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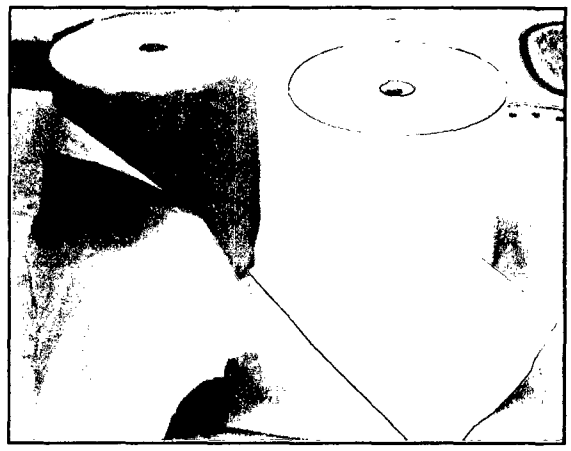
Biotechnological Application of Enzymes for making Paper Pulp from Green Jute/Kenaf (the whole plant)

FINAL REPORT

(January 2001 – December 2003)



International Jute Study Group (IJSG)

and

Bangladesh Chemical Industries Corporation (BCIC)

(Karnaphuli Paper Mills Limited – KPM)

Biotechnological Application of Enzymes for making Paper Pulp from Green Jute/Kenaf (the whole plant)

FINAL REPORT

(January 2001 – December 2003)

Prepared for:

International Jute Study Group (IJSG)
145 Monipuripara, Tejgaon (Near Farmgate)
Dhaka-1215, Bangladesh

Common Fund for Commodities (CFC)
Willemshuis Stadhouderskade 55
1072 AB Amsterdam
The Netherlands

and

United Nations Industrial Development Organization (UNIDO)
Vienna International Centre
P.O. Box 300
A-1400, Vienna, Austria
UNIDO Project: FC/RAS/00/153

Prepared By:

Dr. G. Mohiuddin
Project Leader
International Jute Study Group (IJSG)

and

Managing Director
Karnaphuli Paper Mills Limited (KPM)

Project Associates

Mr. Md. Abdur Razzaque, National Consultant
Dr. Mamunur Rashid, Sr. Researcher
Dr. Mizanur Rahman, Deputy Chief Chemist, KPM
Mr. Sk. Abul Hasib, Researcher, IJSG

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Introduction

Biotechnological Application of Enzymes for making Paper Pulp from Green Jute/Kenaf (the whole plant)

Introduction

The project aims at developing technologies for the use of green jute as raw material for the production of pulp for paper. In developing such a technology, it is intended to reduce the consumption of chemicals through the introduction of eco-friendly production processes, reduce energy cost and cost of production.

Conventional jute products are facing sever competition due to the emergence of synthetic. It is very much imperative for the survival of jute industry to make it competitive by reducing the cost and to use of jute/kenaf in various diversified products. Annual world production of jute and allied bast fibres is around 3 million tons (**Table 1**). In addition to fibre, 6.5 millions tons of sticks are also produced.

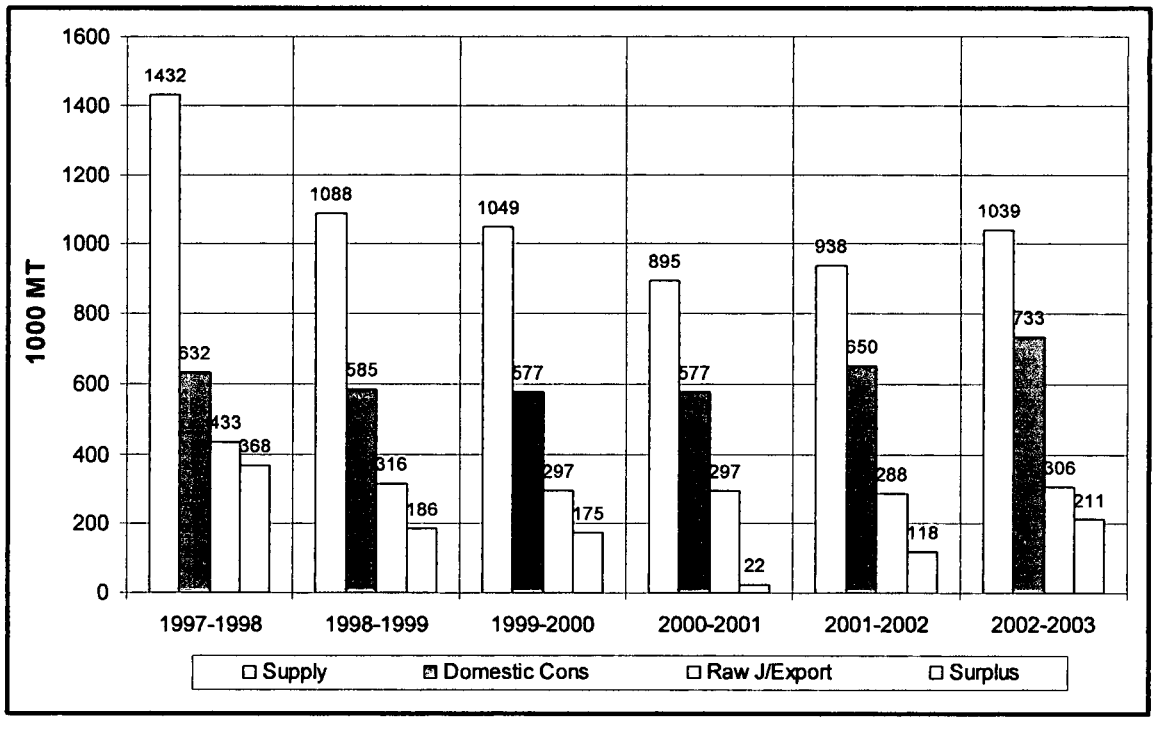
Table 1: World Production of Jute and Kenaf

Country	Area 000 ha	Jute (1000 MT)		Kenaf (1000 MT)	
		Fibre	Stick	Fibre	Stick
World	-	2,612.7	5,225.4	433.7	1,301
Bangladesh	426	777	1,554	--	--
India	1070	1,778	3,556	202	606
China	56	--	--	130	390
Thailand	19	--	--	30	90
Nepal	11	16	32	--	--

Source: *FAO, jute, kenaf and allied fibres June, 2003. CCP: JU/HF/ST/2003/1.

**Calculated from Dempsey James, M. Fibre Crops, 1975. A University of Florida Book

It appears from last five years statistics of Bangladesh that carry over quantity of jute fibre was above 100,000 MT which is about 300,000 MT of dried whole jute plant (jute fibre and jute stick) except in 2000-2001 (FAO-CCP: JU/HF/ST/2003/1).



*Source: CCP: JU/HF/ST/02/1, CCP: JU/HF/ST/01/1, CCP: JU/HF/ST/00/1, CCP: JU/HF/ST/99/1, CCP: JU/HF/ST/98/1.

Fig. I: Availability of Jute fibre as a raw material for Pulp and Paper (000 tons)

Demand for pulp and paper has increased significantly in the jute/kenaf growing countries. With the limited and dwindling forest resources it is not possible to depend on the conventional raw material for pulp and paper. Extensive research works by scientists from various Institutes and pulp and paper mills led to the conclusion that most conventional pulping techniques are suitable for jute/kenaf pulping. The large scale use of green jute in the pulp and paper sector will increase the demand of jute. Induced farmers will go for enhanced cultivation leading to the double cropping as demand will be created. The project also aims at reducing the consumption of chemicals or energy through the introduction of eco-friendly production processes leading to cost savings. As per the planned programme of the project, this new technology is expected to achieve a breakthrough in opening up a new vesta for the jute industry which would generate sustained demand for raw jute/kenaf and will facilitate stabilization of

prices of jute and increase farmers' income. The project is expected to have significant medium to long-term impact on the socio-economic development of the jute producing countries.

This pioneering area of diversified agro-industrial development is also expected to have substantial socio-economic impact on the national economics of producing countries, particularly on rural sector. It will contribute in rural poverty alleviation as jute is grown mostly by marginal and small farmers.

The project specifically aims at developing biotechnological methods which would be applicable in processing of jute into pulp and paper products; i.e. biopulping for the pretreatment of the raw material to achieve savings in chemical or energy consumption and enzyme-aided bleaching for achieving chemical savings in bleaching. The advantages of bioprocesses have been published in various scientific journals. This process is challenging technologies for the industry, especially for the traditional sector, such as pulp and paper industry. It has, however, been clearly reported that biotechnologies can reduce environmental impacts in the pulp and paper sector. Of the process stages studied and developed, the application of enzymes in bleaching are already being used in several (about 25) mills worldwide (producing 500-1000 tons/day pulp), whereas the biopulping technology has so far only been tested in pilot trials, although in quite large scale (about 50 tons/day).

The relevant pulp manufacturing processes are chemical or mechanical processes. The project compares these processes and aims at identifying the most promising methodologies.

The primary beneficiaries of this project from developing countries are Bangladesh, China and India who have been directly involved in the activities of the project. From the consuming countries France and the Netherlands have been participated in the project. It should be noted, however, that the project outcome will be of interest to a large number of countries who would derive benefits from technology transfer emanating from the project.

The project was funded by Common Fund for Commodities (CFC) and co-financed by the Government of France, The European Commission (EC) and the Government of Bangladesh.

The project was implemented with the collaboration of Bangladesh Chemical Industries Corporation (BCIC) and Bangladesh Jute Research Institute (BJRI) in Bangladesh, Agrotechnology & Food Innovations (AFI, former ATO) in The Netherlands, Centre Technique du Papier (CTP), France, Central Pulp & Paper Research Institute (CPPRI) in India and the Institute of Bast Fibre Crops (IBFC) and Yuanjiang Mills in China.

In view of the above this project was initiated with the following five main objectives:

Objective - 1: To identify and collect micro-organisms and processes currently being used in different pulp and paper mills and select suitable ones for jute bio-pulping.

Objective - 2: To develop most suitable enzymes for bio-pulping and bio-bleaching and to apply the same for preparing handsheet at BCIC, AFI, CPPRI, CTP and IBFC.

Objective - 3: To manage the black liquor produced during pulping and effluents generated during bleaching and identify suitable methods for green jute storage.

Objective - 4: Large scale trial application of enzymes.

Objective - 5: Dissemination of project results.

Executive Summary

EXECUTIVE SUMMARY

OBJECTIVE – 1

1.1 To identify and collect micro-organisms and processes currently being used in different pulp and paper mills and select suitable ones for jute bio-pulping.

According to the objective, a computer search was made for biopulping and biobleaching. Approximately 45 references (**Appendix-H**) were found relevant to the project. Such references provided some information about biopulping and biobleaching for selection of known culture for the studies.

On the basis of the information found in the literature the following microorganisms suitable for biopulping were identified.

Ceriporiopsis subvermispora, Phanerochaete chrysosporium, Pleurotus eryngii, Trametes versicolor, Pleurotus ostreatus etc.

On the basis of collected information, communications were made with the institutes concerned with biopulping, biobleaching and making pulp and paper using kenaf and softwood.

Project Leader went on a study tour for 2 weeks as a part of the project activities to visit some of the Institutes/Universities of Canada and USA and to collect suitable microorganisms, other related published literature and scientific papers, exchange ideas and to see biopulping and biobleaching operational procedures. The detailed report is shown in **Appendix-F**.

1.2 Comparative study of different microorganisms used in various pulp and paper mills in Europe, USA and Canada was made. The details report on this task is shown in **Appendix-G**.

1.3 Collection of Microorganisms

Following strains were collected from USDA, FPL, Madison, German Type Culture Collection, University of Wisconsin and University of Greenwich.

P. chrysosporium (5 strains),

C. subvermispora (4 strains),

Pleurotus (1 strain) and

ST (2 strains).

1.4 Isolation of microorganism for biopulping and biobleaching

A. Isolation and screening of microorganisms for biopulping

A large number of fungal strains (about 30 strains) were isolated from different natural habitats and then screening was carried out for isolation of potential ligninolytic fungi. Polymeric dye poly–R478 was used in growth medium to determine the ligninolytic capability of fungal strains, as the bleaching of the dye is associated with ligninolytic capability.

12 fungal strains (isolated and collected) were selected for final screening on solid and liquid media. Out of the 12 strains *F. lignosus* and *C. subvermispora* were exhibited highest percentage of poly–R bleaching. The details on the experimental procedure are shown in **Appendix-A.**

B. Isolation and screening of xylanase degrading microorganisms for biobleaching

30 thermophilic fungal strains were isolated from different habitats for the production of xylanase. All the isolated strains were screened and only three strains were selected for xylanase production. The details on the experimental procedure are shown in **Appendix-A.**

This task (Objective-1) has been accomplished.

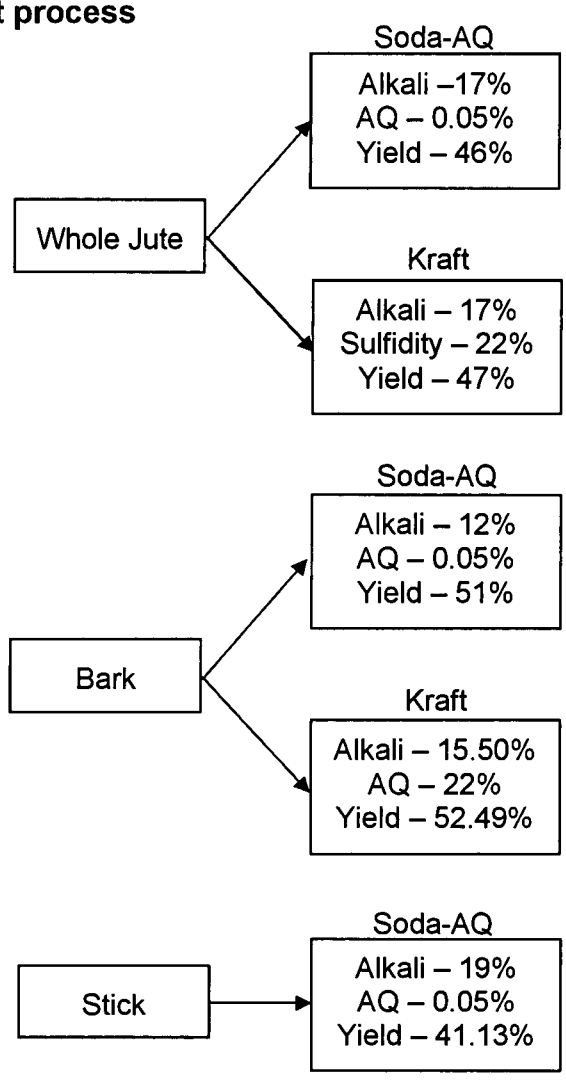
OBJECTIVE – 2

To develop most suitable enzymes for bio-pulping and bio-bleaching and to apply the same for preparing handsheet at BCIC, AFI, CPPRI, CTP and IBFC.

Control pulping Experiments

In order to optimise the conditions of pulping, the liquor ratio with jute chips, AQ dose in Soda-AQ process and requirement of alkali percentage, a number of experiments were conducted at Karnaphuli Paper Mills with a group digester using 60 g materials to produce bleachable grade pulp of Kappa No. 20-22. Whole jute, bark, and stick have been separately used to produce pulp and to make a comparative study. The observations, which resulted in desired kappa No. 20 are presented in the following table:

Optimization of pulping using whole jute, bark and stick in Soda-AQ and kraft process



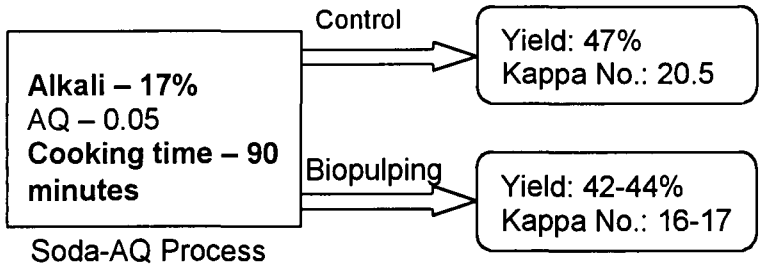
The details on the experimental procedure are shown in **Appendix-B**.

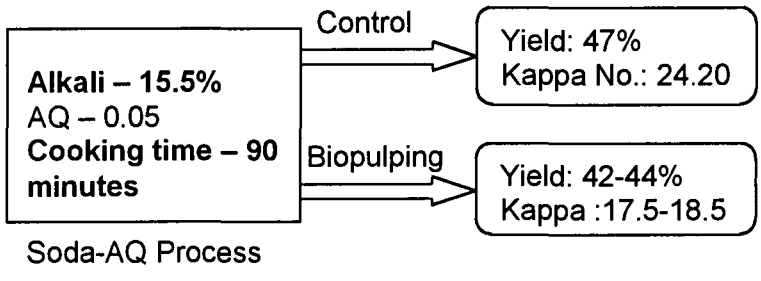
Biopulping Experiments

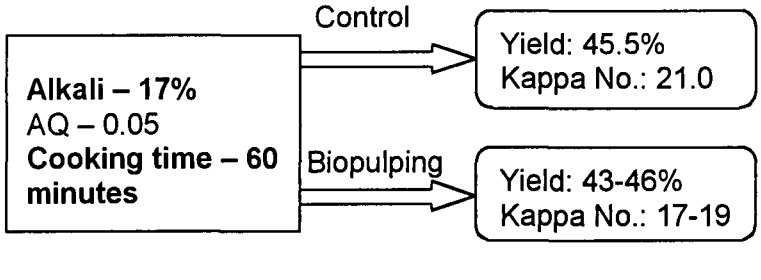
Biopulping experiments were carried out with optimum condition of pulping to reduce chemicals requirement, cooking time and improvement of physical properties of handsheet.

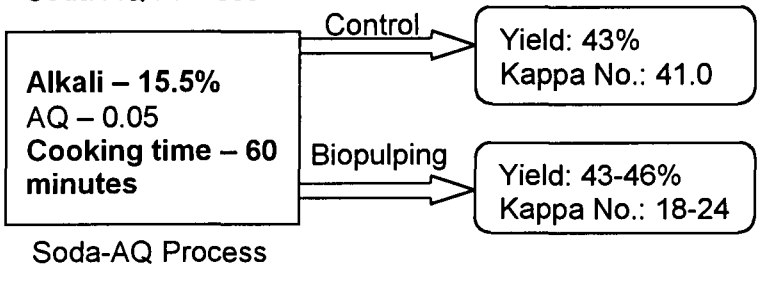
With the optimum conditions obtained at Karnaphuli Paper Mills Ltd. 61 biopulping experiments were conducted for bleachable grade pulp of kappa No. 20 using 4 strains of *P. chrysosporium*, 2 strains of *C. subvermispora*, one strain of *F. lignosus* and 2 strains of ST in Soda-AQ and kraft process.

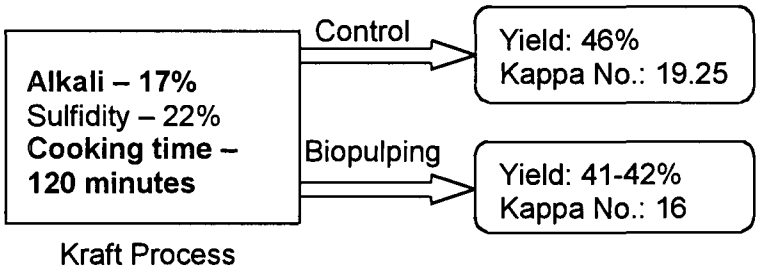
In Soda-AQ process, alkali and cooking time were varied, while in Kraft process only cooking time was varied to see the benefits of biopulping in different conditions. Out of the nine strains, *F. lignosus* and *C. subvermispora* were found to be suitable for biopulping in both the Soda-AQ and kraft processes. The average optimized results of the above mentioned strains in the biopulping process as well as the results of the control process are shown in the following diagrams.



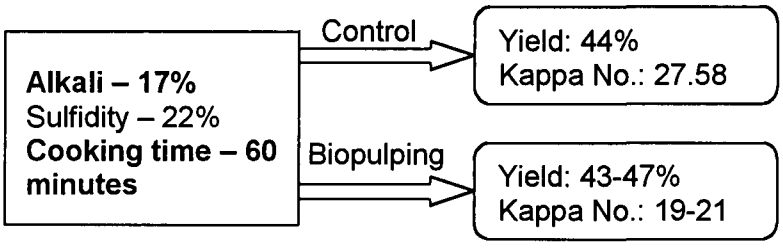








Kraft Process



Kraft Process

By looking at the results, the benefits of the biopulping process can be summarized as:

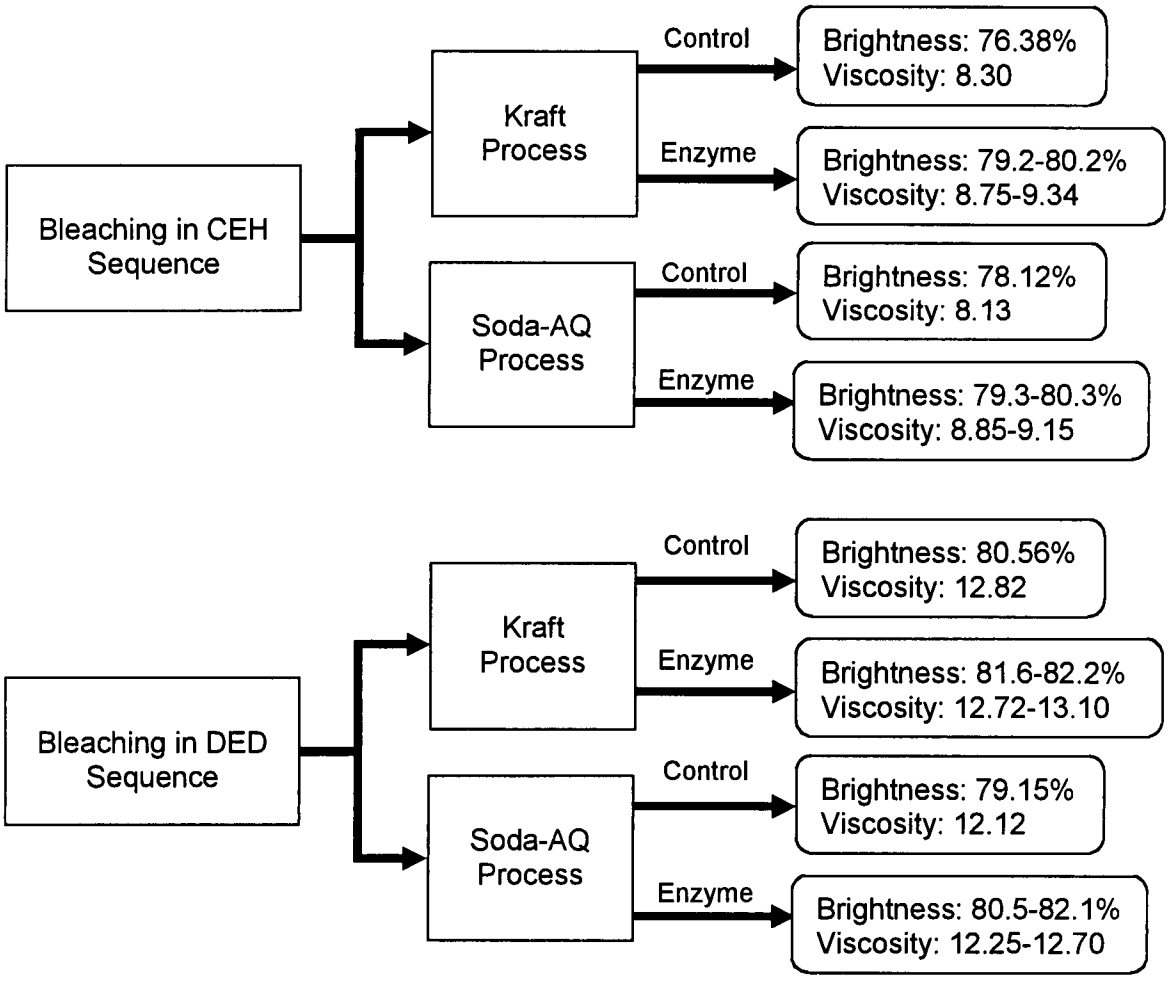
- Less chemical is required in the biopulping process in case of Soda-AQ.
- Cooking cycles can be increased which will facilitate to have more throughputs in the existing mills.
- Physical properties of paper (burst, tear and tensile index) can be improved significantly (20-40%).

