8 weeks. Further, more fungus IZU-154 preferentially degraded the lignin portion compared with the carbohydrate portion, as judged from the relationship between yield & lignin loss in Fig.-4. Fig.-5 further shows the results of refining energy for the fungus treated pulps which was reduced to more than half to that of untreated pulp.

TABLE 3					
Strength & Optical Properties of Beech, MP Before & after Treatment with fungus 120-154.*					
Incubation time, days	**Freeness ml (CSF)**	**Tensile Index N.m/g**	**Burst Index kPa m²/g**	**Tear Index MN-m²/g**	**Brightness %**
		Before H_2O_2 Bleaching			
0	130	8.7	0.35	0.86	40.8
7	160	9.6	0.74	1.65	37.6
14	135	14.6	1.12	1.99	38.1
		After H_2O_2 Bleaching			
0	150	9.6	0.52	1.21	64.7
7	150	19.8	1.05	2.48	65.7
14	140	23.7	1.36	2.71	67.4

Fungal treatment was carried out by inoculating the mycelia without supplemental nutrients.

Results in Table-II further show that 7 days fungal treatment of coarse hardwood mechanical pulp also improved strength properties approximately two folds for both the bleached and unbleached pulps. The brightness of the unbleached coarse MP decreased slightly during fungal treatment however, the fungal treated pulp was brighter than the untreated pulp. Soft wood fungal treatment (mycelia only) of coarse spruce MP for 10 days resulted in a yield loss of 1.5% and a lignin loss of 5.3%. Energy consumption in disc refining of these coarse spruce MP after fungal treatment was compared with that of untreated coarse spruce MP. As seen in Fig.6 & Table-III, the energy consumption to produce 200 ml CSF pulp was about 2600 kWh/tonne for untreated coarse spruce MP while that for biotreated coarse spruce MP was about 1700 kWh/tonne clearly indicating decrease in refining energy in biotreated pulp to approximate 35%. This reduction in refining energy reflects the lignin degradation in coarse MP & thus allowing the individual wood fibres to be separated more easily. This in turn reduces the fibre damage that occur during the refining process.

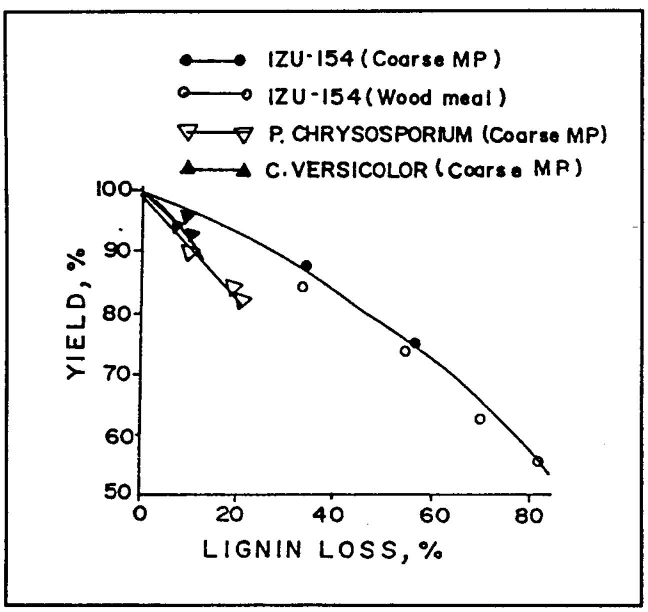


Fig-4 Yield As a Function of Lignin loss During incubation of Hard wood pulps (2 g) with three white-Rot Fungi.

In the United States, a consortium of 13 pulp, paper & chemical manufacturers, alongwith US Forest products Laboratory and the University of Wisconsin, is pursuing the white rot organism route(14). Replingen Sandoz Research Corp. is a company in U.S. that is active in developing recombinant DNA ligninase for pulping & other purposes. Several "Ligninage Club" also exist worldwide.

Number of white rot fungi have been screened and tested in bioreactors with aspen, spruce & lobolly Pine chips. Lignin removal has ranged from 3-37%. Energy requirement in refiner mechanical pulps

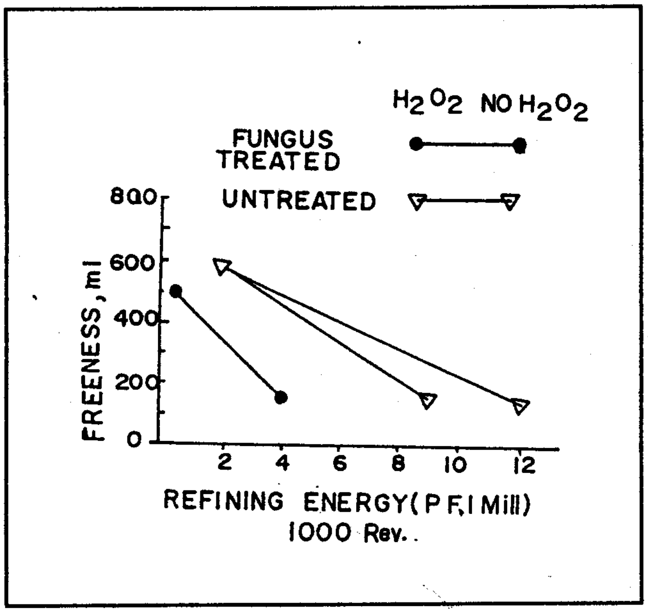


Fig-5 Freeness as a Function of Refining Energy for Fungus Treated & Untreated Coarse Hardwood MP (50 g) Before and after peroxide Bleaching.