The details on the experimental procedure are shown in **Appendix-C**.

Bleaching Experiments

Bleaching experiments were conducted in conventional CEH (chlorine-alkali-hypochlorite), elemental chlorine free (ECF) and OCEH sequences with and without enzyme.

The results of bleaching in CEH and DED sequences following the Kraft and Soda-AQ processes are showing that both brightness and viscosity are improving due to the enzyme treatment (average results of three commercial and developed enzymes of IJSG).



By looking at the results, the benefits of the biobleaching process can be summarized as:

- Reduction of active chlorine 9-12% in CEH and 15-17% in DED.
- Reduction of AOX in the effluent was due to reduced chlorine requirement. The AOX decreased in proportion to the decreasing chlorine usage.

- Increase in effluent's BOD and COD, indicating that the effluent is more amenable to biological degradation. There is an increase in the bleach effluent resulting from the release of low molecular weight xylose from the pulp.
- Application of xylanase in OCEH sequence, before and after oxygen, did not reduce chlorine requirement; but the brightness was improved by 2-3 units when xylanase is used after oxygen in Soda-AQ.
- All the four enzymes, three commercials and one developed, reduce the Kappa no. and improve the brightness.

The details on the experimental procedure are shown in **Appendix-D.**

This task (Objective-2) has been accomplished.

OBJECTIVE - 4

Large scale trial application of enzymes.

Large scale and commercial trial of pulp and paper.

With the optimum conditions obtained at Karnaphuli Paper Mills Limited, IJSG/BCIC scientists conducted one large scale trial for the production of kraft paper (14 MT of dried jute plant which is equivalent to **56 MT** of green jute plant) and one commercial trial for the production of writing paper (80 MT of dried jute plant which is equivalent to **320 MT** of green jute plant) in April, 03 and October, 03 respectively. In these trials stationery digester was used. For the production of kraft paper 12% alkali as Na_2O and 20% sulfidity were used. For the production of writing paper 15.5% alkali as Na_2O and 20% sulfidity were used. Bleaching of the washed, screened and refined pulp was conducted in the conventional bleaching sequence chlorine-alkali-hypochlorite (CEH). After the application of chlorine (0.22 of kappa no. of pulp) the pulp was washed and then alkali was used (2% NaOH). After alkali extraction the pulp was again washed and then hypochlorite (1.5%) was used for final bleaching. Bleached pulp was used for making paper (70 gsm) in paper machine having the speed of 175-200 M/minutes. The details on the experimental procedure are presented in **Appendix-E**.

- Yield of kraft pulp was 52%.
- The physical properties of kraft paper were compared with that of bamboo.
- Physical properties of kraft paper were superior to paper that made from bamboo.
- Yield of pulp for writing paper was 45-48%.
- The physical properties of paper in kraft process were compared with that of bamboo and hard wood.
- Physical properties of the paper were superior to paper that made from bamboo and hard wood.
- The brightness of the paper was 80-83% ISO.

This task (Objective-4) has been accomplished.

Conclusion

- Whole jute and bark (unretted fibre) are suitable as raw material for pulp and paper in both the chemical processes (Soda-AQ and kraft).
- Biotechnological application in chemical pulping require less chemical and improve the physical properties of paper significantly.
- In both the Soda-AQ and kraft processes, cooking time can be reduced (from 120 minutes to 60 minutes in Kraft process and from 90 minutes to 60 minutes in Soda-AQ process). As a result, cooking cycles can be increased which will facilitate to have more throughputs in the existing mill.
- Jute pulp can be bleached in conventional (CEH) and elemental chlorine free (ECF) and OCEH sequences.
- Use of xylanase in these bleaching sequences led to reduction of chlorine. Application of biotechnology will reduce chemical requirement.
- This will make the product cost - effective and environment - friendly.
- With the existing facilities of chemical pulping mill of BCIC, whole jute plant can be used commercially for the production of pulp and paper. Quality of paper is suitable for wrapping and different grades of writing paper. Physical properties of unbleached and writing paper are superior to paper made from bamboo and tropical wood.

Appendix - A

APPENDIX-A

Objective - 1.4: Selection of microorganism for biopulping and biobleaching

Isolation of microorganism for biopulping and biobleaching

A. Isolation and primary selection of lignolytic fungi

Lignin degrading microorganisms, which are biological source of ligninolytic enzymes, are abundantly found in nature. They are very common in compost, rotten wood, saw dust and different forest waste lignocellulosics, where the lignin substances are being decomposed under natural conditions. To search for highly potential fungal strains from wide range of natural habitat, an extensive screening programme was undertaken. The wide varieties of habitats were chosen to cover the search area under different nutritional and environmental conditions.

A large number of fungal strains (about 30 strains) were isolated from different natural habitats and then screening was carried out for isolation of potential ligninolytic fungal strains. Initially fungal strains were isolated on PDA (Potato Dextrose Agar) and MEA (Malt Extract Agar) media. Preliminary screening was carried out by growing fungal isolates on lignin agar medium (containing 0.1% glucose). On the basis of large colony formation and growth rate on Lignin-agar plates, 2 strains were selected for final screening.

MATERIAL AND METHODS

Screening of lignin degrading microorganism .

Polymeric dye was used in growth medium to determine the ligninolytic capability of fungal strains, as the bleaching of the dye is associated with ligninolytic capability of fungal strains. The use of polymeric dye in lignin degrading study of *P. chrysosporium* was first described by Glenn and Gold (1983). There is growing evidence that dye decolourization is correlated with the ligninolytic activity of the fungus and that the efficiency of decolourization is correlated with its ability to degrade lignin model compounds (Platt *et al.,* 1985). Since the 80's the decolourization of some dyes like Remazol Brilliant Blue (RBBR) and several Poly-R dyes (Poly-R 478, Poly-R 411) has been used as an indication of ligninolytic ability of fungal strains. Although this methodology was more or less

neglected for several years, in 1991 Pasti and Crawford proved that the decolourization ability and ligninolytic activity of some microorganisms are correlated. All the ligninolytic white-rot fungi grown on Poly-R medium can decolourize the Poly-R from dark red to yellow.

Experimental procedure for screening of lignin degrading microorganism

The characterization of Poly-R dye bleaching was carried out in two phases. The first phase was on solid media and the second phase was on liquid media with Poly-R. The 12 selected fungal strains were screened on solid media.

Screening ligninolytic fungi in Solid culture

Twelve strains were grown on solid medium containing lignin (with 0.1% glucose) as sole carbon source for enrichment of growth. All these strains were selected primarily on the basis of colony size, shape and growth rate. These strains were then finally screened on Poly-R containing solid media to detect their ligninolytic capability.

Liquid culture with Poly-R

All the 12 strains were cultured on Poly-R containing liquid media at three different pH levels of 3.5, 4.5 and 5.5.

Medium composition for liquid culture with Poly-R

Glucose	:	10 g
Ammonium tartrate	:	0.5g
Potassium dihydrogen phosphate	:	2.2g
Magnesium sulphate	:	0.5g
Calcium chloride	:	0.1g
Thinamine-HCl	:	0.01mg
Poly-R478	:	0.2g
Distilled water	:	1000ml

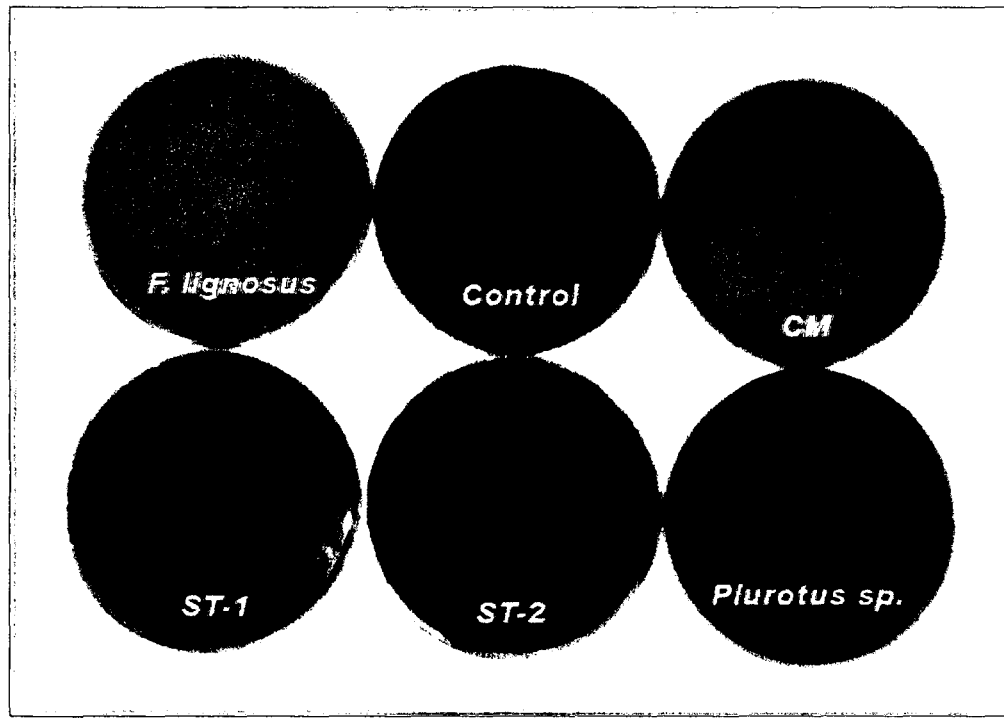
pH of the medium was adjusted to 3.5, 4.5 and 5.5.

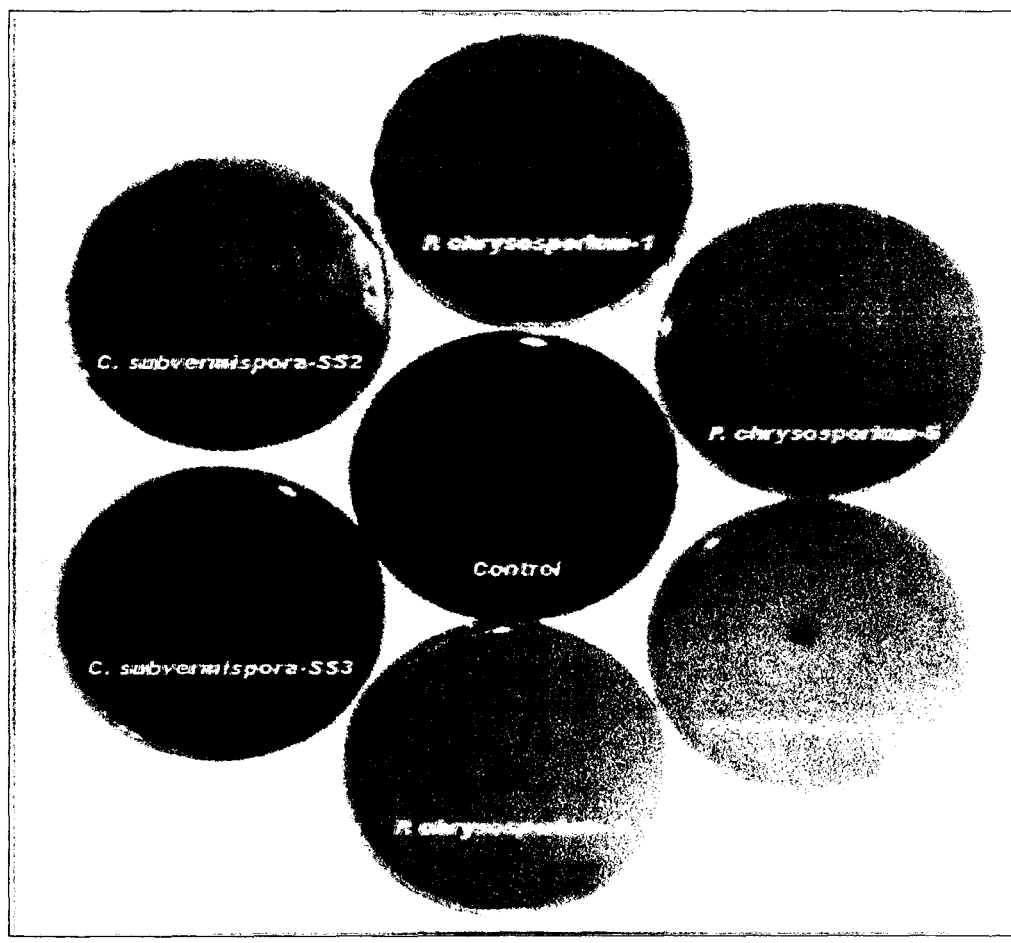
Results

All the strains bleached the red colour of Poly-R to yellow. All the strains bleached to certain extent the red colour of Poly-R to yellow. *F. lignosus* and *P. chrysosporium* started the Poly-R break down 3 days after the inoculation and within 10 days the red colour of Poly-R plates was totally bleached to yellow. However, strain *C. subvermispora*-2, *C. subvermispora*-3, and ST-1 & ST-2 also bleached Poly-R to yellow but they took 15, 18, 24 and 28 days respectively after inoculation.

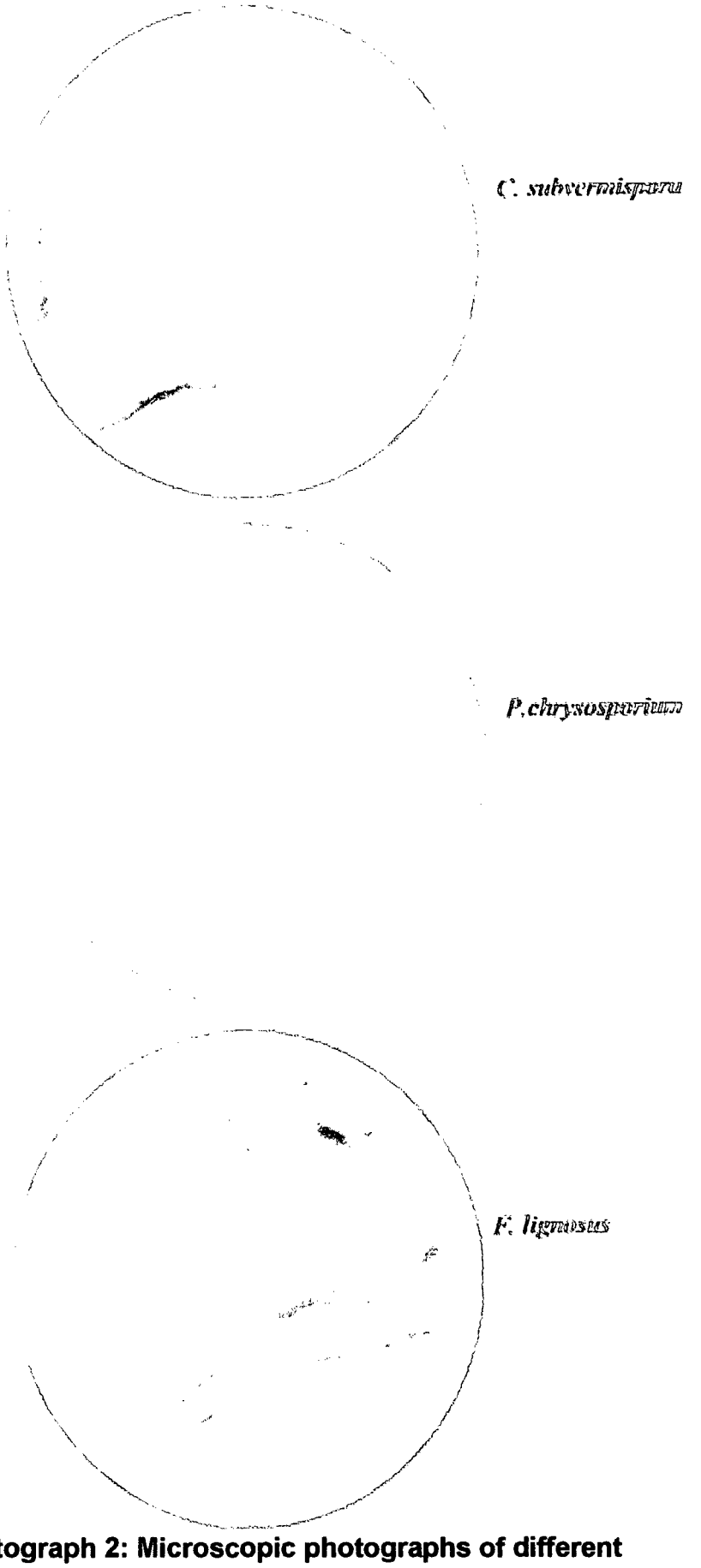
From the experimental results it is clear that the strain *F. lignosus, P. chrysosporium and C. subvermispora*-2 have the highest ligninolytic capability. The other strains of white- rot fungi also bleached the colour to some extent (**Photograph 1**).

F. lignosus and *P. chrysosporium* exhibited the highest Poly-R bleaching capability at pH 5.5. Spectrum of Poly-R is characterised by two picks at 350 nm and 519 nm (**Figure II and III**). Spectral changes of polymeric dye (Poly-R) at different pH levels were scanned and compared with control medium. In liquid media *P. chrysosporium* and *Coriolus sp.* showed highest percentage of Poly-R bleaching at pH 5.5 which was about 75.73 % and 92.23% respectively. *F. lignosus* and *C. subvermispora* exhibited highest percentage of Poly-R bleaching at pH 4.5 which was about 91.44% and 88.73% respectively (**Table 2**).





Photograph 1: Bleaching of Poly-R478 with different fungal strains



Photograph 2: Microscopic photographs of different fungi using for biopulping experiments

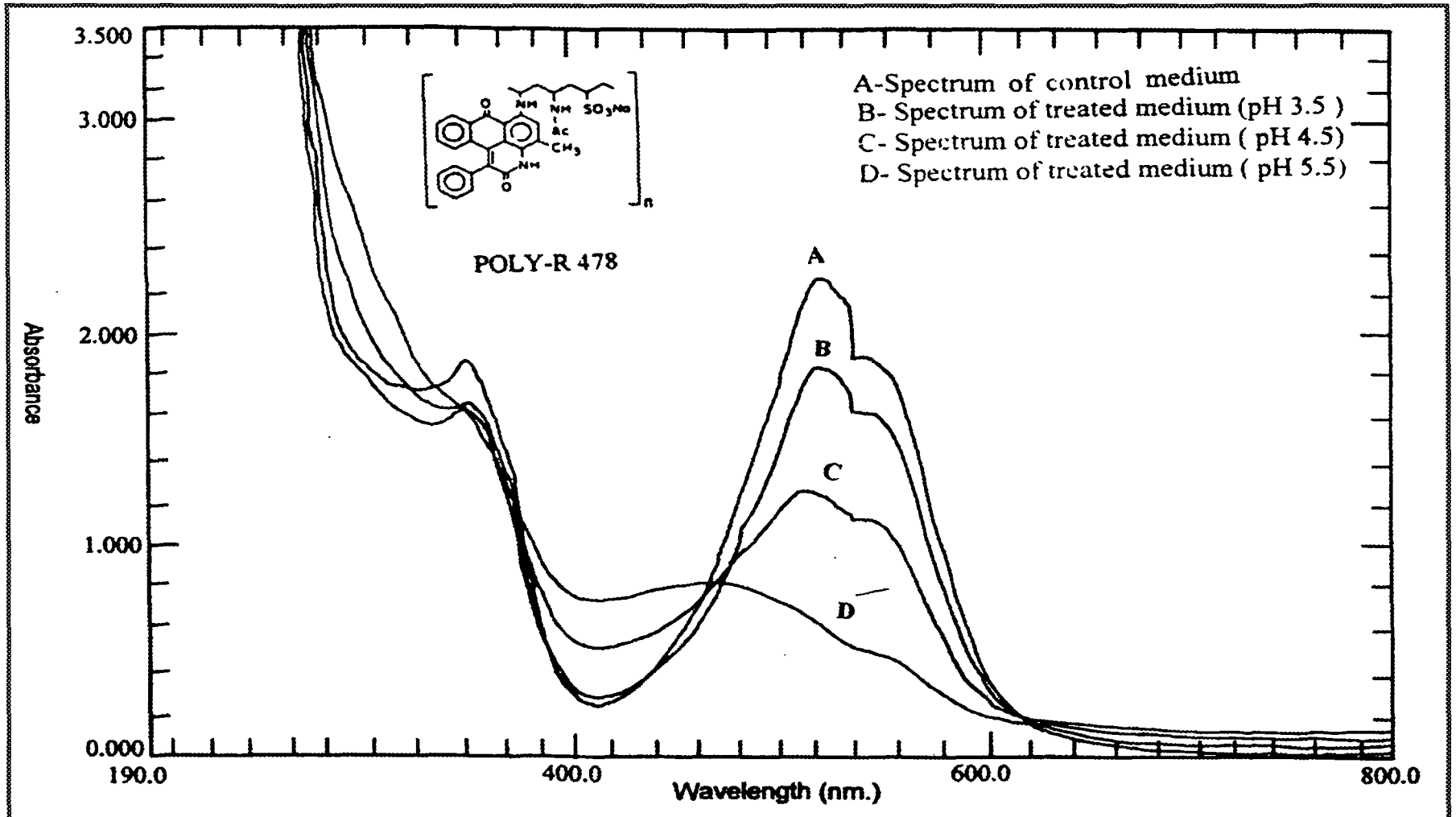


Fig3B: Chemical structure and Visible spectrum of Polymeric dye Poly-R (0.02%) (A) Visible spectrum of Poly-R control medium (before incubation). Spectrum of Poly -R after the treatment (15 days) with *P. chrysosporium* (B) at pH 3.5 (C) at pH 4.5 and (D) at pH 5.5

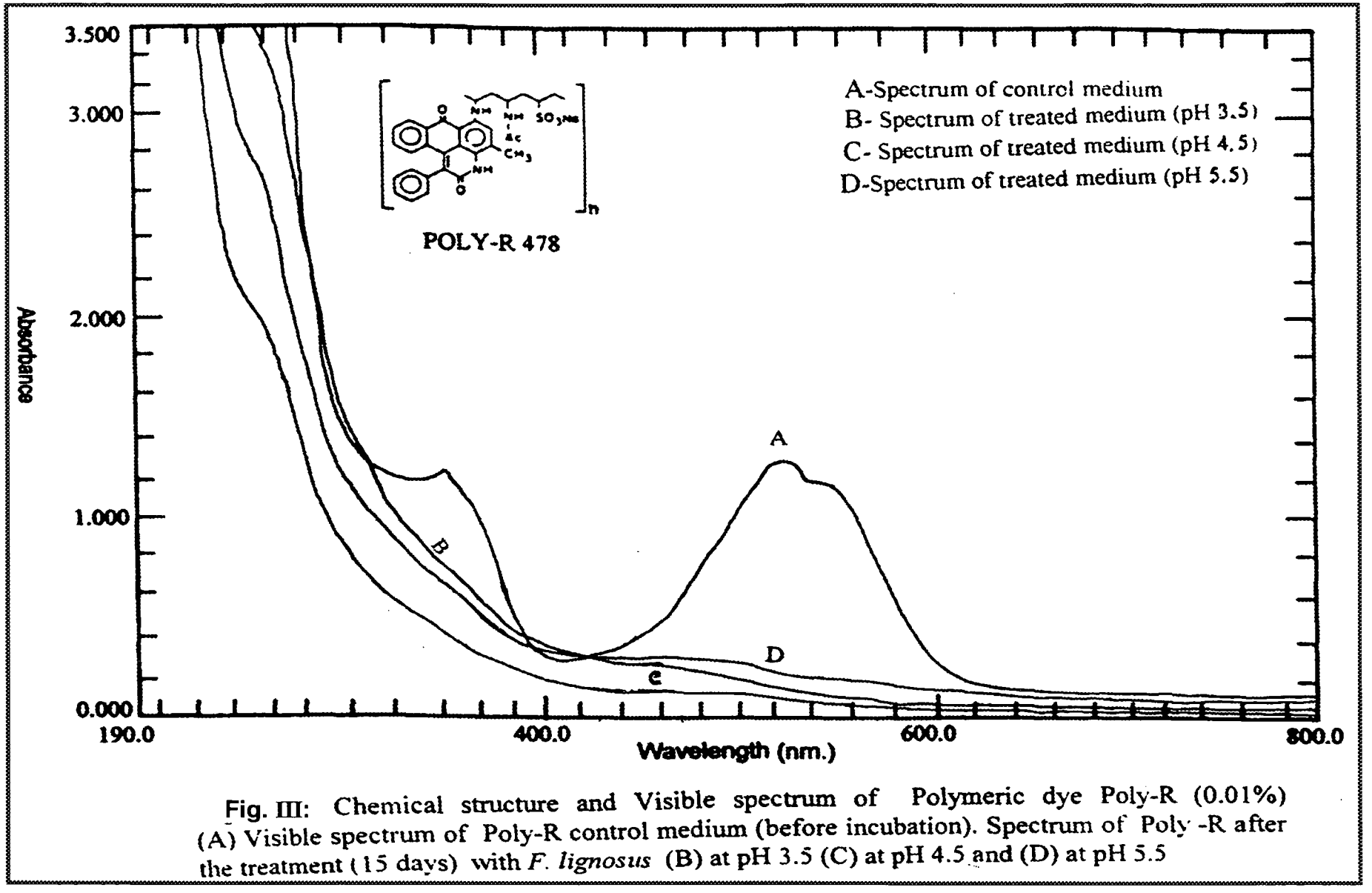


Fig. III: Chemical structure and Visible spectrum of Polymeric dye Poly-R (0.01%) (A) Visible spectrum of Poly-R control medium (before incubation). Spectrum of Poly -R after the treatment (15 days) with *F. lignosus* (B) at pH 3.5 (C) at pH 4.5 and (D) at pH 5.5

Table 2: Poly-R bleaching capability of all white-rot fungal strain in liquid media

Name of strains	Poly-R used in liquid media	Incubation time (days)	Different pH of media	Absorbance at 519 nm	Percentage of Poly-R break down
Control	0.02%	28	pH 3.5	1.262	-
	0.02%	28	pH 4.5	1.438	-
	0.02%	28	pH 5.5	0.804	-
Phanerochaete chrysosporium 1	0.02%	28	pH 3.5	0.490	60.25
	0.02%	28	pH 4.5	0.349	61.17
	0.02%	28	pH 5.5		75.73
Phanerochaete chrysosporium 3	0.02%	28	pH 3.5	1.008	20.13
	0.02%	28	pH 4.5	1.120	22.11
	0.02%	28	pH 5.5	0.581	27.36
Phanerochaete chrysosporium 4	0.02%	28	pH 3.5	1.025	18.78
	0.02%	28	pH 4.5	1.169	18.70
	0.02%	28	pH 5.5	0.356	55.72
Phanerochaete chrysosporium 5	0.02%	28	pH 3.5	1.125	10.85
	0.02%	28	pH 4.5	0.438	69.54
	0.02%	28	pH 5.5	0.285	64.55
Ceriporiopsis subvermispora 2	0.02%	28	pH 3.5	0.708	43.90
	0.02%	28	pH 4.5	0.162	88.73
	0.02%	28	pH 5.5	0.308	61.69
Ceriporiopsis subvermispora 3	0.02%	28	pH 3.5	1.150	8.87
	0.02%	28	pH 4.5	0.901	37.34
	0.02%	28	pH 5.5	0.329	59.08
Ceriporiopsis subvermispora 4	0.02%	28	pH 3.5	0.691	45.24
	0.02%	28	pH 4.5	0.226	84.26
	0.02%	28	pH 5.5	0.371	68.00
Pleurotus eryngii	0.02%	28	pH 3.5	1.200	4.91
	0.02%	28	pH 4.5	1.401	2.25
	0.02%	28	pH 5.5	0.597	25.75
Fomes lignosus	0.02%	28	pH 3.5	0.409	67.59
	0.02%	28	pH 4.5	0.123	91.44
	0.02%	28	pH 5.5	0.088	89.05
Coryolus sp.	0.02%	28	pH 3.5	0.097	92.23
	0.02%	28	pH 4.5	0.190	86.79
	0.02%	28	pH 5.5	0.317	60.57
ST – 1	0.02%	28	pH 3.5	1.005	20.36
	0.02%	28	pH 4.5	1.330	7.51
	0.02%	28	pH 5.5	0.639	20.52
ST – 2	0.02%	28	pH 3.5	1.250	0.96
	0.02%	28	pH 4.5	1.348	6.25
	0.02%	28	pH 5.5	0.623	22.51

B. Isolation and Screening of Xylanase Producing Microorganism

Enzyme activity was demonstrated by the hydrolysis of substrate incorporated, generally as the main carbon source, in a solid agar medium distributed in petridishes. The medium contains all the necessary ingredients (minimal medium) to afford sufficient initial growth of the inoculum. After an appropriate incubation period, the activity can be detected around the colonies by the appearance of zones revealed either by substrate clearances or decoloration.

MATERIAL AND METHODS

Screening of xylanase producing microorganism

Petriplates of the following media composition were prepared for isolation of xylanase producing microorganism.

Carbon source	:	Xylan (larch wood)
Yeast –N-base	:	6.7 g/L
Oxgall	:	10 g/L
Yeast extract	:	2 g/L
Agar	:	15 g/L

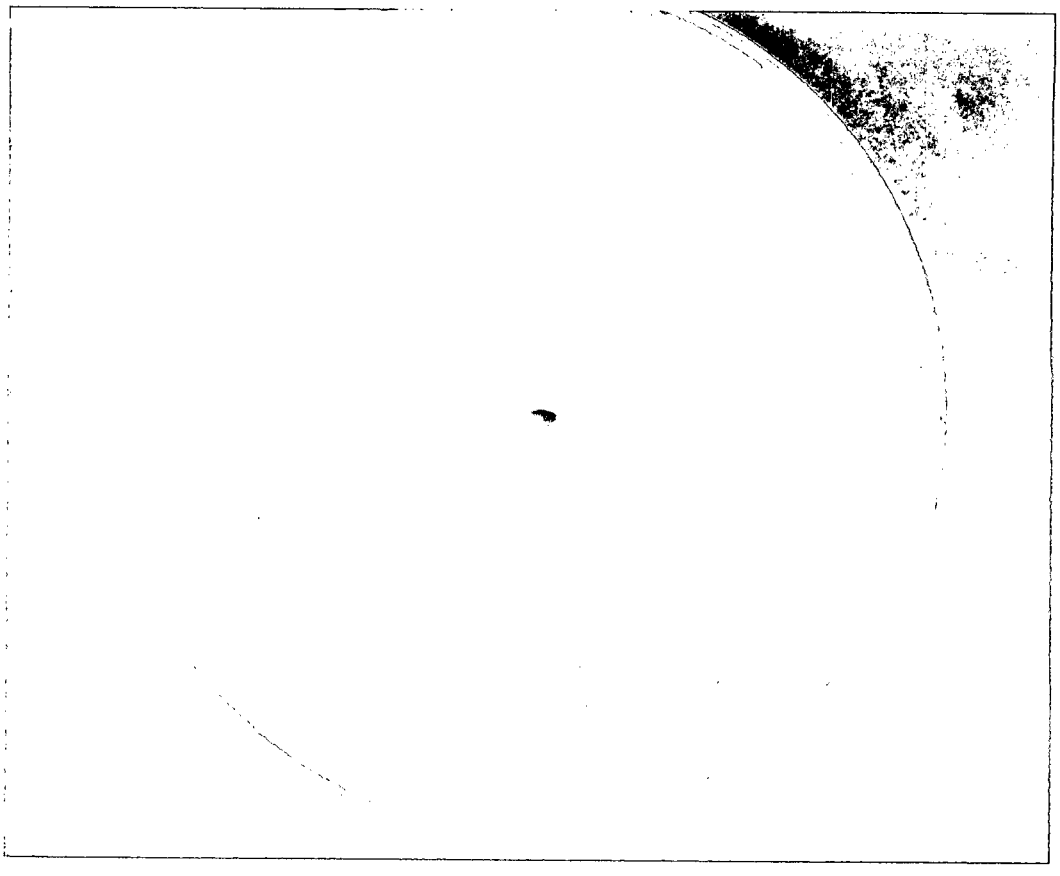
Media of above composition were prepared in distilled water and autoclaved at 121°C for 15 minutes. The sterile liquidified medium was cooled to 45°C-50°C and distributed into petridishes.

The plates were inoculated with isolated and collected microorganisms to be screened for enzyme activity. The plates were incubated at optimum temperature for 3-10 days. Xylan hydrolysis was observed only after staining the plates. To enhance the visibility of hydrolytic zones around the growing colonies, the plates were flooded with 96% ethanol. Although clear zones were visible within 3-4 hours of incubation, prolonged incubation enhances the contrast (**Photograph 3**).

Following thermophilic isolated strains were selected for xylanase production.

Name of the Strains	Isolated/collected
***Thermomyces lanuginosus* RT9**	Locally isolated
***Thermomyces lanuginosus* M3**	Locally isolated
***Thermomyces lanuginosus* M17**	Locally isolated

Out of the three thermophilic microorganisms *Thermomyces lanuginosus* RT9 was isolated from a bin of a Jute mill of Bangladesh and this strain was deposited to DSM (German Type Culture collection). Several papers have been published on the production of xylanase with this strain from the Technical University of Graz, Austria and University of British Columbia has also published paper with this strain. The remaining two were collected recently from decayed wood chips.



Photograph 3: Clear zone of hydrolysis showing xylanase activity by *Thermomyces lanuginosus* M3 on xylan-agar medium

Photograph 4: Microscopic photograph of mycelia of the fungal isolate M3

Photograph 5: Microscopic photograph of spore of the fungal isolate M3

Preparation of Inoculum

The following media composition for the inoculum was prepared.

Yeast extract	0.3%
Malt extract	0.3%
Peptone	0.5%
Dextrose	1.0%
pH	6-8

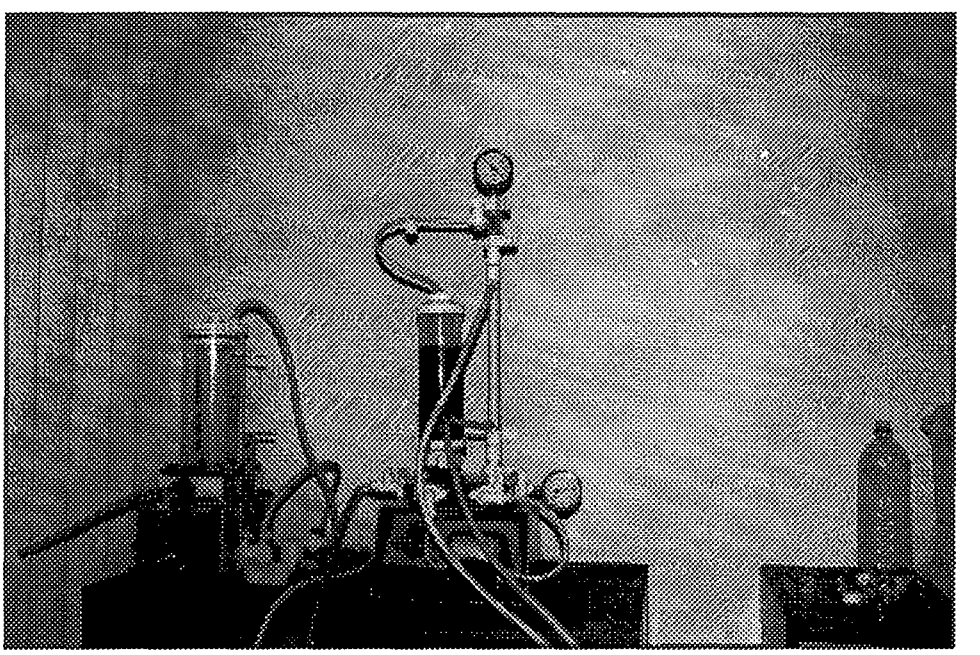
After inoculation microorganisms were grown in a shaker at 100 rpm at 55°C for 3 days.

Preparation of solid state fermentation (SSF)

30 gm of selected lignocellulosic substrate was taken in each of the one litre flask. Distilled water was added to the selected lignocellulosic substrate to give a moisture content of 40 to 100 percent for each replicate. The substrates were mixed thoroughly. The flasks were then properly cotton plugged and autoclaved at 121°C for 20 minutes. Inoculum was then added to the flasks and the flasks were incubated at 55°C for 7 days.

Extraction of Enzyme

Enzyme was extracted by adding 5 volumes of water containing 1% (W/V) NaCl solution in a container and was left for 2 hours with occasional shaking. The slurry was filtered through a sieve of 100 mesh. The filtrate was centrifuged at 6000 rpm. For the concentration of enzyme, quixstand ultrafiltration unit was used with 3,000 mw cut off cartridges (**Photograph 6**).



Photograph 6: Quixstand ultrafiltration unit

Assay of Enzyme

The assay is based upon the increase in reducing groups following the incubation of substrate with the enzyme solution. The enzyme is incubated with carboxymethyl cellulose (CMC) for assaying CMCase activity and with xylan for assaying xylanase activity. The reducing sugar released into solution, following hydrolysis, were measured by dintrosalicylic (DNS) method.

Selection of Carbon sources

The effect of carbon sources on enzyme production was tested using different kinds of lignocullulosic substrates such as wheat bran, saw dust and sugarcane bagasse under SSF. Enzyme production was investigated using three carbon sources. The initial water content of all media was adjusted to 70%. For each substrate duplicate flasks were made. The effect of carbon sources was investigated under solid-state fermentation at 55°C and pH 6.5 for 07 days.

Effect of moisture content on enzyme production

100g wheat bran was mixed with 30ml, 40ml, 50ml, 60m,, 70ml, 80ml, 90ml and 100ml distilled water to make the moisture levels 30, 40, 50, 60, 70, 80, 90 and 100% respectively. 01 ml of spore suspension was added in each flask and mixed properly. All flasks were incubated at 55°C for 7 days. The relative humidity inside the incubator was maintained 60-70%. Enzyme was extracted and assayed.

Effect of different temperature on enzyme production

Solid state fermentation was carried out with isolated fungal strain at different temperatures such as 40, 45, 50, 55 and 60°C. After 7 days of incubation, enzyme was extracted from each flask and assayed.

Effect of initial pH on enzyme production

The effect of initial pH in the culture on the production of enzymes were investigated using 50 gm wheat bran at 55°C. The initial pH of the culture was adjusted to 4.0, 5.0, 6.0, 7.0 and 8.0 for isolated and collected fungal strains.

Enzyme from each flask was extracted after incubation of 07 days and xylanase activity was assayed.

Effect of incubation time on enzyme production

50 gm of wheat bran were taken in each 100 ml conical flask and soaked with 35 ml distilled water. All the flasks were autoclaved and inoculated with 1 ml of fungal spore suspension. The flasks were incubated at the same cultural condition. Enzyme was extracted and assayed at every 24 hours starting from the 3^{rd} day upto 10^{th} day of inoculation.

Thermal stability of xylanase

For determination of thermal stability, the xylanase preparation was incubated in a water bath at constant temperature (50 to 80°C) for 5 hours). Samples were drawn at 1 hour interval. The residual xylanase activity was determined at pH 6.0 and at 50°C.

RESULTS

About 50 different types of thermophilic xylanolytic fungi were isolated and collected from different places of Bangladesh. After preliminary screening and growth performance of the isolated fungi, 10 fungi were selected for final screening. Among the 10 fungi only three fungal strains (**Strain-M3 & M17 and *Thermomyces lanuginosus* RT9**) were finally selected for xylanase production. M3 and M17 were identified **by CABI Bioscience, U.K**. Both the strains ware identified as ***Thermomyces lanuginosus***. RT9 was identified by the **Centraalbureau voor Schimmelcultures**, Baarn, The Netherlands and was identified as *T. lanuginosus*.

Selection of carbon source for xylanase production

Different carbon sources such as wheat bran, saw dust, sugarcane bagasse, etc. were used. Results are presented in the **Table 3.** All these three strains showed maximum xylanase activities (124.25, 145.75 & 133.10 IU/ml by RT9, M3 and M17 respectively) when enzyme was extracted by using wheat bran as a carbon source.

Table 3: Enzyme activity on different carbon sources

Carbon source	Microorganisms	Enzyme activity IU/ml	
		CMCase	Xylanase
Wheat bran	Thermomyces lanuginosus RT9	5.00	124.25
	M3	3.18	145.75
	M17	0.00	133.10
Sugarcane bagasse	Thermomyces lanuginosus RT9	5.75	42.10
	M3	10.18	31.95
	M17	0.00	31.60
Saw dust	Thermomyces lanuginosus RT9	3.05	91.52
	M3	5.15	112.20
	M17	0.00	101.72

Effect of moisture content on enzyme production

After selection of carbon source, wheat bran was used for enzyme production in solid state fermentation. Results are shown in the **Table 4**. The rate of xylanase production was found to gradually increase with the increase of moisture level from 50% to 70%. Maximum xylanase activity was found at 70% moisture level for all the fungi.

Table 4: Effect of moisture content on enzyme production

Moisture content (%)	Fungal strains	Enzyme activity IU/ml	
		CMCase	Xylanase
40	Thermomyces lanuginosus RT9	0.00	0.00
	M3	0.00	0.00
	M17	0.00	0.00
50	Thermomyces lanuginosus RT9	0.10	10.25
	M3	0.00	12.20
	M17	0.00	10.12
60	Thermomyces lanuginosus RT9	3.50	112.75
	M3	4.50	132.50
	M17	0.00	91.78
70	Thermomyces lanuginosus RT9	8.70	122.50
	M3	10.52	156.00
	M17	0.00	113.90
80	Thermomyces lanuginosus RT9	9.25	111.55
	M3	8.25	134.50
	M17	0.00	94.20
90	Thermomyces lanuginosus RT9	1.15	20.90
	M3	1.05	52.35
	M17	0.00	12.52
100	Thermomyces lanuginosus RT9	0.00	0.00
	M3	0.00	0.75
	M17	0.00	0.50

It was also found that no enzyme was produced with these strains at low moisture levels (>40%). It became clear from the results that moisture content is an important factor for enzyme production.

Effect of different temperature on enzyme production

Since all the fungal isolates are thermophilic organisms it was of interest to determine the effect of growth temperature on xylanase production. In solid state fermentation with wheat bran, xylanase was produced over a range of temperature form 45°C to 70°C. But the optimum temperature of enzyme production was found to be 55°C for all the three strains (**Table 5**).

Table 5: Effect of different temperature on enzyme production

Temperature °C	Fungal strains	Enzyme activity IU/ml	
		CMCase	Xylanase
40	Thermomyces lanuginosus RT9	0.00	0.00
	M3	0.00	0.00
	M17	0.00	0.00
45	Thermomyces lanuginosus RT9	3.15	41.42
	M3	5.18	51.30
	M17	0.00	31.25
50	Thermomyces lanuginosus RT9	0.35	93.50
	M3	7.26	125.23
	M17	10.00	73.53
55	Thermomyces lanuginosus RT9	5.40	123.70
	M3	11.44	145.71
	M17	0.00	103.55
60	Thermomyces lanuginosus RT9	0.25	52.25
	M3	1.16	74.20
	M17	0.00	62.21

Effect of initial pH for enzyme production

To determine the optimum initial pH of the media, the pH of wheat bran was adjusted at different pH levels. All the three organisms were found to secrete

xylanase at a broad range of pH (5.0-8.0) and maximum xylanase was found at pH 6.0 – 7.0. Results are shown in **Table 6**. The pH optimization for xylanase production was supported by the results of the other workers.

Table 6: Effect of initial pH on enzyme production

pH	Fungal strains	Enzyme activity IU/ml	
		CMCase	Xylanase
4.0	Thermomyces lanuginosus RT9	0.05	21.00
	M3	0.00	31.02
	M17	0.00	20.50
5.0	Thermomyces lanuginosus RT9	2.12	31.75
	M3	4.25	42.06
	M17	0.00	31.20
6.0	Thermomyces lanuginosus RT9	4.40	113.35
	M3	6.30	166.27
	M17	0.00	74.00
7.0	Thermomyces lanuginosus RT9	2.25	92.80
	M3	4.36	104.55
	M17	0.00	63.57
8.0	Thermomyces lanuginosus RT9	0.00	11.30
	M3	0.00	15.25
	M17	0.00	6.00

Effect of incubation time on enzyme production

All the flasks were incubated at the previously obtained optimum conditions. Enzyme was extracted and assayed after 2, 4, 6, 8, and 10 days of incubation. Results are presented in **Table 7**.

From the results it was observed that the xylanase activity could be achieved after five days of incubation. It indicates that prolonged incubation is not required for xylanase production.

Table 7: Effect of incubation time on enzyme production

Incubation days	Fungal strains	Enzyme activity IU/ml	
		CMCase	Xylanase
3	Thermomyces lanuginosus RT9	0.00	10.00
	M13	0.00	15.20
	M17	0.00	0.22
5	Thermomyces lanuginosus RT9	0.27	73.05
	M3	1.05	125.80
	M17	0.00	63.50
7	Thermomyces lanuginosus RT9	4.50	44.50
	M3	2.70	106.20
	M17	0.00	44.00
10	Thermomyces lanuginosus RT9	6.52	33.20
	M3	7.60	85.50
	M17	0.00	23.80

Thermal stability of xylanase

Thermal stability of xylanase produced from three fungi were studied (**Table 8**). All the enzymes from the 3 strains were pre-incubated separately for 5 hours at different temperature (50^0C - 80^0C). Enzymes from all the three sources after treatment for 5 hours at 50^0C, 100% of the activities were retained. All the enzymes after incubating at 60^0C and 70^0C up to 2 hours, 100% of the activities were retained while at 80^0C RT9 retained only 10% activity. All the enzymes at 80^0C after incubating for 5 hours lost their activities. These findings demonstrate the high stability of enzyme at 50^0C and 60^0C for 2 hours. However, enzyme extracted from M3 and M17 retained their 80% activities up to 5 hours at 70^0C.

Table 8: Thermal stability of xylanase

Temperature °C	Fungal strains	Xylanase activity IU/ml at different time interval (Hours)				
		1	2	3	4	5
50	RT9	112.10	112.1	112.0	111.9	111.85
	M3	135.60	135.5	135.0	135.0	134.0
	M17	62.60	62.40	62.35	62.33	62.00
60	RT9	112.15	112.0	101.0	91.96	74.80
	M3	135.56	135.4	135.1	135.0	134.50
	M17	62.65	62.52	62.45	62.25	52.11
70	RT9	72.00	51.98	18.81	10.72	3.22
	M3	125.25	125.2	115.0	104.8	84.50
	M17	65.50	62.38	60.12	52.00	45.89
80	RT9	52.00	41.80	21.52	9.10	1.72
	M3	95.50	90.12	52.45	33.26	10.23
	M17	52.15	45.02	31.61	10.02	2.56

Conclusion

- Among the 3 isolated strains, M3 was found most suitable for the production of enzyme using wheat bran as a raw material in SSF.

- Thermal stability of enzyme is also a desired property for use in industrial processes. Xylanase from M3 was found to be stable up to 60^0C for 3 hours.

Appendix - B

APPENDIX-B

Optimization of pulping in chemical process (both Soda-AQ and Kraft Process)

The pulp and paper industry normally uses chemical or mechanical methods or a combination of the two methods to produce pulp of desired character. Chemical pulping accounts for about 75% of the world pulp production. Out of four Pulp and Paper mills of BCIC, North Bengal Paper Mills (NBPM) and Sylhet Pulp and Paper Mills (SPPM) use Soda Process where as Karnaphuli Paper Mills (KPM) uses Kraft Process. Considering the prevailing situation in the pulp and paper industry of Bangladesh experiments were conducted in both Soda-AQ & Kraft processes.