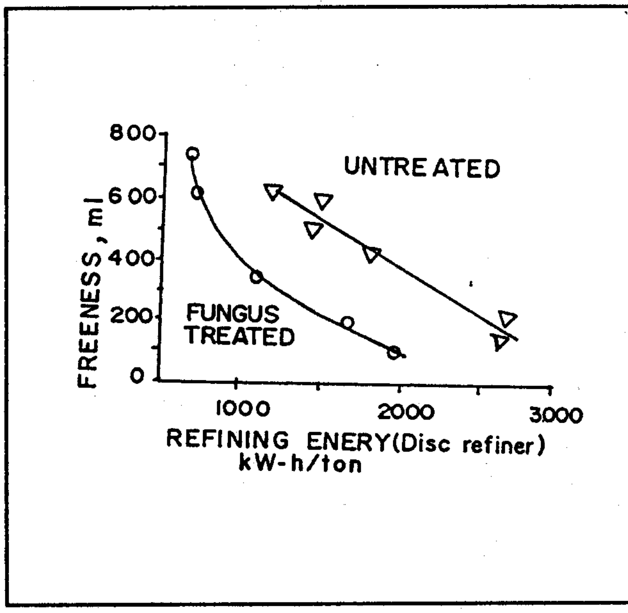


Fig-6 Freeness as a Function of Refining Energy for Fungus Treated & Untreated Spruce MP (1 kg.)

TABLE 4

Properties of Wheat- straw Biomechanical (BMP) abd Biosemichemical Pulps (BSCP), Compared with semichemical pulps (SCP) and control straw (CON)

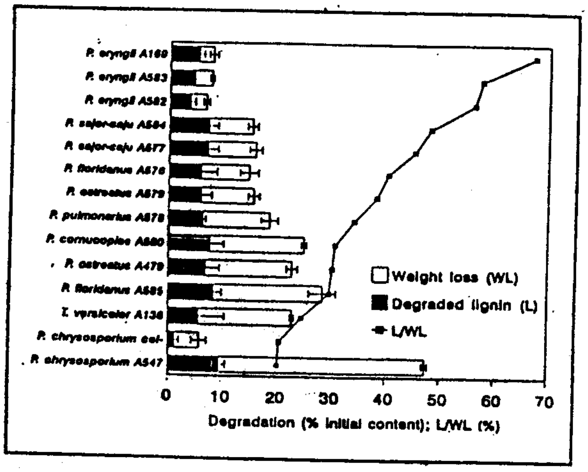
Straw Pulp types

	CON[1]	BMP[1]	BSCP	SCP[1]	SCP
Yield (%)	100	100[2]	69[2]	72	68
Energy (kWh/t)	2145	651	319	576	540
Refining Degree					
c (°SR)	68	68	68	68	68
WRV (g/g)	1.53	1.87	2.40	3.14	2.98
iCMT (Nm²/g)	--	--	1.10	2.11	1.73
iSCT (Nm/g)	--	--	24.9	26.9	28.6
Burst (kPm²g)	--	--	1.19	--	2.66
SST (g/L)	--	--	19	18	23
COD (g/L)	--	--	18	13	18

CON = Control (refined untreated straw); BMP = Biomechanical pulp (Straw treated with P. eryngii and refined); BSCP = Biosemichemical pulp (Straw treated with P eryngii, cooked for 1 h and refined); SCPI = Semichemical pulp after 1 h soda cooking in a laboratory digester and refining; SCP = Semichemical pulp from SAICA (3 h cooking and refining); WRV = Water retention value; iCMT = Concora medium TEST indes; iSCT = Short Compression Test index; SST = Total suspended solids, COD= Chemical oxygen demand.

1. *Due to poor properties of pulps, handsheets could only be prepared after mixing with 50% of waste paper pulp (see text).*

2. *Weight loss caused during SSF (6-7%) should be deducted.*



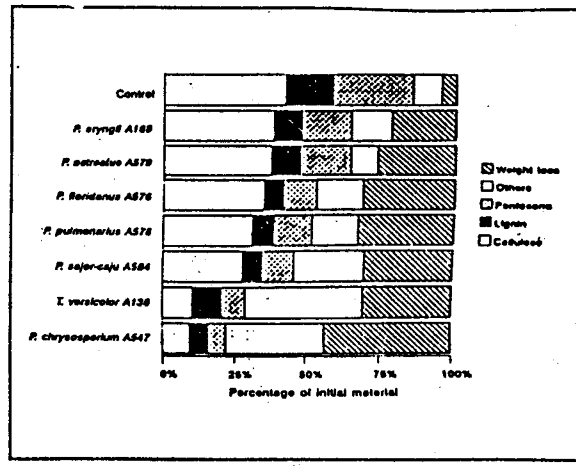


Fig-7 Weight loss, fraction corresponding to lignin removal, and L/WL ratio after wheat-straw treatment with different fungal strains under SSF conditions. Standard deviations are shown.

Fig-8 Modification of wheat-straw constituents and weight loss by different fungi (after 40-day period of SSF) and control.

decreased approximately up to 50%. Burst, tear & tensile strength of the hand sheets increased. Brightness & light scattering decreased. Total substance losses remained at less than 10% only.

BIOPULPING OF AGRO-BASED RAW MATERIALS

Biopulping of bagasse has been studied by Swedish Pulp & Paper Research Institute & at a Cuban laboratory were in nearly 40% energy savings have been realized by employing a cellulaseless mutant followed by cold soda lime treatment. Patent on the basis of above results have been filed in US, Japan & UK for biopulping using unidentified fungi. Several studies on biopulping of non-woody materials including rice straw, kenaf, jute and reed canary grass have been published (15-19).

Work of solid state fermentation with fungi from the genus Pleurotus was investigated for wheat straw biolpulping and the results compared with those obtained with other lignolytic fungi. Chemical analysis & microscopy observation revealed the potential of Pleurotus species especially Pleurotus eryngii for selective removal of lignin from straw, resulting in separation of fibres similar to that obtained by chemical pulping. On the contrary, the well known lignolytic fungus Phanerochaete chrysosporium strongly hydrolysed polysaccharides & destroyed fibres. Fungal pretreatment resulted in a decrease in energy consumption during mechanical refining of the pretreated straw, the highest savings being obtained with P. eryngii. Moreover, when alkaline cooking was combined with the biological pretreatment, mechanical properties of the resulting paper board could be improved with respect to those obtained after biomechanical pulping, maintaining energy savings during refining.

The constituents & weight loss by different fungi and fractions corresponding to lignin removal and L/WL ratio after wheat straw treatment with different fungal strain under SSF condition are shown in Fig.7 & Fig. 8 respectively. Table-IV further shows the properties of wheat straw Bio-mechanical(BMP) and Bio- semimechanical pulps (BSCP) pretreated with P. eryngii compared with semichemical pulps (SCP) and control straw. Results clearly showed that handsheets made of this BSCP presented much better mechanical properties (They were prepared mixing with waste paper pulp) than those previously obtained by refining fungal pretreated straw (BMP). Cooking yield was comparable to those obtained after semi-chemical pulping in this case. Most positive effect of fungal treatment was also the lower energy consumption to obtain similar beating degrees. Comparison of pollution parameters from these two processes showed similar COD values and SST contents decreased after fungal treatment. The positive effect of fungal pretreatment in bleachability of wheat straw pulp has been described (21, 22).

A similar study with respect to decrease in refining energy (nearly 50%) has been reported after alkaline pulping of wheat straw treated with a crude enzyme preparation from the lignolytic fungi, lestinale edodes (23).

To sum up biopulping research is currently following two tracks.

-The first is to use the white rot organism as pretreating agents for subsequent mechanical pulping.

-The second is to use the isolated ligninase enzymes.

In the first case the challenge still is to optimise the organism for the fibrous raw materials being treated & thus bring down the treatment time. In the second case the thrust seems to be to produce the enzymes via recombinant DNA techniques and then to reconstitute an enzyme system that will duplicate in vivo lignin degradation.

PERSPECTIVE OF BIOPULPING IN INDIAN PAPER INDUSTRY:

Preliminary work on biological delignification has been carried out/ is being carried out in our country primarily for improving the nutritious status of decay animals, production of protein rich food & other related areas however, very little work has been reported in the area of biopulping. Although some studies were initiated on biodegradation of Dendrocalamus strictus (Bamboo) at Forest Research Institute Laboratory, Dehradun to see the effect of particular strains of white-rot fungi on bamboo dust. In this case treatment of bamboo dust with white rot fungi Coriolus hirsutus and Daedalia flavida over a period of 4-12 weeks of time resulted in appreciable loss of lignin and holocelluloses (24). Thus lignin degradation might allow easy separation of individual fibres during chemical/mechanical pulping resulting in decreased energy/chemical requirements.

Biotechnological application have yet to be recognised by Indian Paper Industry but begin has been made by some of the Paper Industry Associations and the paper industries. Central Pulp & Paper Research Institute has started its activities in the area of biotechnology in colloboration with some of the Institutions engaged in the respective area, paper industries and paper industry association. Some of the identified potential areas where the Institute is trying to explore the posibilities of bitechnological applications from developmental stage to demonstration scale, are:

- Storage of raw materials,
- Enzymatic prebleaching
- Bio-deinking
- Slime control in paper making and
- Effluent treatment

A more substantial impact of research and development in the area of biotechnology in Pulp & Paper Industry would make it possible to evaluate how promising such biotechnological approaches really are.

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