In order to optimise the conditions of pulping, the liquor ratio with jute chips, AQ dose in Soda-AQ process and requirement of alkali percentage, a number of experiments were conducted at Karnaphuli Paper Mills with a group digester using 60 g materials to produce bleachable grade pulp of Kappa No. 20-22 (which is considered to be suitable for good quality pulp) with two chemical processes.

Material and Methods

Chemical analysis of whole jute, bark, core and bamboo

Complete chemical analysis of whole jute, bark (bast fibre), core (stick) and bamboo were carried out and the results are shown in **Table 9**. From the results it has been observed that holocellulose content in the bark is higher than the whole jute and stick. Fibre length of bark is higher than those of whole jute and stick.

As a result yield of pulp and strength properties found to be superior with bark as a raw material.

Table 9: Chemical Composition of whole Jute, Bark and Bamboo

Chemical Composition	Whole Jute	Jute bark	Core	Bamboo (*Melocanna bacifera*)
Holocellulose	77	81.5	74.5	60
α-Cellulose %	41.5	51.5	36.3	50
β- Cellulose%	19.5	14.5	25.0	-
λ- Cellulose%	16.0	12.5	13.2	-
Klasson Lignin%	20	15	24	26
Ash content%	3.5	4.0	1.8	3.4
*Ave. fibre length mm	-	2.5	0.8 -1.00	2.8 -

- From the published results obtained at KPM.

Pulping in Soda-AQ and Kraft process (Laboratory scale)

Experimental:

Soda-AQ Process

In order to optimise the liquor ratio with jute chips, AQ dose in Soda-AQ process and requirement of alkali percentage, a number of experiments were conducted at Karnaphuli Paper Mills with a group digester (OY.SANTASALO-SOHLBERGAB, Helsinki, Finland) using 60g materials to produce pulp with Kappa No. 20-22. Different percentage of alkali and AQ were used. Rise of temperature from room temperature to cooking temperature (170^0C) was 90 minutes. Cooking time was 90 minutes for Soda-AQ process. In case of kraft process cooking time was 120 minutes.

Similar experiments were also carried out with bark and stick separately.

The chemical and physical properties of the pulp were analyzed according to TAPPI test methods: T-236 cm 85 (Kappa number), T-205 sp 95 (hand sheet preparation), T-403 om 97 (burst index), T-414 om 98 (tear index), T-404 cm 92 (tensile index), T-220 sp 96 (density), T-227 om 94 (freeness of pulp, ^{0}SR), T-230 om 89 (viscosity), T-423 om 89 (fold No.) etc.

Kraft Process

Similar experiments were carried out to optimise alkali charge (using different percentage of alkali) and sulfidity (20%, 22% and 25%) in Kraft process using whole jute and bark separately to produce pulp with Kappa No. 20-22.

Results

From our experimental results obtained at KPM, it has been observed that the liquor ratio 1:5 and alkali percentage 17% (as Na_2O) have been found to be most effective for getting required Kappa No. (**Tables 10 and 11**).

Similarly 12% alkali (Na_2O) and 0.05% AQ were suitable for bark (**Table 12**) and 19% alkali (Na_2O) and 0.05% AQ with a liquor ratio 1:5 was found to be suitable for stick (**Table 13**).

It has been found that 17% alkali (as Na_2O) with 22% sulfidity suitable for producing pulp with required Kappa No. (20-22%) from whole jute.

It has also been observed that 15.5% alkali and 22% sulfidity is suitable for bark to produce pulp with Kappa No. 20–22 (**Table 14**).

Table 10: Pulping of Whole Jute in Soda-AQ Process (using different liquor ratio)

Material	Na_2O (%)	AQ (%)	M:L	pH of liquor	K. No.	S. reject %	Yield %	Burst Index KPa m^2/g	Tear Index mNm^2/g	Tensile index kN/m	Density Kg/m^3	Freeness °SR
Jute Chips	17	0.1	1:4	11.45	15.5	Nil	45.34	2.59	8.86	38.17	510	16
Jute Chips	**17**	**0.1**	**1:5**	**11.24**	**15.3**	**Nil**	**45.00**	**2.80**	**9.25**	**39.65**	**517**	**17**
Jute Chips	17	0.1	1:6	11.35	14.8	Nil	45.12	2.53	7.15	36.68	515	17

* Rising time from 30 to 170°C 90 minutes, cooking temperature 170°C, cooking for 90 minutes, Liquor ratio 1:5, H Factor 1600

Table 11: Pulping of Whole Jute in Soda-AQ Process (using different % of Alkali & AQ)

Material	Na_2O (%)	AQ (%)	pH of liquor	Kappa No.	S. reject %	Yield %	Burst Index KPa m^2/g	Tear Index mNm^2/g	Tensile index kN/m	Density Kg/m^3	Free-ness °SR
Jute Chips	17.0	0.1	**11.24**	**15.3**	Nil	**45.1**	**2.80**	**9.25**	**39.65**	**517**	**17**
Jute Chips	**17.0**	**0.05**	**10.81**	**22.1**	**Nil**	**47.00**	**2.53**	**9.60**	**45.21**	**520**	**17**
Jute Chips	15.5	0.1	10.84	22.2	Nil	46.12	2.45	9.64	35.39	513	17
Jute Chips	15.5	0.05	10.02	26.4	Nil	47.23	2.33	9.80	30.93	508	17
Jute Chips	14.0	0.1	10.40	28.4	1.51	48.25	1.62	13.20	29.59	509	16
Jute Chips	14.0	0.05	9.75	31.0	5.10	49.10	1.40	13.10	25.09	504	16

* Rising time from 30 to 170°C 90 minutes, cooking temperature 170°C, cooking for 90 minutes, Liquor ratio 1:5, H Factor 1600

Table 12: Pulping of Jute Bark in Soda-AQ Process

Material	Na_2O (%)	AQ (%)	pH of liquor	K. No	S. reject %	Yield %	Burst Index KPa m^2/g	Tear Index mNm^2/g	Tensile index kN/m	Density Kg/m^3	Free-ness °SR
Bark	13	0.1	10.26	12.52	nil	49.97	3.90	20.46	57.07	520	15
Bark	13	0.05	9.95	15.96	nil	50.68	3.71	20.21	55.25	522	16
Bark	12	0.1	9.85	16.15	nil	52.22	3.47	19.78	65.83	517	16
Bark	**12**	**0.05**	**9.45**	**18.90**	**nil**	**51.00**	**3.87**	**18.60**	**60.32**	**518**	**15**
Bark	11	0.1	9.56	24.39	nil	53.00	3.27	20.66	54.46	518	16
Bark	11	0.05	9.25	28.15	1.10	53.85	3.65	15.35	45.60	519	16

* Rising time from 30 to 170°C 90 minutes, cooking temperature 170°C, cooking for 90 minutes, Liquor ratio 1:5, H Factor 1600

Table 13: Pulping of Jute Stick in Soda-AQ Process

Material	Na_2O (%)	AQ (%)	pH of liquor	K. No.	S. reject %	Yield %	Burst Index KPa m^2/g	Tear Index mNm^2/g	Tensile index kN/m	Density Kg/m^3	Free-ness °SR
Stick	16	0.1	10.10	26.30	1.20	43.15	2.12	12.13	55.23	490	18
Stick	16	0.05	9.65	29.36	2.25	43.70	2.36	10.23	45.20	500	18
Stick	17	0.1	10.73	23.18	nil	42.19	3.75	14.42	65.01	495	20
Stick	17	0.05	9.85	25.22	1.00	42.25	3.05	12.66	53.50	498	18
Stick	18	0.1	11.38	19.38	nil	40.71	3.76	11.52	68.00	496	23
Stick	**18**	**0.05**	**10.95**	**20.80**	**nil**	**41.13**	**3.65**	**11.22**	**60.35**	**500**	**19**

* Rising time from 30 to 170°C 90 minutes, cooking temperature 170°C, cooking for 90 minutes, Liquor ratio 1:5, H Factor 1600

Table 14a: Pulping of Whole Jute in Kraft Process

Na_2O (%)	Sulfidit %	pH of liquor	K. No.	S. reject %	Yield %	Burst Index KPa m^2/g	Tear Index mNm^2/g	Tensile index kN/m	Density Kg/m^3	Free-ness ^oSR
14	20	9.5	30.01	3.6	47.4	2.99	11.35	54.88	525	14
15.5	20	9.9	24.47	3.1	49.52	1.71	14.33	39.53	522	16
17	20	9.88	23.45	Nil	41.46	3.24	15.56	47.30	530	17
14	22	9.75	28.65	1.5	46.74	2.99	11.35	54.88	518	14
15.5	22	10.01	24.58	1.25	49.53	3.24	14.53	59.44	523	16
17	**22**	**10.55**	**19.05**	**Nil**	**47.00**	**3.81**	**13.41**	**67.77**	**525**	**18**
14	25	9.6	28.25	3.5	48.53	1.82	11.35	43.85	520	15
15.5	25	9.83	22.91	0.2	43	2.53	16.43	53.03	522	15
17	25	10.66	18.81	Nil	42	3.29	17.54	50.10	526	14

Table 14b: Pulping of Bark in Kraft Process

Na2O (%)	Sulfidity %	pH of liquor	K. No.	S. reject %	Yield %	Burst Index KPa m2/g	Tear Index mNm2/g	Tensile index kN/m	Density Kg/m3	Free-ness ^oSR
14	20	9.6	18.47	0.6	49.41	2.42	11.10	38.14	521	15
15.5	20	10.10	17.47	Nil	50.7	3.13	10.00	46.70	523	16
17	20	10.3	16.3	Nil	45.56	3.40	15.56	50.60	527	16
14	22	9.5	18.64	4	47.15	1.37	13.41	47.07	524	14
15.5	**22**	**10.2**	**17.88**	**Nil**	**52.49**	**2.25**	**14.00**	**52.85**	**521**	**15**
17	22	10.8	15.38	Nil	46.61	3.55	18.95	54.65	519	15
14	25	9.77	20.20	Nil	50.8	1.73	13.25	40.30	517	14
15.5	25	9.97	15.50	Nil	50	2.22	13.04	43.93	519	14
17	25	10.76	12.00	Nil	44	2.73	12.47	46.29	518	14

* Rising time from 30 to 170^{0}C 90 minutes, cooking temperature 170^{0}C, cooking for 120 minutes, Liquor ratio 1:5, H Factor 1600

Storage of green jute plants

The harvesting period of jute is in the monsoon when humidity is very high in Bangladesh and West Bengal of India. Moreover, continuous sunshine is not available for a long time and most of the time it rains.

For this reason if the harvested whole jute plants are stacked then there is a possibility of microbial degradation. Moreover, even fungicide cannot be sprayed because it would be washed away by the rain. The problem could be solved in the following way.

- If the jute is harvested at the end of September (it is done in some parts of Bangladesh) then there will be no problem of getting continuous sunshine which will help in getting the plants dried.
- If the jute plants are kept in a bundle in horizontal position there will be generation of heat which will degrade the plants.
- If jute bundles are kept in vertical position and if there is movement of air then 40- 50% moisture will be removed within 2/3 days.
- For easy transportation, the simple decortification method developed at BJRI may be applied. It has been demonstrated and calculated that an amount of additional Tk.3400.00 equivalent to USD 60.00 will be required for decortication of 12 tons of green jute plants.
- After harvesting jute plants are kept in a bundle of 12 inches. After the preparation of the bundles these bundles were kept in a vertical position for 5-6 days. After 07 days leave were removed.
- Some plants were attacked by fungus. The growth of fungi and plant degradation were stopped by using fungicide (Dithane M45).
- When the moisture content of the plant was about 18-20% plants were kept in bamboo frame in horizontal condition. While keeping the plant in horizontal position provision of ventilation for free movement of air was made. It may be mentioned that if the lignocellulosic materials contain more than 25% moisture then there is a heat generation due to the growth of microorganism. This generation of heat is due to the exothermic metabolic reaction. It happens with wood chips, jute chips and jute fibre when moisture contain is above 25%.

Appendix - C

APPENDIX-C

Objective 2.1: To develop suitable microorganism for biopulping and enzymes for biobleaching and to apply the same at BCIC, AFI, CTP, CPPRI and IBFC.

Optimization of biopulping in chemical process.

Experimental

Sample preparation

Fresh culture of three strains of ***Ceriporiopsis subvermispora*** (from FPL of USDA Forest Service), four strains of ***Phanerochaete chrysosporium*** (three from FPL of USDA Forest Service, one from DSM, Germany), one strain of ***Fomes lignosus*** (locally isolated strain) and one strain of ST-2 (from Central Pulp and Paper Research Institute, India) were used.

It may be noted that ***P. chrysosporium*** is the most widely used strain for biopulping and that ***P. chrysosporium-3*** (known as *BKMF 1767*) is the most widely studied strain. Considering the climate in jute and kenaf growing areas mesophilic ***P. chrysosporium*** having an optimum temperature of 35°-38°C was collected. Of the locally isolated strains, ***F. lignosus*** was found suitable for biopulping.

The composition of the inoculum media was 0.3% malt extract, 0.3% yeast extract and 1% glucose. The strain was allowed to grow in stationary condition for 7 days. Normally the strains are inoculated in several conical flasks. The biomass weight of one or two conical flask was taken to determine the required biomass for particular quantity of jute chips.

For biopulping, initially dried whole jute plants (***Corchorus olitorius***) were cut into small pieces (2-3 cm). 350 gm of the dried samples were put into polythene bags and 700 ml of water containing 35 mg of KH_2PO_4, 70mg of NaH_2PO_4, 150 mg of

$MgSO_4$, 35µg of $CaCl_2$, 35µg $FeSO_4$, 3µg $ZnSO_4$, 7µg $CuSO_4$ and 3µg $MnSO_4$ was added. The bags were autoclaved at 121°C for 15 minutes. Separate bags were then inoculated with 10 plugs of fresh culture of ***C. subvermispora*** (1, 2, 3), ***P. chrysosporium*** (1, 3, 4, 5), ***F. lignosus*** and ST-2 and 14 gm of glucose was added. The bags were fitted with inlet and outlet tubes for aeration and incubated at 30 °C for 14 days. Total moisture content of the jute chips including the media was 66.6%. 0.8 gm of biomass per kg of jute chips was used. Control experiments were carried out without fungus under the same conditions.

Biopulping in the Soda-AQ Process - variation of AQ charge

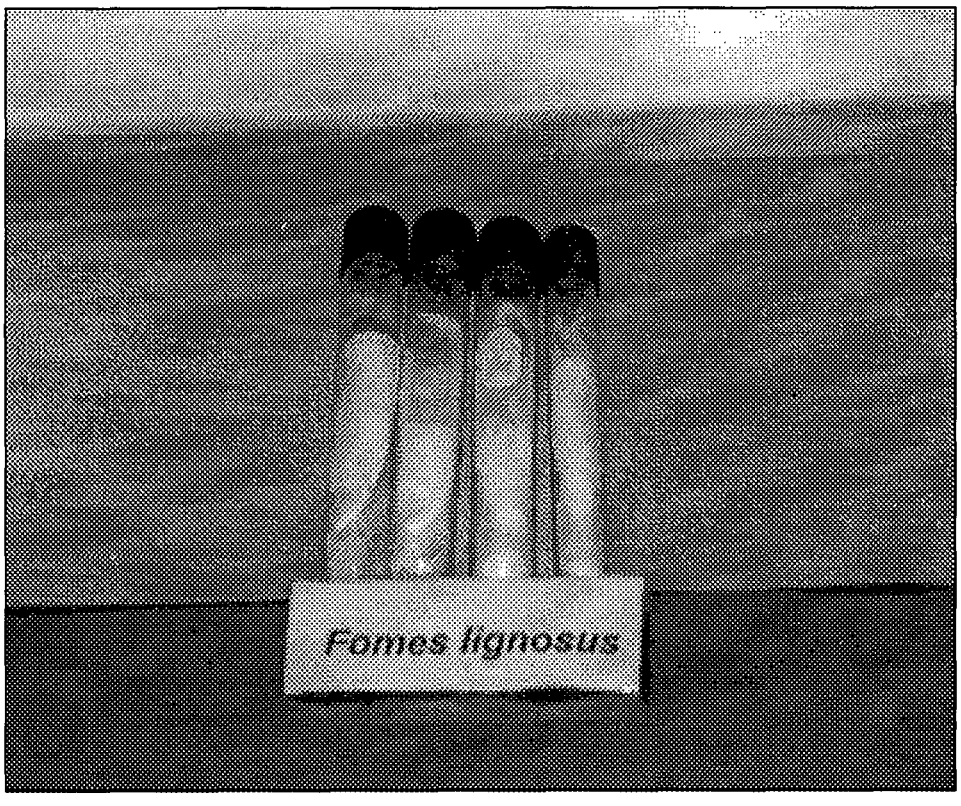
On the basis of that optimum condition obtained at BCIC biopulping was conducted at IJSG and KPM using ***P. chrysosporium, F. lignosus, C. subvermispora*** and ST–2. Laboratory scale pulping trials were conducted on the control chips and chips treated with the nine fungal strains using 170°C for 90 minutes, liquor ratio of 1:5, 17% alkali charge as Na_20 and varying the AQ charge from 0.05 to 0.1%. The temperature increased from an initial temperature of 35°C over 90 minutes.

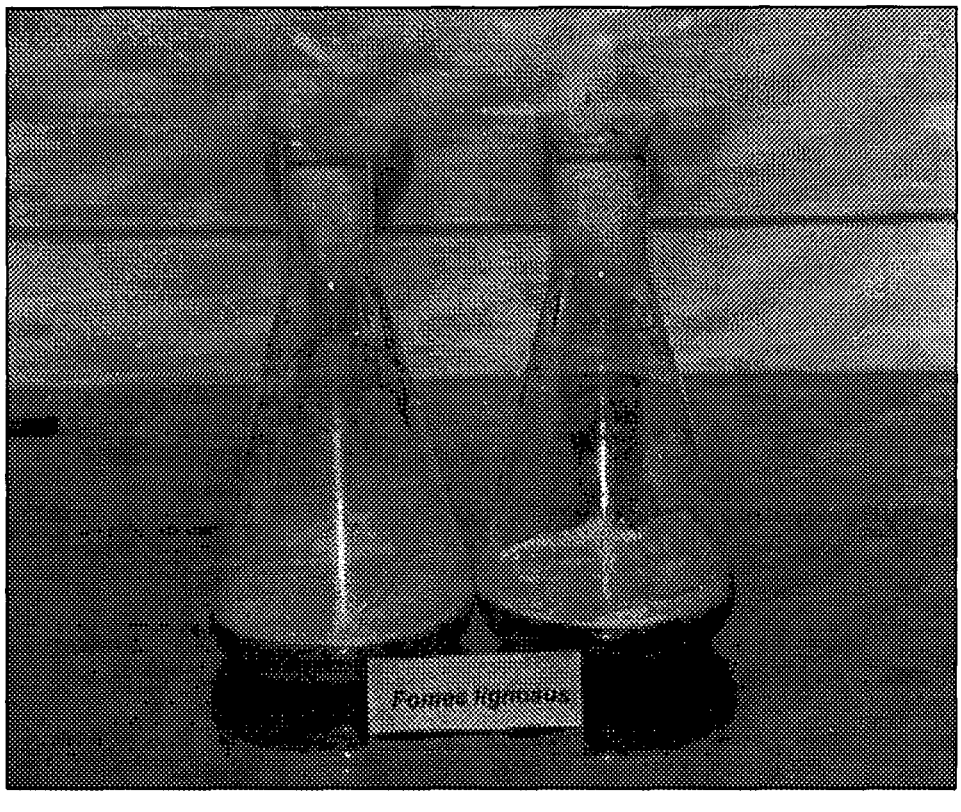
Biopulping in the Soda-AQ Process - reduced alkali charge

To determine the effect of varying the alkali charge, control chips and chips treated with seven fungal strains were pulped using an 15.5% alkali charge as Na_2O with all other parameters kept same as before.

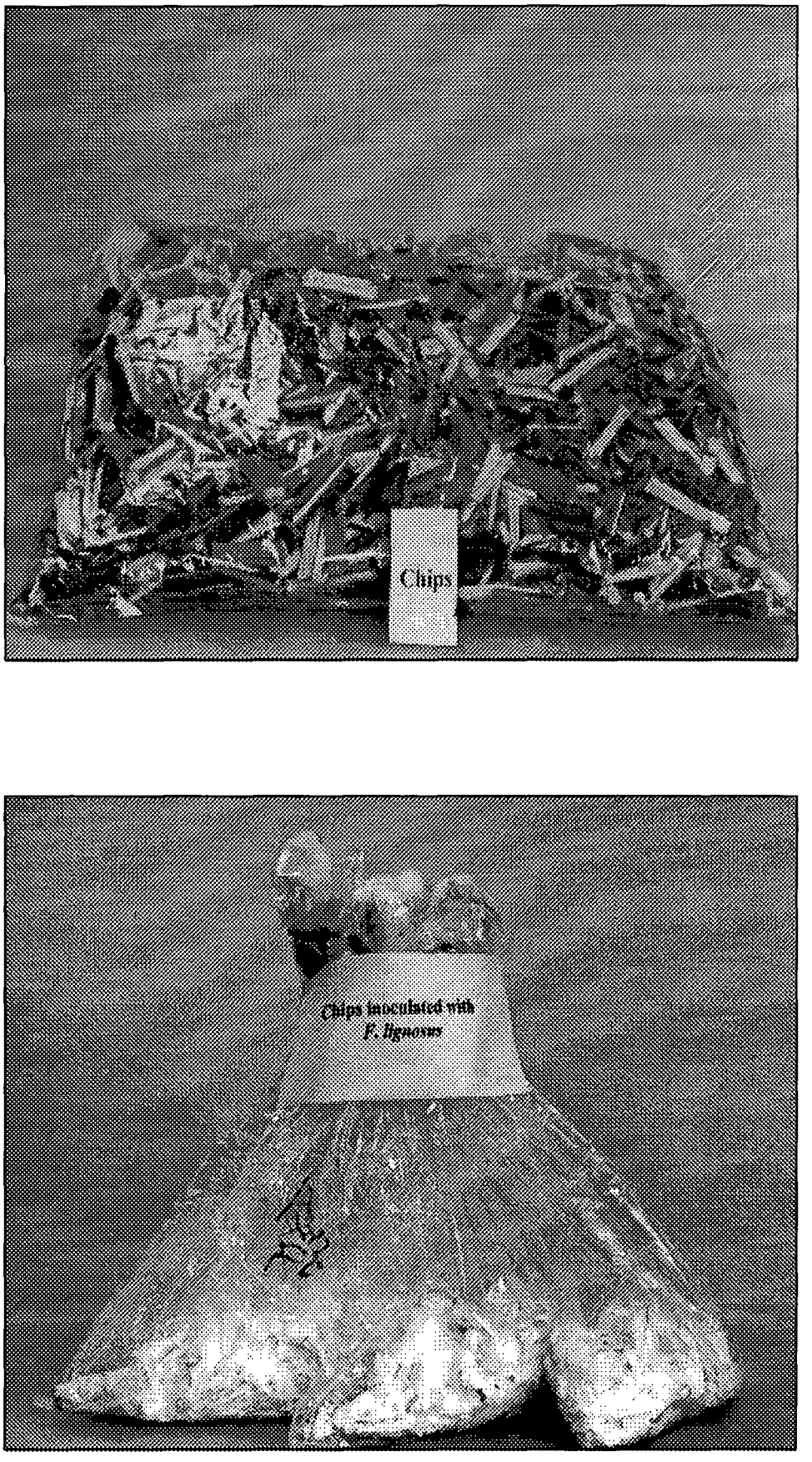
Biopulping in the Soda-AQ Process - reduced cooking time

To determine the effect of reducing the cooking time, control chips and chips treated with four fungal strains were pulped using a reduced cooking time of 60 minutes instead of 90 minutes. Experiments were carried out at both 15.5% and 17% active alkali as Na_2O and varying the AQ charge from 0.05 to 0.1%. All other parameters were kept same as before.





Photograph 7: Inoculums preparation for biopulping experiments



Photograph 8: Jute chips treated with *F. lignosus* for biopulping

The four selected fungal strains were ***P. chrysosporium-1, P. chrysosporium-3, C. subvermispora-2*** and ***F. lignosus*** based on their better performance regarding Kappa number reduction and improved physical properties.

Biopulping in the Kraft Process

Experiments were also conducted with four strains, ***P. chrysosporium-1, P. chrysosporium-3, C. subvermispora-2*** and ***F. lignosus***, using the Kraft process. Experiments were done varying the incubation period (7, 14 and 21 days). Since satisfactory results were obtained after 14 days of incubation, these results are presented in this paper.

Laboratory scale pulping trials were conducted using 170°C for 120 minutes, liquor ratio of 1:5, 17% alkali charge as Na_2O and 22% sulfidity. We also conducted experiments reducing the cooking time from 120 minutes to 90, 60 and 30 minutes.

Weight, Holocellulose and lignin loss at different incubation period of treated and untreated jute chips

Among the 8 strains, 4 strains (***P. chrysosporium-1, P. chrysosporium-3, C. subvermispora*** and ***F. lignosus)*** have been found to reduce Kappa no. and improve the physical properties of pulp using different percentage of alkali and also to reduce cooking time. Weight loss, lignin loss and holocellulose losses of treated samples were studied in different incubation time. The results are shown in **Table 21**.

Testing

All pulps were tested according to TAPPI Test Methods: T-236 cm 85 (Kappa number), T-205 sp. 95 (hand sheet preparation), T-403 ohm 97 (burst index), T-414 om 98 (tear index), T-404 cm 92 (tensile index) and T-220 sp. 96 (density), etc.

Results

Results of biotreated and untreated pulp in the Soda-AQ process (using 17% Alkali as Na_2O) are given in **Tables 15 and 16**. It appears that of the nine fungal strains, ***P. chrysosporium-1, P. chrysosporium-3, C. subvermispora-2*** and ***F. lignosus*** are more effective in reducing the pulp Kappa number by 3-4 units. The important point is that due to the reduced Kappa number of the treated samples, less chemicals will be required in the bleaching process.

After reducing the alkali charge from 17% to 15.5% as Na_2O (**Tables 17 and 18**), we can still achieve the desired Kappa number of 20 with improved physical properties. Yield loss is more or less same as with 17% alkali charge.

Figures III and IV show Kappa number as a function of alkali charge with control pulp and biotreated pulp using the Soda-AQ and kraft processes, respectively. For any Kappa number, the pulp yield was higher for the control pulp than for the biotreated pulp. But, at the same alkali charge, the Kappa number reduced significantly in biotreated pulp. Among the four microbial strains ***C. subvermispora*** and ***F. lignosus*** are found most suitable strain for biopulping in both the Soda-AQ and Kraft processes.

For biomechanical pulping, various authors have reported 4-5% yield loss, and reduced brightness. But they also report an energy reduction around 30-35% and improved physical properties for the biotreated samples compared to control mechanical pulping. In our case, however, we found that biochemical pulping using all nine fungal strains gave improved brightness (**Tables 16, 18 and 20**).

Physical properties of biochemical pulp improved significantly, pulp produced both the Soda-AQ (**Tables 16 and 18**) and Kraft processes (**Table 20**). The burst index of all pulps from treated chips was 20-25% higher than that of control pulp. Akhter and Leatham also found improved burst index when they produced biomechanical pulp.

The tensile strength of Soda-AQ biochemical pulp increased about 20-30%. Akhter reported that the tensile strength of biomechanical pulp increased by 17-27% using ***C. subvermispora***. Myers also found improved tensile properties

after refiner mechanical pulping with small diameter softwood and aspen treated with ***C. subvermispora*** and ***P. chrysosporium***, and Kohler also found the same result with ***Ophiostoma piliferum***.

From the experimented result 40-50% improvement in tear index for Soda-AQ biochemical pulp. Harmohinder, Blanchette and Scott also reported on improved tear index by fungal pretreatment using different raw materials in both chemical and mechanical pulping. Myers found that tear strength decreased after fungal pretreatment when applied to lodge pole pine but tear index increased when applied to aspen.

From results on biopulping in the Kraft process, it appears that there is practically no difference in yield loss for chips treated with ***C. subvermispora*** compared to the control, if the cooking time is reduce from 120 minutes to 60 minutes. But the physical properties of hand sheet improved significantly with the treated chips. For 90 minute cooking with ***C. subvermispora***, the yield loss was negligible compared to the control. When cooking time was reduced from 120 to 60 minutes yield loss was 3.4% with treated chips of ***F. lignosus***. From these results, it appears that chips treated with ***C.subvermispora*** and ***F.lignosus*** are most suitable for biopulping in the Kraft process. Cooking time using biotreated chips can be significantly reduced in both the processes (Soda-AQ and Kraft) and will allow increased throughput in the existing mills. Bajpai also reported on reduced cooking time for Kraft pulped eucalyptus chips pretreated with ***C.subvermispora*** and on improvements in physical properties.

From our result it has been revealed that there is no significant loss of holocellulose, **cellulose and hemicellulose** after treatment with ***C. subvermispora*** and ***F. lignosus*** for 07 days.

After 14 days of treatment with ***C. subvermispora*** no significant loss of holocellulose, and **cellulose** was observed. But lignin loss was found to be 11%. But in case of treatment of jute chips with ***P. chrysosporium-1*** there was a loss 20% in holocellulose, 12% in **cellulose** and 32% in **hemicellulose** along with 18% of Lignin. Similar results were observed sample treated with ***P. chrysosporium-3***.

After treatment of 21 days loss of holocellulose, **cellulose** and **hemicellulose** are much higher than treatment for 14 days with both ***P. chrysosporium-1*** and ***P. chrysosporium-3*** and ***Fomes lignosus***.

Considering all these experiment it can be concluded that in respect of delignification or reduction of Kappa No. treatment of jute chips for 14 days may be sufficient with all strains.

Weight loss of samples (different wood) treated with ***P. chrysosporium*** and ***C. subvermispora*** have been observed by different workers. Prof. A. Hatakka (Dept. of Chemistry & Microbiology, Univ. of Helsinki) in a report mentioned that minimum weight loss has been observed with ***C. subvermispora*** treated samples kept for 0-15 days. She also observed maximum weight loss with ***P. chrysosporium***.

Similarly Dr. Andre Ferraz (Department de Biotechnologia, Brazil) also observed minimum loss of weight with samples treated with ***C. subvermispora*** up to 15 days.

Table 15: Biopulping Jute Chips in the Soda-AQ Process - 17% alkali

Treatment	AQ %	Liquor pH	Unscreened yield %	Screen rejects %	Kappa No.
Control (untreated)	**0.05**	**12.1**	**47.50**	**0.50**	**20.50**
	0.10	12.2	47.10	0.20	18.00
P. chrysosporium-1	0.05	11.5	40.00	0.80	16.98
	0.10	11.6	42.00	0.50	14.94
P. chrysosporium-3	0.05	11.5	42.29	4.30	18.70
	0.10	11.6	43.15	0.50	17.30
P. chrysosporium-4	0.05	11.2	44.00	0.60	24.10
	0.10	11.9	44.15	0.60	20.00
P. chrysosporium-5	0.05	11.9	43.02	2.35	23.30
	0.10	11.5	42.94	1.00	19.39
C. subvermispora-1	0.05	11.2	44.18	1.50	29.00
	0.10	11.3	44.25	0.50	27.00
C. subvermispora-2	**0.05**	**11.7**	**44.20**	**0.00**	**16.00**
	0.10	11.8	44.50	0.00	15.50
C. subvermispora-3	0.05	11.2	46.00	3.00	28.00
	0.10	11.3	45.00	3.10	26.00
F. lignosus	**0.05**	**11.6**	**42.12**	**0.00**	**17.50**
	0.10	11.7	43.25	0.00	15.60
ST-2	0.05	11.6	42.00	0.50	22.00
	0.10	10.8	41.00	0.00	18.00

Rising time - 90 minutes, cooking time - 90 minutes, cooking temperature - 170°C, liquor ratio - 1:5

Table 16: Handsheet Physical Properties of Biochemical Soda-AQ Jute Pulp-17% alkali

Treatment	A.Q %	Burst Index $KPam^2/g$	Tear Index MNm^2/g	Tensile Index Nm/g	Freeness °SR	Brightness % (elripho)
Control (untreated)	**0.05**	**2.78**	**8.55**	**41.04**	**15**	**23.3**
	0.10	2.85	13.00	49.00	16	25.6
P. chrysosporium-1	0.05	3.28	13.01	59.43	21	28.4
	0.10	3.87	12.00	62.32	24	31.8
P. chrysosporium-3	0.05	3.35	11.56	56.00	21	28.3
	0.10	4.40	14.00	65.00	23	30.9
P. chrysosporium-4	0.05	2.85	9.60	40.30	15	22.4
	0.10	2.96	10.50	42.30	17	24.7
P. chrysosporium-5	0.05	2.78	9.50	41.10	16	23.7
	0.10	3.01	12.20	42.00	17	26.1
C. subvermispora-1	0.05	2.60	8.35	40.00	15	23.3
	0.10	2.70	13.00	43.00	16	26.0
C. subvermispora-2	**0.05**	**3.68**	**15.30**	**57.12**	**20**	**29.8**
	0.10	3.78	15.50	58.10	20	33.4
C. subvermispora-3	0.05	2.30	15.00	42.00	15	21.9
	0.10	2.60	16.00	43.00	16	22.5
F. lignosus	**0.05**	**3.56**	**14.80**	**51.00**	**21**	**29.6**
	0.10	3.25	15.60	58.52	21	33.2
ST-2	0.05	3.12	8.88	40.00	17	23.1
	0.10	3.45	8.62	40.00	18	27.6

Table 17: Biopulping Jute Chips in the Soda-AQ Process - 15.5% alkali

Treatment	AQ %	Liquor pH	Unscreened yield %	Screen reject %	Kappa No.
Control (untreated)	**0.05**	**12.00**	**48.00**	**0.85**	**24.20**
	0.10	12.10	47.60	0.49	22.86
P. chrysosporium-1	0.05	11.40	43.50	0.58	22.60
	0.10	11.25	41.20	0.22	20.50
P. chrysosporium-3	0.05	11.80	41.20	0.52	24.24
	0.10	11.60	42.50	0.21	19.80
P. chrysosporium-4	0.05	11.90	43.55	0.60	21.00
	0.10	11.00	43.00	0.00	21.00
P. chrysosporium-5	0.05	11.00	43.02	2.30	23.00
	0.10	11.50	43.00	1.00	19.00
C. subvermispora-2	**0.05**	**11.50**	**44.25**	**0.00**	**18.73**
	0.10	11.40	44.83	0.00	17.26
C. subvermispora-3	0.05	11.10	46.00	3.50	28.00
	0.10	11.20	46.00	3.50	27.00
F. lignosus	**0.05**	**11.60**	**42.40**	**0.65**	**17.50**
	0.10	11.70	43.20	0.42	17.38

Rising time - 90 minutes, cooking time - 90 minutes, cooking temperature - 170°C, liquor ratio - 1:5

Table 18: Handsheet Physical Properties of Biochemical Soda-AQ Jute Pulp-15.5% alkali

Treatment	AQ %	Burst Index $KPam^2/g$	Tear Index mNm^2/g	Tensile Index Nm/g	Freeness °SR	Brightness % (elripho)
Control (untreated)	**0.05**	**2.07**	**13.41**	**44.68**	**14**	**22.1**
	0.10	2.18	14.94	40.66	15	22.8
P. chrysosporium-1	0.05	3.37	14.50	61.00	19	26.2
	0.10	3.61	13.78	67.67	21	27.3
P. chrysosporium-3	0.05	3.26	11.82	45.15	21	23.2
	0.10	3.26	13.07	48.58	22	27.8
P. chrysosporium-4	0.05	1.60	13.00	36.00	15	25.5
	0.10	2.60	14.00	38.00	16	26.5
P. chrysosporium-5	0.05	2.20	10.00	33.00	14	24.4
	0.10	2.40	11.00	37.00	14	28.3
C. subvermispora-2	**0.05**	**3.58**	**14.33**	**58.76**	**20**	**27.7**
	0.10	3.60	14.25	59.28	21	31.1
C. subvermispora-3	0.05	2.10	13.00	40.00	15	20.0
	0.10	2.40	14.00	42.00	15	21.2
F. lignosus	**0.05**	**2.87**	**12.20**	**51.52**	**21**	**28.7**
	0.10	3.11	11.94	55.10	23	29.0

Table 19: Biopulping Jute Chips in the Soda-AQ Process - reduced cooking time 60 minutes

Treatment	Alkali %	AQ %	Liquor pH	Unscreened Yield %	Screen reject %	Kappa No.
Control (untreated)	17.0	**0.05**	**12.25**	**46.80**	**1.38**	**21.00**
		0.10	12.38	46.00	1.26	20.00
	15.5	**0.05**	**12.04**	**48.00**	**5.00**	**41.00**
		0.10	12.18	46.80	2.00	27.40
P. chrysosporium-1	17.0	0.05	11.60	43.00	0.00	18.93
		0.10	11.60	42.00	0.00	15.60
	15.5	0.05	11.50	42.00	1.00	22.20
		0.10	11.70	43.00	0.00	20.50
P. chrysosporium-3	17.0	0.05	11.50	43.00	0.00	19.80
		0.10	11.60	41.00	0.00	18.50
	15.5	0.05	11.60	41.00	0.50	24.00
		0.10	11.70	41.00	0.00	20.05
C. subvermispora-2	17.0	**0.05**	**11.70**	**46.80**	**0.00**	**17.32**
		0.10	11.80	43.36	0.00	16.92
	15.5	**0.05**	**11.60**	**46.66**	**0.00**	**18.18**
		0.10	11.60	45.70	0.00	17.40
F. lignosus	17.0	**0.05**	**11.70**	**43.60**	**0.00**	**19.35**
		0.10	11.80	42.42	0.00	15.60
	15.5	**0.05**	**11.60**	**43.00**	**0.00**	**24.00**
		0.10	11.80	42.00	0.00	20.00

Rising time - 90 minutes, cooking temperature - 170°C, liquor ratio - 1:5

Table 20: Biopulping Jute Chips in the Kraft Process - 17% alkali, 22% sulfidity

Treatment	Cooking time (min)	Screened yield %	K. No.	Burst Index kPam^2/g	Tear index mNm^2 /g	Tensile index Nm/g	Free-ness °SR	Bright-ness % (elripho)
Control	**120**	**46.00**	**19.25**	**2.74**	**8.76**	**47.07**	**15**	**26.10**
	90	45.55	21.70	2.37	12.76	46.00	16	24.46
	60	**44.00**	**27.58**	**2.85**	**12.32**	**49.24**	**15**	**21.93**
P.chrysosporium-1	120	40.12	16.40	3.15	10.13	51.85	21	31.32
	90	42.03	18.25	3.70	12.21	55.70	21	29.05
	60	42.75	22.50	2.96	14.17	53.82	19	25.55
P.chrysosporium-3	120	41.37	17.10	2.87	12.02	53.20	20	30.10
	90	42.56	19.75	3.11	13.17	50.55	20	28.65
	60	44.00	23.06	2.63	14.25	50.45	19	25.86
C.subvermispora-2	**120**	**42.12**	**16.12**	**3.25**	**11.30**	**55.36**	**21**	**33.12**
	90	44.25	18.65	3.18	14.38	56.63	23	29.35
	60	**46.92**	**21.22**	**3.14**	**13.87**	**55.30**	**20**	**27.75**
F.lignosus	**120**	**41.07**	**15.81**	**3.02**	**11.51**	**59.51**	**24**	**35.00**
	90	43.54	17.57	3.50	13.91	58.39	21	31.10
	60	**43.00**	**19.33**	**3.26**	**14.53**	**54.65**	**20**	**27.33**
	30	45.29	27.80	2.85	16.38	47.18	18	22.25

Rising time - 90 minutes, cooking temperature - 170°C, liquor ratio - 1:5

Table 21: Weight and component of jute chips (treated & untreated) by different fungal strain

Jute chips treated with	Incubation week	Weight loss %	Holocellulose %	Cellulose %	Hemicellulose %	Lignin %
Water	0	00.00	70.80	47.48	22.52	22.50
	1	01.10	70.15	47.52	22.50	22.35
	2	01.25	69.88	47.62	22.40	22.30
	3	01.63	69.68	47.40	22.41	22.00
P. chrysosporium-1	1	05.24	59.98	42.44	19.35	22.00
	2	10.90	56.90	41.75	15.18	18.48
	3	18.70	52.18	39.93	12.25	13.83
P. chrysosporium-3	1	04.04	61.20	43.00	18.20	21.90
	2	11.90	55.70	41.35	14.30	19.03
	3	19.06	51.09	40.81	11.28	14.78
C. subvermispora	1	01.31	70.30	47.15	20.37	21.97
	2	02.98	69.00	46.05	19.66	20.00
	3	03.32	68.33	46.12	18.50	19.06
F. lignosus	1	07.28	66.56	45.88	20.37	21.75
	2	09.10	61.87	42.82	18.94	20.02
	3	16.86	55.96	40.51	15.10	17.43

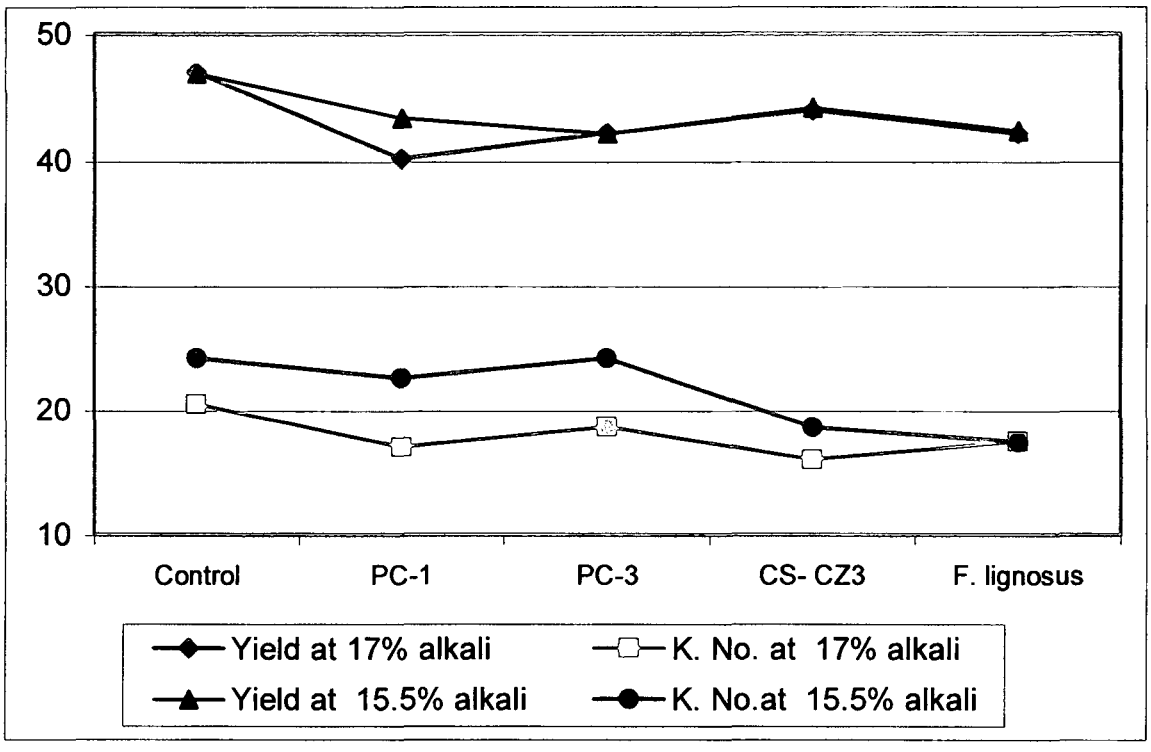


Fig. IV: Yield versus Kappa No. of pulps from control and biotreated chips in Soda-AQ process

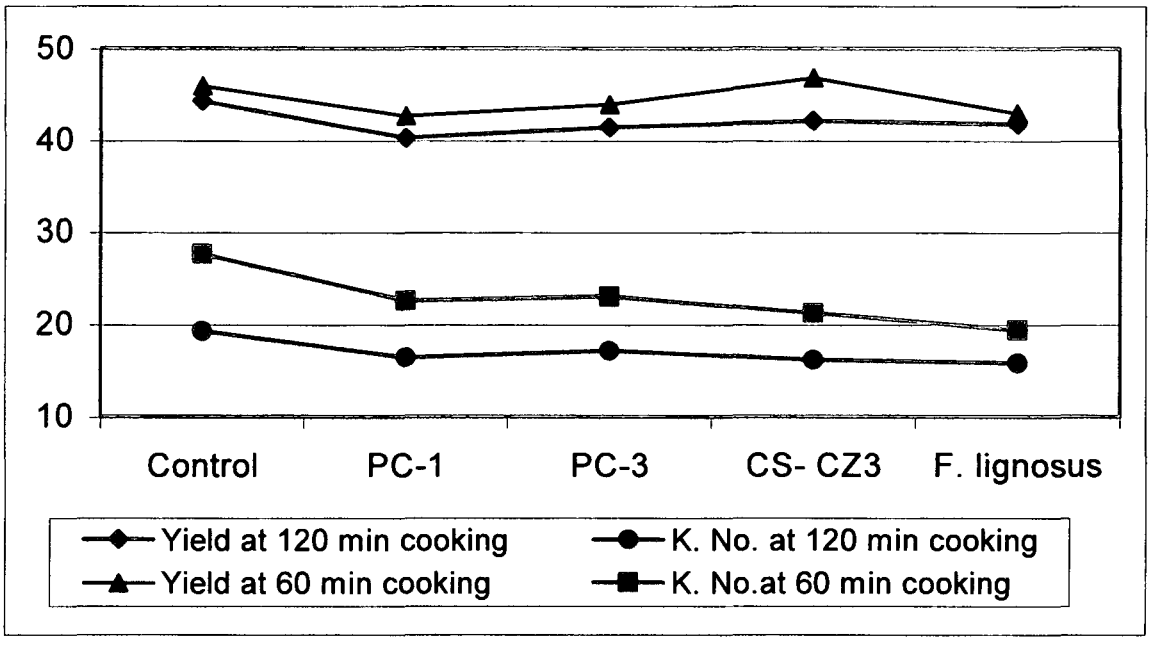


Fig. V: Yield versus Kappa no. of pulps from control and treated chips in Kraft process

Conclusion

- Among the 9 fungal strains, *F. lignosus* and *C. subvermispora* were found suitable for biopulping in both the Soda-AQ and kraft process.
- It can reduce the kappa no. (15%) with same alkali charge.
- Desired kappa no. can also be obtained by reducing the alkali charge (9%).
- In both the Soda-AQ and kraft processes, cooking time can be reduced (from 120 minutes to 60 minutes in Kraft process and from 90 minutes to 60 minutes in Soda-AQ process). As a result, cooking cycles can be increased which will facilitate to have more throughputs in the existing mill.
- Physical properties of paper (burst, tear and tensile index) can be improved significantly (20-40%) in biopulping process to get a better quality paper.

Appendix - D

APPENDIX-D

Bleaching of pulp with and without (three commercial and one developed) enzyme

Material and Methods

Bleaching Experiment

Bleaching experiments of Soda-AQ and kraft pulps were conducted in CEH, DED and OCEH sequences with and without enzyme to reach 80% ISO brightness. The conditions of these sequences (for both Soda-AQ and kraft pulp) are presented in **Table 22**.

Table 22: Conditions of pulp bleaching using various sequences

Sequence	Symbol	Chemical charge	Reaction time (min.)	Temperature ^0C	pH	Consis-tency %
CEH	C	(Kappa No. x 0.22)%	60	25	2.5-3	03
	E	NaOH 2%	60	70	11-12	10
	H	Ca-hypo (as active Cl_2) 1.5%	120	40	10-11	08
DED	D1	70% of total active chlorine	60	70	3.5-4.0	8
	E	NaOH 2%	60	70	11-12	10
	D2	30% of total active chlorine	60	70	3.5-4.0	8
OCEH	O	O_2 at 5 bars	60	100	11-12	10
	C	(Kappa No. x 0.22)%	60	25	2.50	3
	E	NaOH 2%	60	70	11-12	10
	H	Ca-hypo (as active Cl_2) 0.75%	120	40	10.5-11	7
Xylanase	X	2 IU/g od pulp	60	50	5-8	10

CEH:

CEH sequence was conducted in conventional conditions. Cl_2 was applied according to kappa no. (% Cl_2 applied = kappa no. x 0.22). 2% NaOH was used for alkali extraction stage. 1.5% Ca-hypochlorite (as active Cl_2) was used to reach 80% ISO brightness.

DED:

The DED sequence was carried out with and without enzyme in both the Soda-AQ and kraft process. Three commercial enzymes and one of the developed enzymes of IJSG were used separately.

OCEH:

In OCEH bleaching experiment, O_2 was applied in the pulp from a gas cylinder using a closed chamber. Five bar pressure at a temperature of 100^0C were kept for O_2 reaction with the pulp. 2% NaOH was used for maintaining the alkaline conditions (pH 11-12). 0.05% $MgSO_4$ was used as stabilizer. Enzyme was applied separately both before and after O_2 treatment.

Xylanase treatment:

Xylanase treatment of pulp was conducted in various sequences according to the condition mentioned in **Table 22**.

Comparison of commercial and developed enzyme (IJSG)

Three commercial enzymes i.e. **Biobrite, Pulpzyme and Ecopulp and one developed enzyme** (IJSG) were applied separately in different bleaching sequences. Prior to enzyme application, xylanase activity of commercial enzymes and developed enzyme were optimized at different pH. Requirement of enzyme (IU), reduction of kappa number and release of xylose were also studied with different commercial and developed enzyme. After optimization of enzyme with different IU, 2 IU/gm pulp was used which was found suitable for reduction of kappa No.

Results

Brightness in CEH and DED sequence

The bleaching response in CEH sequence found to be suitable for Soda-AQ pulp than kraft pulp (**Table 24 and 25**). In order to reach the target brightness 80% ISO, a complementary hypochlorite stage was required for both Soda-AQ and kraft pulp, with and without enzyme treatment. But pretreatment with xylanase increase the brightness by 1-3 units in both the pulps even with the reduced chlorine application (**Table 24 and 25**). There was reduction of kappa No. of pulp (1.1 -2.8 units) of unbleached kraft or Soda-AQ jute pulps after enzyme treatment (**Figure III**).

There was increase of brightness in DED sequences by 2/3 units in both Soda-AQ and kraft pulp. In DED sequence, maximum ClO_2 was consumed in D_1 stage. Bleaching response of kraft pulp in DED sequence was higher than that of Soda-AQ (**Table 26 and 27**).

Bleaching response in DED bleaching sequence found suitable for jute pulp (both Soda-AQ & kraft) than conventional CEH sequence (**Table 26 and 27**).

Yield & viscosity

Bleaching yield of Soda-AQ and kraft pulp was the same. Viscosity of enzyme treated pulp found to be higher than that of the control pulp. Viscosity of DED bleached pulp (Soda-AQ and kraft) was higher than those of CEH and OCEH bleached pulp (**Table 26, 27, 28 and 31**). It indicated that ClO_2 did not degrade the cellulosic component of pulp in DED sequence. Physical properties of control and enzyme treated pulp were found to be the same (**Table 24, 25, 26 and 27**).

Benefit of enzyme and oxygen application in CEH, DED and OCEH sequence

Use of xylanase (commercial & developed enzyme) in different bleaching sequences led to a reduction of chlorine (**Table 24, 25, 26 and 27**) due to the reduction of kappa No. (**Figure III**). But the benefit of enzyme application depends on type of pulp (Soda-AQ/Kraft) and bleaching sequences.

Results from the various bleaching sequence with Soda-AQ and kraft pulp showed that the reduction of active chlorine were 09-12% in CEH and 15-17% in DED.

Reduction of AOX

Xylanase pretreatment has led to the reduction in effluent AOX due to reduced chlorine requirement. It decreased AOX in proportion to the decreasing chlorine usage. The level of AOX in effluent was found to be significantly lower (10-14%) for all the xylanase pretreatment (commercial and developed enzyme – **Table 36**). It also improved the brightness of pulp in all the sequences.

Effluent's BOD and COD were increased, indicating that the effluent was more amenable to biological degradation. There is an increase in the bleach effluent resulting from the release of low molecular weight xylanase from the pulp. This BOD is of an easily degraded nature in secondary treatment system. But the ratio of COD/BOD of enzyme treated effluent found to be less (<6) than that of control bleaching effluent indicating an easier biodegradability. This may be due to the reduction of chemical requirement for bleaching by enzymatic pretreatment.

Xylanase increase efficiency of bleaching by making lignin accessible to the bleach chemicals. Advantage of xylanase is that it dose not need any investment in equipment – no pumps, washing, etc. It may be applied in – line ahead of bleaching sequences.

Oxygen delignification (OCEH) found to be suitable for reducing the chlorine requirement (45%) in conventional CEH sequence. Brightness of OCEH bleached pulp was found higher (3-6%) than that of CEH bleached pulp (**Table 28 and 31**). 40-45% chlorine can be reduced with the application of oxygen. Application of xylanase in OCEH sequence, before and after oxygen did not reduce chlorine requirement (**Table 29, 30, 32 and 33**) but the brightness was improved by 2-3 units when xylanase is used after O_2 (**Table 30 and 32**) in Soda-AQ.

Among the three commercial enzymes and developed enzyme, Biobrite was found to be the most suitable in reducing kappa No. (**Figure VII**). However, the bleaching response found to be the same with all the enzymes.

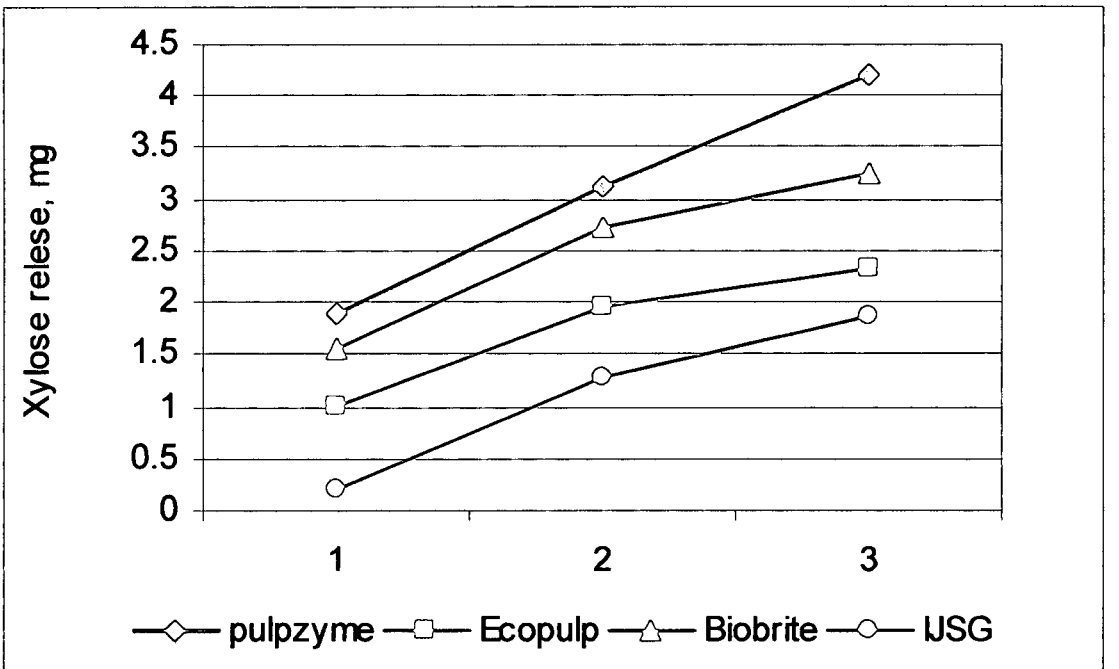


Figure VI: Xylose release with different IU/g pulp

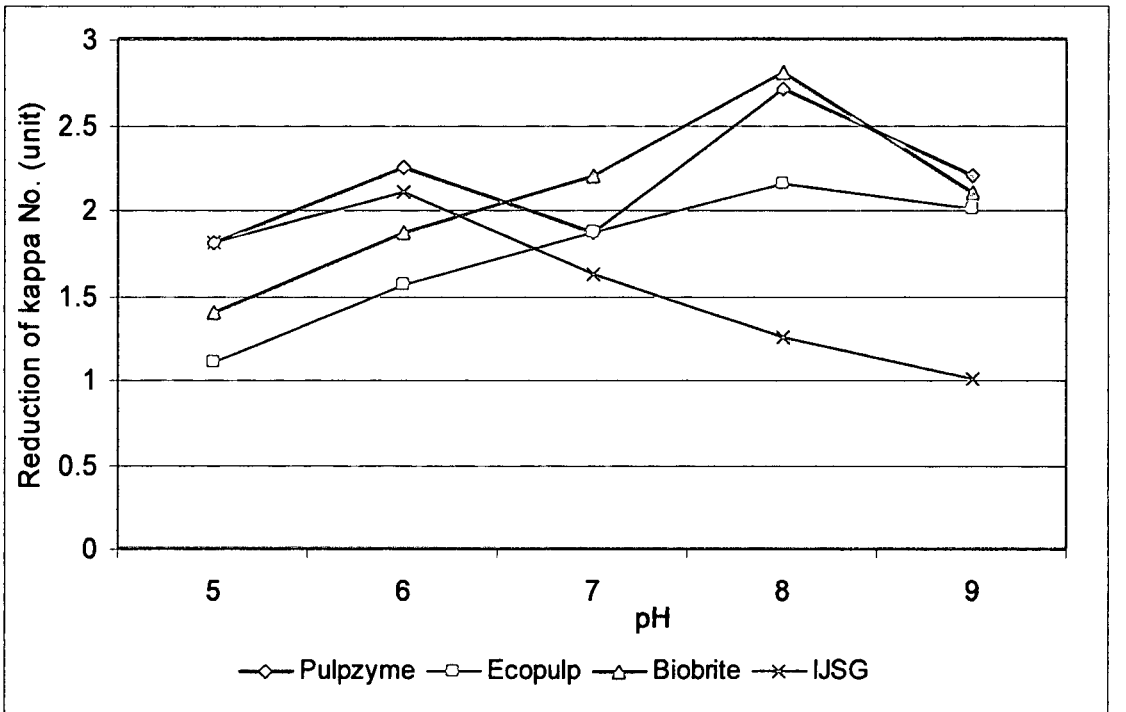


Figure VII: Reduction of kappa No. of kraft pulp at different pH using commercial and developed enzyme

Table 23: Optimization of IU/g of pulp using different commercial & developed enzymes at pH 5.5

Enzyme	IU/g	Initial kappa No.	Kappa No. after treatment
Pulpzyme	1	20.10	19.40
	2	20.10	17.95
	3	20.10	16.62
Ecopulp	1	20.10	19.70
	2	20.10	17.85
	3	20.10	17.32
Biobrite	1	20.10	18.78
	2	20.10	17.47
	3	20.10	16.25
IJSG	1	20.10	19.62
	2	20.10	18.00
	3	20.10	17.30

Table 24: Results of Bleaching Experiments in CEH Sequence with and without Enzyme of Kraft Pulp

Stages	Conditions	Control (without enzyme)	Enzymes			
			X_1	X_2	X_3	X_4
X	Final pH	7.50	6.50	8.10	8.00	8.10
	Initial Kappa No.	**20.10**	**20.10**	**20.10**	**20.10**	**20.10**
	Final kappa No.	**20.00**	**18.00**	**17.80**	**17.90**	**17.50**
C	Chlorine %	**4.4**	**3.96**	**3.92**	**3.94**	**3.85**
	Final pH	3.00	2.90	3.10	3.00	2.95
	Residual Cl_2 in effluent %	0.58	0.60	0.65	0.62	0.62
	Brightness %, ISO	41.26	43.63	43.53	43.60	43.70
E	Final pH	12.40	12.40	12.30	12.40	12.20
	Brightness %, ISO	39.20	40.15	40.35	40.50	40.40
H	Final pH	10.65	10.70	10.60	10.65	10.60
	Residual Cl_2 in effluent %	0.59	0.60	0.60	0.70	0.65
	Brightness %, ISO	**76.38**	**79.24**	**80.25**	**79.80**	**80.00**
	Bleaching yield %	95.70	94.50	94.50	94.32	94.33
	Viscosity, mPa, s	**8.30**	**9.34**	**8.80**	**9.10**	**8.75**

X_1 = develop enzyme, X_2 = pulpzyme, X_3 = Ecopulp, X_4 = Biobrite

Table 25: Results of Bleaching Experiments in CEH Sequence with and without Enzyme of Soda-AQ Pulp

Stages	Conditions	Control (without enzyme)	Enzymes			
			X_1	X_2	X_3	X_4
X	Final pH	7.00	6.50	8.00	8.05	8.00
	Initial Kappa No.	19.50	19.50	19.50	19.50	19.50
	Final kappa No.	**19.35**	**17.65**	**17.10**	**17.55**	**17.25**
C	Chlorine %	**4.40**	**3.88**	**3.76**	**3.86**	**3.79**
	Final pH	2.80	3.00	3.10	3.00	2.95
	Residual Cl_2 in effluent %	0.58	0.55	0.58	0.63	0.62
	Brightness %, ISO	42.05	45.10	45.00	44.80	45.05
E	Final pH	12.50	12.45	12.50	12.50	12.40
	Brightness %, ISO	39.55	40.70	40.60	40.50	40.85
H	Final pH	10.65	10.80	10.75	10.80	10.80
	Residual Cl_2 in effluent %	0.59	0.55	0.57	0.50	0.60
	Brightness %, ISO	**78.12**	**79.28**	**80.22**	**79.73**	**80.32**
	Bleaching yield %	95.10	95.27	94.50	94.75	94.35
	Viscosity, mPa, s	**8.13**	**9.05**	**9.00**	**9.15**	**8.85**

X_1 = develop enzyme, X_2 = pulpzyme, X_3 = Ecopulp, X_4 = Biobrite

Table 26: Results of Bleaching Experiments in DED Sequence with and without Enzyme of Kraft Pulp

Stages	Conditions	Control (without enzyme)	Enzymes			
			X_1	X_2	X_3	X_4
X	Final pH	7.70	6.50	8.00	8.10	8.10
	Initial Kappa No.	20.26	20.05	20.05	20.05	20.05
	Final kappa No.	20.05	18.48	17.95	18.20	17.90
D	ClO_2 (as active Cl_2) %	**3.08**	**2.84**	**2.76**	**2.80**	**2.76**
	Final pH	4.70	4.45	4.50	4.50	4.55
	Residual Cl_2 in effluent %	0.52	0.60	0.60	0.58	0.55
	Brightness %, ISO	43.25	44.45	44.50	44.36	44.60
E	Final pH	12.50	12.45	12.40	12.45	12.50
	Brightness %, ISO	40.30	41.22	41.36	42.55	42.50
D	ClO_2 (as active Cl_2) %	**1.32**	**1.21**	**1.18**	**1.20**	**1.18**
	Final pH	4.20	4.30	4.25	4.20	4.25
	Residual Cl_2 in effluent %	0.46	0.50	0.52	0.55	0.52
	Brightness %, ISO	**80.56**	**81.70**	**81.70**	**81.60**	**82.20**
	Bleaching Yield %	**95.64**	**95.20**	**95.28**	**94.65**	**94.85**
	Viscosity, mpa, s	**12.82**	**13.08**	**12.80**	**12.70**	**13.10**

X_1 = develop enzyme, X_2 = pulpzyme, X_3 = Ecopulp, X_4 = Biobrite

Table 27: Results of Control Bleaching and Biobleaching Experiments in DED Sequence of Soda-AQ Pulp

Stages	Conditions	Control (without enzyme)	Enzymes			
			X_1	X_2	X_3	X_4
X	Final pH	7.00	**6.60**	8.05	8.00	8.10
	Initial Kappa No.	20.89	20.89	20.89	20.89	20.89
	Final kappa No.	20.80	19.10	18.88	18.65	18.30
D	ClO_2 (as active Cl_2) %	**3.20**	**2.94**	**2.89**	**2.89**	**2.87**
	Final pH	3.50	3.40	3.00	3.25	3.30
	Residual Cl_2 in effluent %	0.55	0.52	0.65	0.50	.055
	Brightness %, ISO	41.25	43.18	43.30	43.20	43.25
E	Final pH	12.52	12.52	12.62	12.60	12.60
	Brightness %, ISO	39.22	41.20	41.50	41.33	41.50
D	ClO_2 (as active Cl_2) %	**1.36**	**1.26**	**1.23**	**1.23**	**1.23**
	Final pH	3.52	3.40	3.60	3.40	3.20
	Residual Cl_2 in effluent %	0.58	0.62	0.60	0.55	0.53
	Brightness %, ISO	**79.15**	**80.51**	**81.20**	**81.10**	**82.10**
	Bleaching Yield %	**95.12**	**94.89**	**95.50**	**94.70**	**94.72**
	Viscosity, mpa, s	**12.12**	**12.70**	**12.25**	**12.50**	**12.69**

X_1 = develop enzyme, X_2 = pulpzyme, X_3 = Ecopulp, X_4 = Biobrite

Table 28: Results of Bleaching Experiments in OCEH Sequence of Kraft Pulp

Stages	Conditions	
O	Initial kappa No.	21.05
	Final kappa No.	10.45
C	Chlorine %	2.29
	Final pH	2.60
	Residual Cl_2 in effluent %	0.52
	Brightness %, ISO	64.25
E	Final pH	12.21
	Brightness %, ISO	60.80
H	Final pH	10.63
	Residual Cl_2 in effluent %	0.33
	Brightness %, ISO	**82.40**
	Bleaching yield %	94.33
	Viscosity, mPa, s	**7.97**

Table 29: Results of Bleaching Experiments in OXCEH Sequence of Kraft Pulp

Stages	Conditions	
O	As O stage of OCEH	--
X	pH	6.70
	Initial Kappa No.	10.45
	Final kappa No.	10.01
C	Chlorine %	2.20
	Final pH	2.62
	Residual Cl_2 in effluent %	0.55
	Brightness %, ISO	63.50
E	Final pH	12.62
	Brightness %, ISO	60.20
H	Final pH	10.70
	Residual Cl_2 in effluent %	0.37
	Brightness %, ISO	**83.43**
	Bleaching yield %	**94.51**
	Viscosity, mPa, s	**8.53**

Table 30: Results of Bleaching Experiments in XOCEH Sequence of Kraft Pulp

Stages	Conditions	
X	Final pH	5.80
	Initial Kappa No.	21.10
	Final kappa No.	19.57
O	O_2 pressure bars	5.00
	Kappa No.	10.39
C	Chlorine %	2.28
	Final pH	2.60
	Residual Cl_2 in effluent %	0.63
	Brightness %, ISO	65.25
E	Final pH	12.62
	Brightness	61.20
H	Final pH	10.81
	Residual Cl_2 in effluent %	0.29
	Brightness %, ISO	**83.65**
	Bleaching yield %	**94.30**
	Viscosity, mPa, s	**8.53**

Table 31: Results of Bleaching Experiments in OCEH Sequence of Soda-AQ Pulp

Stages	Conditions	
O	Initial kappa No. Final kappa No.	20.05 10.17
C	Chlorine % Final pH Residual Cl_2 in effluent % Brightness %, ISO	2.23 2.53 0.51 63.55
E	Final pH Brightness %, ISO	12.58 58.40
H	Final pH Residual Cl_2 in effluent % Brightness %, ISO Bleaching yield % Viscosity, mPa, s	10.30 0.36 **81.36** **94.13** **8.63**

Table 32: Results of Bleaching Experiments in OXCEH Sequence of Soda-AQ Pulp

Stages	Conditions	
O	As OCEH sequences	
X	PH Initial Kappa No. Final kappa No.	6.50 10.17 10.01
C	Chlorine % Final pH Residual Cl_2 in effluent % Brightness %, ISO	2.20 2.32 0.55 64.65
E	Final pH Time, Minutes	12.62 60.00
H	Final pH Residual Cl_2 in effluent % Brightness %, ISO Bleaching yield % Viscosity, mPa, s	10.51 0.35 **83.56** **94.89** **9.43**

Table 33: Results of Bleaching Experiments in XOCEH Sequence of Soda-AQ Pulp

Stages	Conditions	
X	Final pH Initial Kappa No. Final kappa No.	6.50 20.89 19.07
O	As OCEH sequences	
C	Chlorine % Final pH Residual Cl_2 in effluent % Brightness %, ISO	2.09 3.25 0.51 64.35
E	Final pH Brightness %, ISO	12.00 60.25
H	Final pH Residual Cl_2 in effluent % Brightness %, ISO Bleaching yield % Viscosity, mPa, s	11.53 0.39 **82.65** **94.30** **8.15**

Table 34: Physical properties of bleached pulp using different bleaching sequences (Soda-AQ pulp)

Bleaching sequence	Burst Index KPa m^2/g	Tear Index mN m^2/g	Tensile Index KN/m	Freeness ^{0}SR
CEH	2.18	12.14	41.72	15
X_1CHE	2.25	15.20	41.77	16
X_2CEH	2.40	13.85	42.50	16
X_3CEH	2.10	11.25	41.50	15
X_4CEH	2.31	13.90	43.25	16
DED	2.72	13.63	40.80	15
X_1DED	2.35	14.43	41.65	15
X_2DED	3.15	12.33	41.50	16
X_3DED	2.75	12.50	41.00	15
X_4DED	3.24	12.50	42.45	16
OCEH	2.67	16.81	44.50	15
OXCEH	3.02	13.10	43.20	16
XOCEH	3.05	13.10	44.47	17

X_1 = develop enzyme, X_2 = pulpzyme, X_3 Ecopulp and X_4 = Biobrite.

Table 35: Physical properties of bleached kraft pulp using different bleaching sequences

Bleaching sequence	Burst Index KPa m^2/g	Tear Index mN m^2/g	Tensile Index KN/m	Freeness ^{0}SR
CEH	2.70	11.69	40.39	15
X_1CHE	2.91	12.80	42.92	16
X_2CEH	2.73	12.50	42.21	16
X_3CEH	2.80	11.92	41.30	15
X_4CEH	3.10	11.76	42.00	16
DED	2.90	11.65	40.39	16
X_1DED	2.50	15.80	47.52	16
X_2DED	3.25	12.88	47.50	17
X_3DED	3.10	12.55	46.66	16
X_4DED	3.44	13.15	50.33	17
OCEH	3.17	13.05	41.50	16
OX_1CEH	3.02	13.10	43.20	17
X_1OCEH	3.05	13.10	44.47	17

X_1 = develop enzyme, X_2 = pulpzyme, X_3 = Ecopulp, X_4 = Biobrite

Table 36: Effluent analysis (DED bleaching effluent)

Enzyme	BOD, Kg/ton	COD, Kg/ton	BOD/COD	AOX, Kg/ton
Pulpzyme	9.0	41.26	4.587	0.987
Ecopulp	8.2	40.90	4.987	0.981
Biobrite	10.6	42.40	3.981	0.960
IJSG	9.2	41.15	4.472	1.001
Control (without enzyme treatment)	7.0	38.50	5.50	1.105

Conclusion

The results of bleaching in CEH and DED sequences following the Kraft and Soda-AQ processes are showing that both brightness and viscosity are improving due to the enzyme treatment.

The benefits of the biobleaching process can be summarized as:

- Reduction of active chlorine 09-12% in CEH and 15-17% in DED.
- Reduction of AOX in the effluent was due to reduced chlorine requirement. The AOX decreased in proportion to the decreasing chlorine usage.
- Increase in effluent's BOD and COD, indicating that the effluent is more amenable to biological degradation. There is an increase in the bleach effluent resulting from the release of low molecular weight xylose from the pulp.
- Application of xylanase in OCEH sequence, before and after oxygen, did not reduce chlorine requirement; but the brightness was improved by 2-3 units when xylanase is used after oxygen in Soda-AQ.
- All the four enzymes, three commercials and one developed, reduce the Kappa No. and improve the brightness.

Appendix - E

APPENDIX-E

Objective-4: Large scale trial application of pulp and paper using whole jute plant / kenaf to determine the physical characteristics of pulp and paper and to evaluate and compare the results.

Material and Methods

Large scale and commercial trial of jute plant for the production of pulp and paper

Procurement

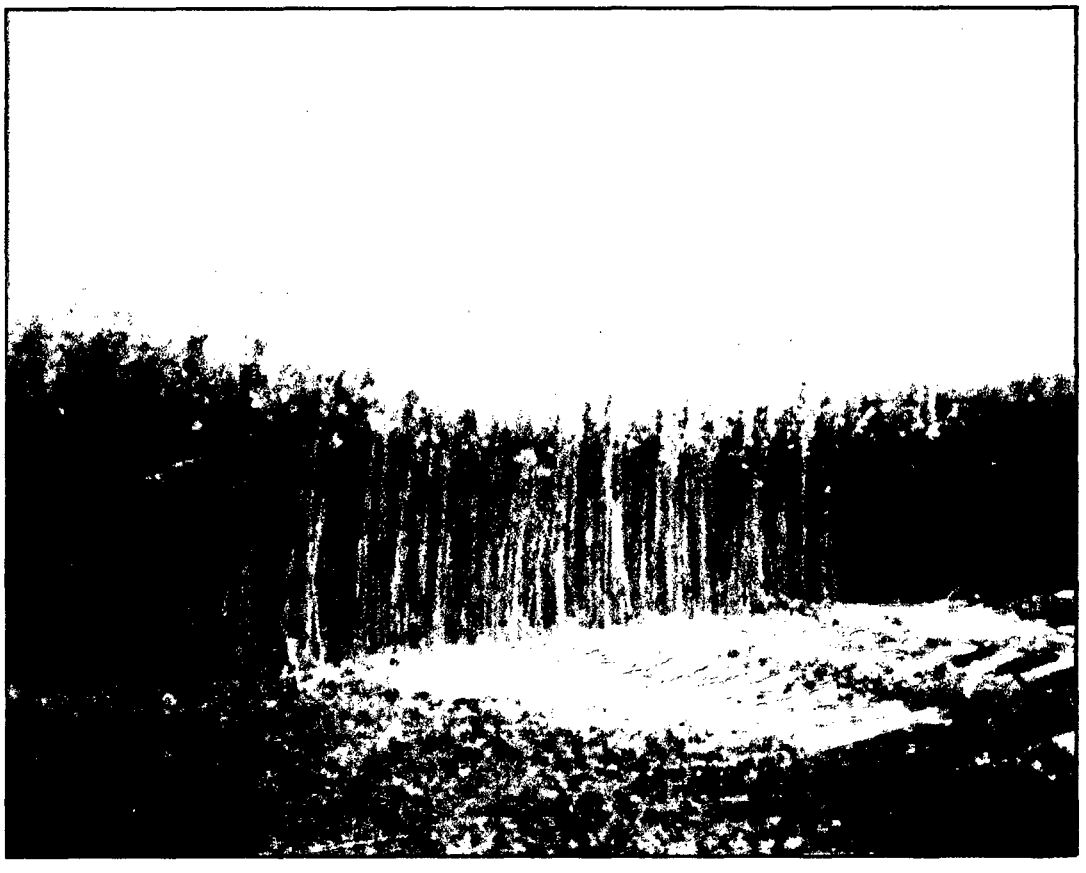
For the procurement of jute plant, scientists of IJSG/BCIC visited several jute-growing areas of Bangladesh. For jute fibre, there are several agencies and local traders. Jute plants are not generally sold in the open market. In order to procure the jute plant farmers were contacted and negotiated for supplying green jute plant. While fixing the price of jute plant normal sale proceed of jute fibre and that of stick were counted together and from this amount cost of normal retting was deducted. After thorough discussion with them (farmers), price of jute plant was fixed at Tk. 1,050/ton (after defoliation).

Harvesting and Defoliation

After harvesting jute plants are kept in a bundle of 12 inches diameter. After the preparation of the bundles, these bundles were kept in a vertical position for 5-6 days. After 7 days leave were removed.

Transportation

These jute plants were then transported to KPM by road. It may be mentioned here that harvesting period of jute is in the monsoon (Mid July to end of September) when humidity is very high in Bangladesh and West Bengal of India. Moreover continuous sunshine is not available for a long time and most of the time it rains. After transportation of jute plant at KPM the plants were kept in small bundle in vertical position for free movement of air to reduce moisture content. Scientists also requested farmers to keep the plant in vertical position to reduce moisture content before transportation.

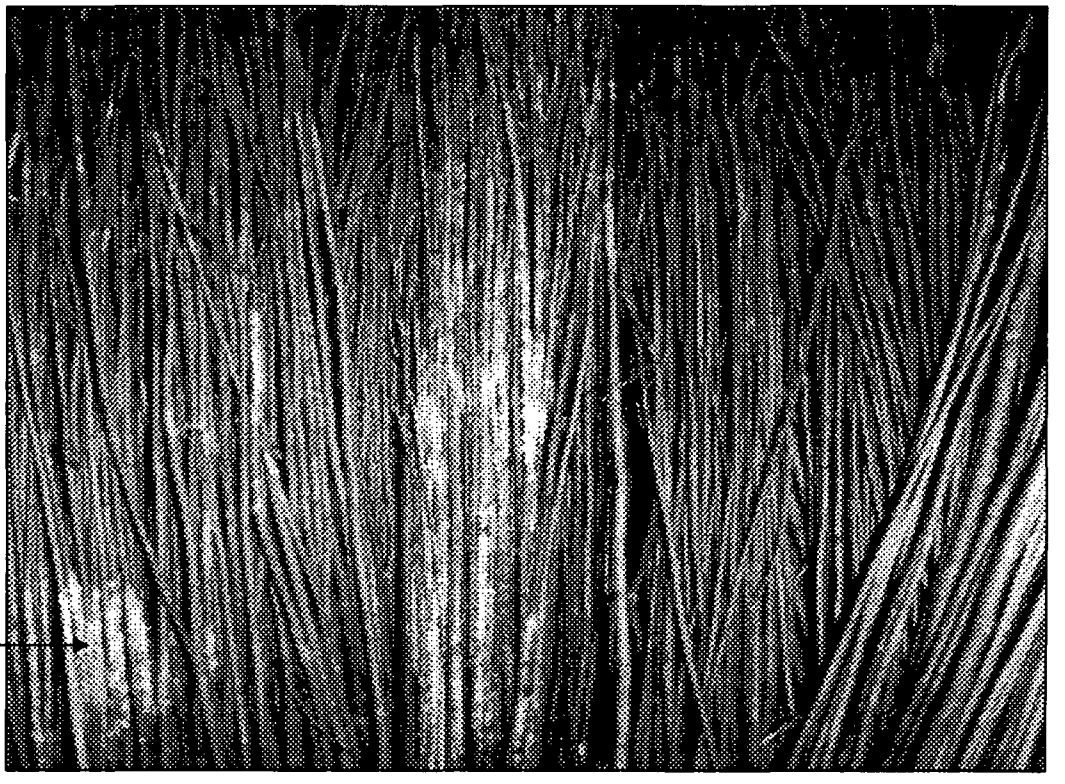


Jute Field

Jute plants for transportation

Application of Fungicide

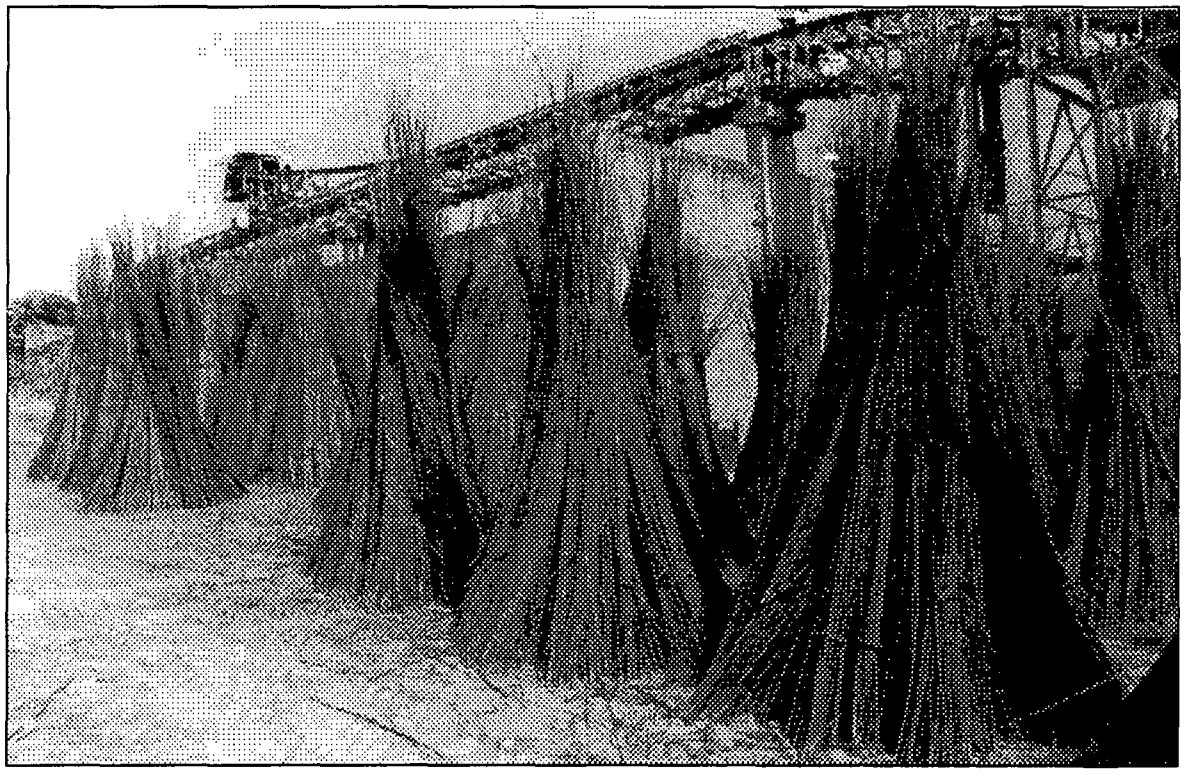
About 05-10% of the plants were attacked by fungus. The growth of fungi and degradation were stopped by the application of fungicide (Diathen M45).

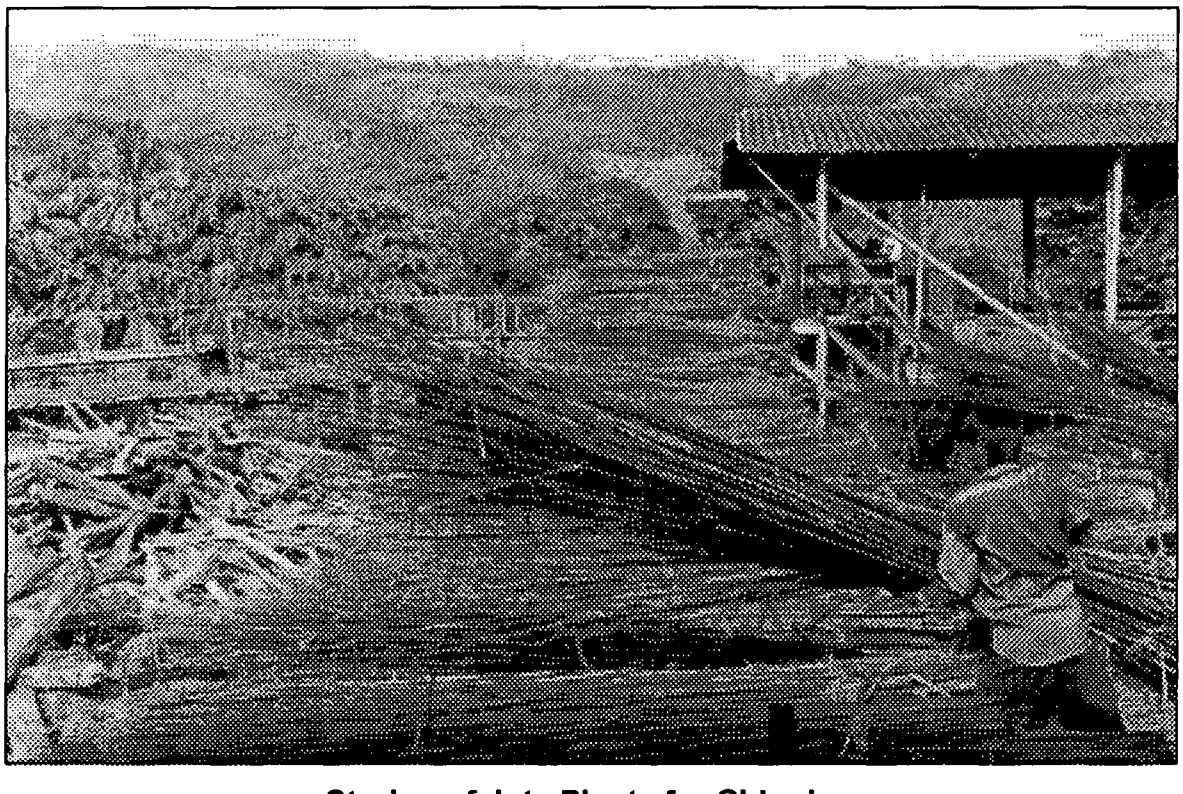


Infected Jute Plant

Storing of the Jute Plant

When the moisture content of the plant was about 18-20%, plants were kept in bamboo frame in horizontal condition. While keeping the plant in horizontal position provision of ventilation for free movement of air was made. It may be mentioned that if the lignocellulosic materials contain more than 25% moisture then there is a heat generation due to the growth of microorganism. This generation of heat is due to the exothermic metabolic reaction. It happens with wood chips, jute chips and jute fibre when moisture contain is above 25%.





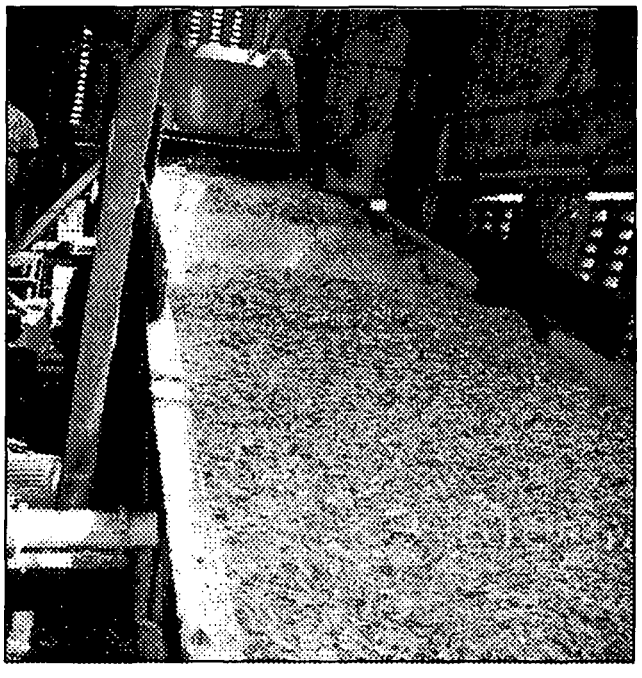
Storing of Jute Plants for Chipping

Chipping of Jute Plants

In the past users faced some problems for chipping. We used Pallmann Chipper which is normally used for chipping bamboo and hard wood. Jute plants were chipped with the same Pallmann Chipper after adjustment of knife and other necessary parameters. Before chipping moisture content of the jute plant was 25-30%. Plant could not be chipped properly if moisture content was below 20%.

Chips Washing

Jute chips were washed is a vibrating screen and these chips were kept in the silo prior to cooking.



Cooking

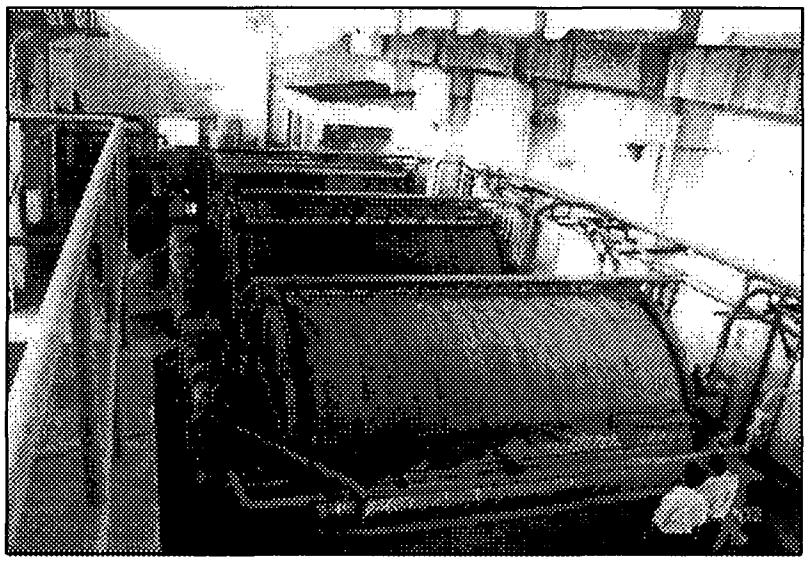
After the optimization of pulping conditions, IJSG/BCIC scientists conducted one large-scale trial for the production of kraft brown paper (using 14 MT of dried jute plant that is equivalent to **56 MT** of green jute plant), another commercial trial for the production of writing and printing paper (using 80 MT of dried jute plant that is equivalent to **320 MT** of green jute plant) in April, 03 and October, 03 respectively. The capacity of digester is 100 M^3. Subsequently the pulp was washed, screened and bleaching. Cooking conditions were presented in **Table 37**.

Table 37: Cooking conditions of jute plants during commercial trial

Sl.#	Cooking condition	Units	Writing & printing	Kraft (Brown)
1.	OD jute chips taken in each digester	MT	15	15
2.	Material: Liquor	--	1:4	1:4
3.	Active alkali as Na_2O	%	16	12
4.	Sulphidity	%	20	20
5.	Cooking temperature	^0C	170	170
6.	Cooking time at 170^0C	Minute	120	90
7.	Steaming time	Minute	90	90
8.	Blowing	Kg/cm^2	5-6	5-6

Pulp washing and screening

The pulp was washed in a three stages rotary vacuum drum filter. Washed pulp was screened in Johnson vibrating screener.

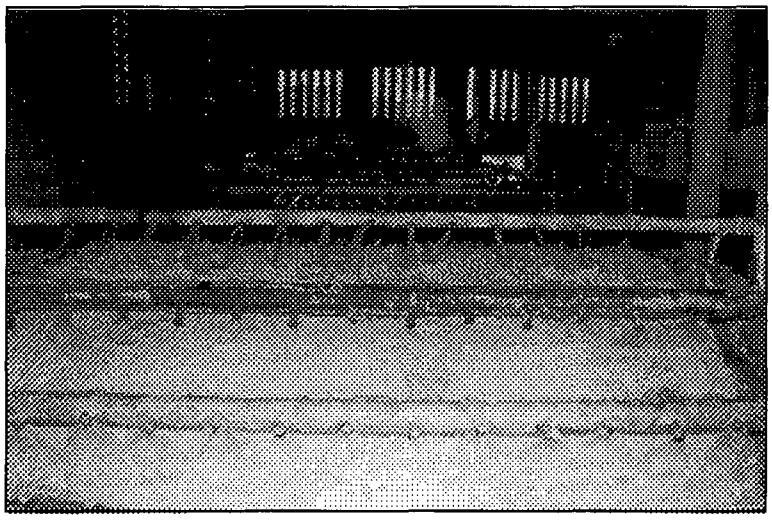


3 stage pulp washing

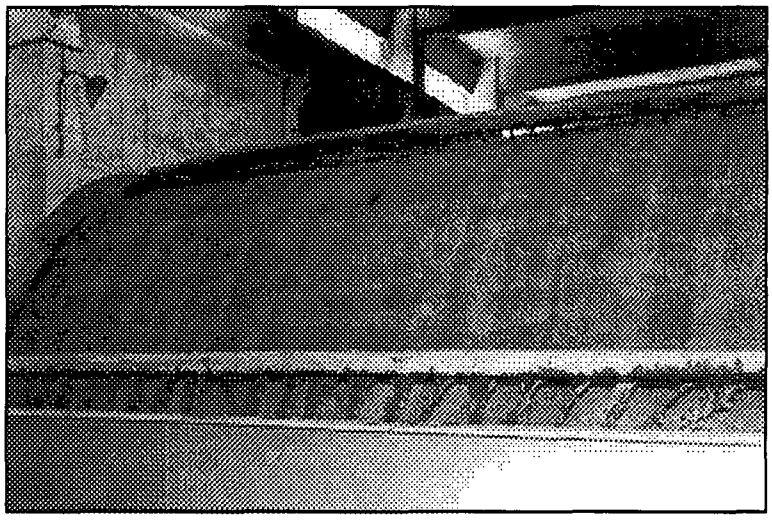
Bleaching

The screened chemical pulp was bleached in the conventional CEHH bleaching sequence i.e. chlorine–alkali–hypochlorite. The detailed bleaching conditions are presented in the **table 38**.

C stage



E stage



H stage

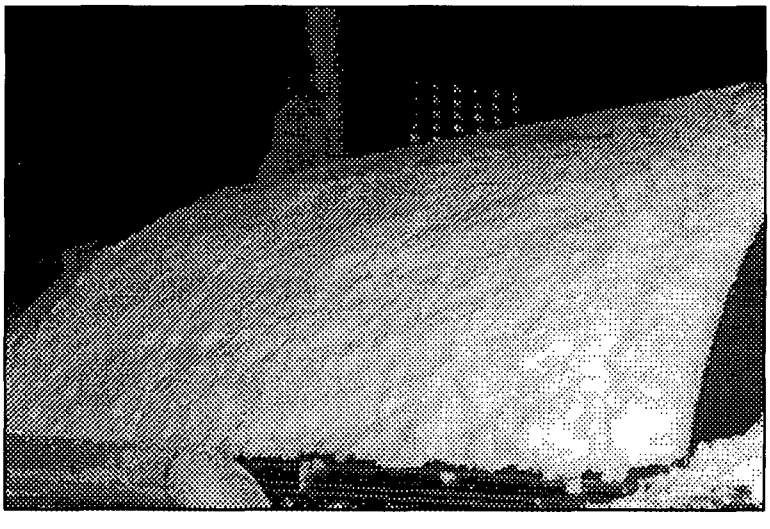
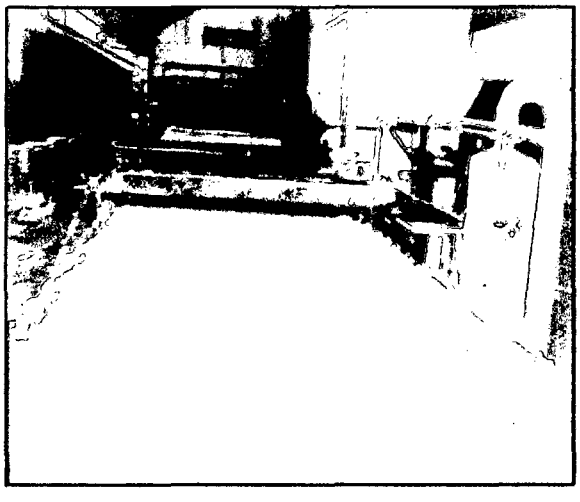


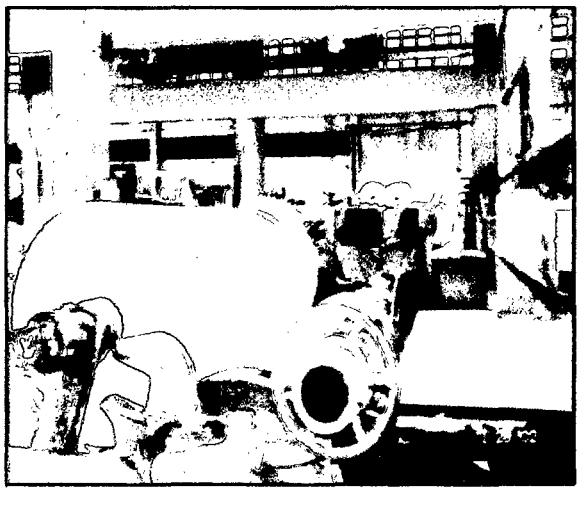
Table 38: Bleaching conditions c pulp from whole jute plant

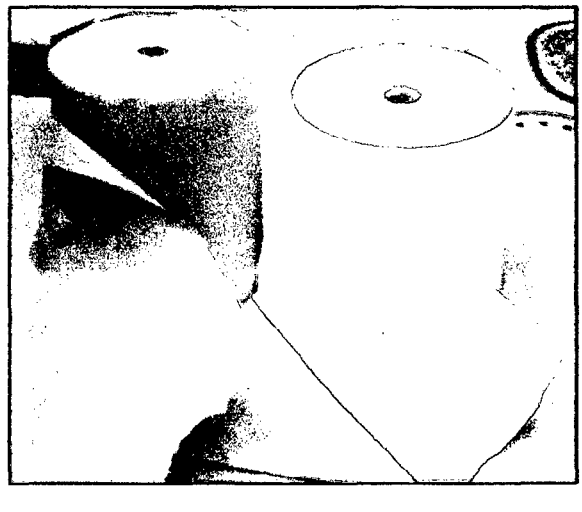
Parameters	Units	Chlorination 1 hr at 25^{0}C	Alkali extraction 1 hr at 65^{0}C	Hypo stage H_1 1 hr at 40^{0}C	Hypo stage H_2 2 hrs at 40^{0}C
Cl_2 (kappa No.x 0.22)	%	4.6	--	--	--
NaOH	%	--	2.0	--	--
Active Cl_2 from Hypo	%	--	--	1.5	1.0
Consistency	%	2.5	03	03	03
Residual Cl_2	%	0.8	--	0.54	0.30
Residual pH	--	2.2	10.85	8.8	8.9
Relative viscosity	--	6.24	5.32	4.83	4.60
Brightness	% ISO	41.0	43.2	75.2	80.0

Paper Making

These bleached pulps were refined in a conical disk refiner and double disc refiner (DDR) parallelly and adding sizing & filler materials before entering into head box. Then 70 GSM paper was made at PM-2 at KPM (WALMSLEYS (BURY) Ltd., England). The speed of the paper machine was 200 m/min. The brightness of the paper was 78 ISO.







Results

Yield, kappa No. and physical properties of unbleached and bleached paper are presented in **table 39 and 40**. Physical properties of both the papers made from jute were compared with the paper made from bamboo.

Table 39: Comparison of yield and physical properties of unbleached paper from whole jute plant and bamboo

Material	Yield %	Kappa No.	Burst Index $KPam^2/g$	Tear Index MNm^2/g	Tensile Index Nm/g
Whole Jute Plant	55	46	2.9	10.08	60.83
Bamboo	45	44	2.2	8.5	42.5

Table 40: Comparison of yield and physical properties of writing and printing paper from whole jute plant and bamboo

Raw material	Yield %	Kappa No.	Burst Index KPa m2/g	Tear Index mNm2/g	Tensile index kN/m	Freeness °SR
Whole jute plant	45	21	3.64	13.71	28.51	33
Bamboo	45	22	2.28	10.50	25.03	35

Conclusion

- The yield of screened chemical pulp was 45% and screened kraft pulp was 55%.

- With the existing facilities of chemical pulping mill of BCIC, whole jute plant can be used commercially for the production of pulp and paper. Quality of paper is suitable for wrapping and different grades of writing paper. Physical properties of unbleached and writing paper are superior to paper made from bamboo and tropical wood.

Appendix - F

APPENDIX-F

Tour Report of the Project Leader

Visit to IOGEN Corporation

In the first leg of tour of Project Leader, he visited Iogen Corporation at Ottawa and met **Dr. Jeffrey S. Tolan**. Iogen Corporation develops; manufacturers, and markets industrial enzymes to be used to modify and improve the processing of natural fibres, bleach pulp and paper in textiles and animal feed industries. Based in Ottawa, Ontario, Canada, Iogen is a privately owned Canadian Company whose enzyme products are sold around the world and used in the diverse range of applications. During my visit I discussed with him about the details of production and applications of enzymes in biobleaching of pulp and paper and also collected some brochure.

Xylanase enzyme of Iogen Corporation was introduced commercially in 1991. These enzymes are now being used in 11-15 paper mills in N. America. They are producing Xylanase using the fungus ***Trichoderma sp***. It may be used in bleaching plants in 3, 4 or 5 stages with chlorine, chlorinedioxide, hydrogenperoxide, oxidation extraction or combination of these. It has been mentioned that instead of CEH sequence better results are obtained with CED, DED or DEDED sequences. Enzyme can also be used OXDP or OXZP sequence [O = Oxygen, X = Xylanase, P = Peroxide, D = Chlorine dioxide, Z = Ozone].

The enzymes act on the xylan, a portion of the hemicellulose. The xylanase action opens up the surface of the pulp, but does not actually bleach the pulp. Somewhat surprisingly, xylanase does not change the kinetics or efficiency of the Do or DC stage. However, once the pulp has been through a Do or DC stage, the oxidized lignin is more easily removed from an enzyme-treated pulp in the alkaline extraction stages. The surface of the pulp allows lignin of higher molecular weight to be removed from the pulp than would be possible from a pulp without enzyme treatment. These results in a decrease of up to 20% in the amount of oxidizing chemicals required to bleach the pulp.

Visit to Institute of Armand Frappier

Project Leader visited Institute of Armand Frappier which is one of the prestigious Institute of Canada. He has a contact with this institute since 1990. He met **Dr. Rolf Morosoli** who is head of Microbiology and Biotechnology. They are using *Streptomyees lividance* as xylanase producing organism. They have also produced several mutants with this organism. He has promised me to send some enzymes for comparison with enzymes that will be produced in our laboratory or enzyme plant.

ANDRITZ Sprout-Bauer Pilot Plant and Research and Development Laboratory.

Sprout-Bauer's pilot plant process equipment laboratory in Springfield, Ohio, is equipped for research development and testing of various pulping processes and related applications. He met **Dr. Eric Chao Xu** who appraised me about their recent development of P-RC APMP process to use jute, kenaf and other non-wood materials as pulp and paper.

Generally speaking, alkaline peroxide refiner mechanical pulping process, where H_2O_2 and alkali in various proportions together with various amount of peroxide stabilizer are used before or during refining. In some cases chips are pretreated, by applying all the chemicals on chips before refining. Others are relying on applying alkaline peroxide at the refiner and have no pre-treatment prior to the alkaline peroxide application. These two types of the alkaline peroxide refiner pulping concepts, however, are not fully effective in the utilization of chemical and mechanical pulping processes.

They are using P-RC APMP, because if alkaline peroxide is used at the refiner, the high temperature would decompose H_2O_2 if the pH is too high. In order to balance the hydrolysis and the peroxide decomposition reaction, a low temperature preconditioning is needed to allow the chemical liquor have a higher pH initially to consume some of the hydrolytic reaction, and to have a certain degree of bleaching. In the meantime, peroxide does not suffer considerable decomposition. After the preconditioning, the pH is reduced, which helps to stabilize the peroxide in the refiner. In this way, a relatively lesser amount of the hydrolysis reactions would occur during refining, so the pH would not drop too fast.

The second reason for applying the chemicals prior to refining is to allow the chemicals to be better distributed across each individual chip's structure before being subjected to a very high temperature in the refiner.

The process is to have a mild ***P*reconditioning, P**, on the alkaline peroxide impregnated lignocellulose material, and to use ***R*efiner Chemical treatment, RC**, for the major, or at least some, bleaching reactions. The name for the process is, therefore, **P-RC**, to emphasize the importance of the preconditioning, the refiner chemical treatment and proper distribution of chemical reactions between the two. It is different from the conventional APMP, or APP, process which aims at doing all the bleaching on chips before refining; and the refiner bleaching, which has no preconditioning and does all the bleaching in the refiner.

The major difference in terms of process design, between P-RC APMP and conventional APMP, or APP, is that the latter has no HC (high consistency being 20-40%) retention after the primary refining, and the pulp is diluted in an interstage washing chest instead. The interstage washing stops any potential bleaching reactions, and hence lowers the bleaching efficiency. In addition, the early designs for the APP process all had a plug screw feeder (PSF) to feed the primary refiner, which could cause further losses in chemical and process efficiency by logging the alkaline peroxide chemical residuals at the PSF.

For industrial operations, a typical simplified process flow sheet for P-RC process is showed in **Figure 1**. The raw materials, or chips, are first impregnated with chemicals. The total impregnation system may have one to three stages, but a 2-stage in general is sufficient for many applications.

Figure 1. Simplified P-RC Process Flow sheet

In a multi-stage impregnation, the first stage generally receives less chemicals than the later stages, and most of bleaching chemicals are applied at the last stage. At each stage of the impregnation, chips are thermally treated for 10-30 minutes first, and then squeezed by a high compression chip press, normally 4:1 compression ratio. Chemical liquor is applied at the discharge end of the press. The pressure release on the chips at the discharge helps the chemicals to penetrate into the chips.

In their Pilot plant they have used water soaked, hammermilled whole jute and was pressed using an Andritz 560GS Impressafiner at 4:1 compression ratio. Chemical liquor containing NaOH, H_2O_2, dtpa, $MgSO_4$ and silicate ware applied at the discharge of the press. A total of three runs with different chemical charges were performed. H_2O_2 varied from 2.5–2.9%, and total alkali from 2.9 to 3.5%. After the materials were impregnated with the chemicals, it was allowed for 20 minutes retention without steaming before being refined.

Refining: An Andritz 91cm diameter Model 401 atmospheric Double Disc Refiner was used for refining. Two stages refining were used for all the runs. There was 15 minutes retention after the primary and before the secondary. Different energies were applied at the secondary to establish a power curve for each run.
In their Pilot plant they have used kenaf and jute as raw materials for pulp and paper. The results from their investigations have shown that the jute APMP pulps have:

1) A better tensile /density or bulk/tensile property than aspen APMP pulps;
2) Strength properties comparable to, or better than, most hard wood pulps;
3) Significantly higher light scattering than aspen APMP pulps; and
4) A similar intrinsic light scattering properties as kenaf APMP pulps, but better tensile/density property. Jute may be easily APMP bleached to 70% ISO or higher brightness without post-bleaching. Comparison with aspen APMP pulp suggest that the jute APMP pulp has a good potential for applications such as printing, writing, tissue and paperboard grades.

Visit to USDA Forest Product Laboratory (FPL) at Madison.

Biopulping is defined as the treatment of wood with lignin degrading fungus prior to pulping.

The biopulping was first introduced by **Professor Karl-Eric L. Erikson** of Swedish Forest Product Laboratory (who has recently retired as a Professor of Biochemistry is an eminent scholar of biotechnology in the University of Georgia). Simultaneously, Kent Kirk also started biopulping at the USDA Forest Product Laboratory (FPL). Various laboratories in the world are using the following white rot fungi for biopulping:

Ceriporiopsis subvermispora, Phanerochaete chrysosporium, Pleurotus eryngii, Pleurotus ostreatus, Trametes hirsuta, Sterum hirsutum *and* Trametes versicolor.

After a decade of research, FPL and Biopulping International Inc. have been able to develop the pulping process and they have taken the lead in commercializing biopulping technology. They have also developed an extensive technology package which includes the patent licensing agreements, supply of fungal inoculums, design and supply of appropriate equipment. I met **Dr. Masood Akhtar**, Head of Biopulping International Inc. He and his colleagues showed me the various developments at FPL and Biochemical International Inc.

They are using a fungus ***C. subvermispora*** that performed biopulping very effectively on both hardwood and softwood. They have selected this fungus as a lignin degrader and was chosen after screening several hundred species of fungi and their strains. The whole process is shown diagrammatically in **Figure 2**.

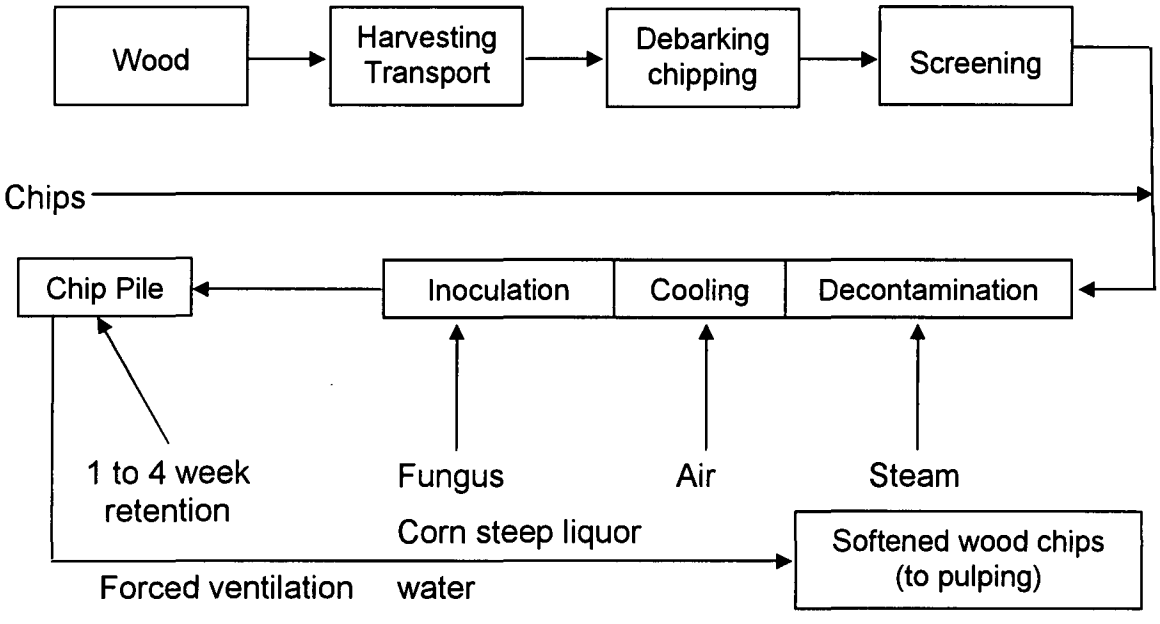


Figure 2: Overview of the biopulping process showing how the biotreatment process fits into an existing mill's wood handling system.

They have used the biopulping in Thermomechanical process. They found that, in the control treatment, the energy requirement is 3033 kwt./h./ton whereas the energy requirement of the fungal treated sample is 2030 kwt./h/ton. In the TMP

process 30% energy saving and significant strength properties were observed. But fungal pretreatment reduced the brightness that can be restored with 60% more H_2O_2 in the bleached liquor. The cause of this darkness was indicated due to the production of quinones.

Based on 33% energy savings and 5% reduction in kraft pulp in the final product a saving of about US$ 5 million can be realized. The biopulping process also reduced the pitch content. Biopulping was also used in the kraft process in which cooking time can be reduced from 90 to 30 minutes without affecting the quality of final product.

Apart from wood plant they have used 6 different non-wood plant, such as kenaf, bagasse, corn stalk, wheat straw, rice straw and flax. Of these kenaf showed the most promising fibrous materials. Their results with mechanical pulping of the whole kenaf indicated that fungal pretreatment saved 36% electrical energy and improved paper strength properties significantly compared to the control(**Table 1**). During kraft pulping the fungal pretreatment had a profound positive effect on the resulting brightness; for both whole and bast kenaf biokraft pulps, brightness ranged from 86 to 88% compared to 78 to 81% for similarly bleached controls.

Parameters	Control	Treatment
Freeness	170	180
Energy requirement (wt./h/kg o.d. material)	2344	1501
Energy savings (% over the control)	-	36
Bust Index (kN/g)	0.65	1.10
Tear Index (mNm^2/g)	2.85	3.39
Tensile Index (Nm/g)	15.3	23.5
TEA Index (j/g)	0.16	0.26

Table 1: Biochemical pulping of whole kenaf with ***C. subvermispora*** (2 weeks treatment).

The mechanical properties of bast kenaf biofibres were close to those of soft wood kraft pulp and far superior to those of hardwood kraft pulp. Current research is focused on process optimization, engineering/ scale up, and economics. From

Dr. Masood Akhtar Project Leader received two strains of ***C. subvermispora*** and a wild strain of Portugal and he has also collected a video cassette on biopulping process from him.

Project Leader met **Dr. Aziz Ahmed**, Pulping and Bleaching Department, who is working in FPL. He is working with kenaf for a long time. He found kraft pulp of whole kenaf and bast kenaf to be comparable to hard wood kraft and soft wood kraft pulping process. The bleachability of pulp in DED, DEDED and DEDP stages was evaluated. The mechanical properties of fungi treated whole kenaf kraft pulp were similar to those of the controls. The mechanical properties of the bast fibre pulp (fungi treated or control) showed significantly high tear values when compared with whole kenaf. The brightness of biokraft bast fibre was about 8% higher than control for a similar three (DED) or four (DEDP) stage bleaching. The brightness of biokraft pulp of whole kenaf and bast kenaf was in the range of 86-88% compared to 78 to 81% for control with similar bleaching treatment. Whole kenaf pulp yield was in the range of 42 to 44% with kappa numbers between 17-22, whereas, bast kenaf pulp yield was between 50 to 52% with the kappa numbers of 12-14. That means, kraft pulp needs substantially less chemicals during bleaching in comparison to soft wood kraft pulp. The mechanical properties of kenaf kraft pulp, especially the bast, are closed to soft wood pulp and far superior to those of hard wood pulp. **Dr. Aziz Ahmed** has already submitted a project to use whole kenaf for pulping without the use of sulfur and chlorine. Project Leader also met **Dr. Suki C. Croan** of Mycology Department, FPL, and collected few stains for biopulping.

Visit to Georgia Institute of Technology

Project Leader went to Georgia Institute of Technology and met **Professor Jeffery S. Hsieh** who is the Professor of Chemical Engineering and Director, Pulp and Paper Engineering. At present they are using kenaf core in their pilot plant. They use kenaf in TMP and CTMP process. The conclusions of their result may be summarized as follows:

- Pine TMP pulp had a lower unbleached brightness compared to kenaf core TMP pulp. After bleaching the higher brightness margin was maintained.
- Kenaf core TMP demonstrated easier bleachability compared to pine TMP.
- Tensile strength at a given level of bulk was higher for the pine TMP pulp than for the kenaf core TMP pulp.
- For kenaf TMP pulp tensile strength increased with increased bleaching and a higher brightness.
- For the pine TMP pulp tensile strength decreased with the increased bleaching and a higher brightness.
- For pine TMP pulp hand sheet bulk increased with increased bleaching and a higher brightness.
- For kenaf TMP pulp, hand sheet bulk decreased with increased bleaching and the higher brightness.
- The strength and brightness values obtained for bleached kenaf core TMP were comparable to those obtained earlier or hardwood pulp.
- During discussion **Prof. Jeffery S. Hsieh** told that as the strength of the core (stick) of kenaf is comparatively low, so they would blend 30-40% of bast (bark) with kenaf core for better quality pulp and paper.

Visit to University of Georgia at Athens

At the Georgia University at Athens, Project Leader met **Dr. Rolf Adolphson**, Director, Pilot Plant, Department of Biochemistry and Molecular Biology. It may be mentioned that University of Georgia recently established two pilot plants, one for bleaching pulp and another for drinking and recycling of paper. In their pilot plant EnZone process which combine oxygen and enzymatic delignification of hard wood pulp with ozone treatment and a final peroxide bleaching stage to produce pulp of equal brightness as compared to conventional chlorinedioxide bleached pulp. An alkaline extraction stage is inserted between the enzyme and ozone stages to obtain fully bleached softwood kraft pulp. This plant was set up by world renowned biotechnologist **Prof. Karl-Erik L. Erikson** who has recently retired as Prof. of Biochemistry. In addition to that they used DEDED sequence in the bleaching process.

Project Leader has a long discussion with him regarding the exchange of microorganisms for mutual interest. It may be mentioned here that the Thermophilic organism that was isolated in our laboratory and which was deposited to German Type Culture is now being used in various laboratories in different countries. This is by far the best suitable thermophilic micoorganism for xylanase production. Very recently we have isolated a few more thermophilic fungi having better xylanase activity. It may be further mentioned here that normally it takes about 10 to 15 months for making genetic improvement of a strain. But he showed interest and he would try to do this work for us, if possible, in a shortest possible time. He is also interested to exchange microorganisms suitable for biopulping and biobleaching. He will send me very shortly a strain of ***P. chrysosporium*** for lignin degradation and a genetically modified strain for xylanase production.

It would not be out of place to mention here that we are producing some mutant strains by using Gama ray at the Atomic Energy Commission of Bangladesh.

In the biopulping project we have five objectives and we have to address four major problems:

1. Storing of green jute/kenaf. Although initially this was assigned to CTP, AREL requested for some additional fund for this activity. As there is no sufficient fund, it was not possible to allocate more funds. Storing of jute/kenaf, it is very important to know the microorganisms responsible for degradation of jute or kenaf and to use fungicide according to the necessity. So BJRI will take up the responsibility for storage of jute and IBFC, China, will take the responsibility for kenaf.

2. **Biopulping**: For biopulping a number of microorganisms have been collected and isolated. In FPL, Madison, the microorganisms were selected on the basis of the reduction of kappa number, reduction of energy requirement and improvement of the physical properties of pulp. In our case, primary selection of microorganism would be on the basis of fading of dyes (Poly-R or cellulose Azure) and the organisms which secrete less

cellulytic enzymes and have more lignolytic activities. The first attempt for biopulping was made at the Swedish Pulp and paper Research Institute by **Prof. Kirl-Erik L. Erikson** using ***P. chrysosporium.*** He also produced cellulose less mutants of this strains. But these mutants showed slow degradation.

One fungus, ***C. subvermispora***, was originally isolated from rotting pine-logs in Southern Chile where farmers had found that fungi could eliminate lignin so specifically that rotted logs – containing almost only cellulose – could be fed directly to the cows. I had the opportunity to discuss the activities of this strain, with Prof. Kirl Eric while we were in Boston. He told me that most likely the fungus lacks the enzyme cellobiohydrolase, necessary for degradation of crystalline cellulose by the white rot fungi. This ***C. subvermispora*** was adopted by the researchers at the Forest Product laboratory (FPL), Madison, Wisconsin and allowed them to develop a biopulping process for production of both mechanical and kraft processes.

Project Leader has already collected several strains of ***C. subvermispora*** and ***P. chrysosporium.*** Unfortunately some of these strains are suitable/effective at low temperature (26-28°C) which may not be suitable in Bangladesh/India during summer. We also have isolated some local strains having similar activities.

After screening, suitable strains would be applied both in mechanical and chemical pulping processes.

We have already reported in the last TAPPI conference held in Boston about biopulping in soda process. So in order to find out the effectiveness of biopulping in different processes, biopulping should be tried in chemical (soda/kraft) and mechanical (TMP/APMP) processes. Prior to the application of biopulping at BCIC, ATO-DLO, CTP, CPPRI and IBFC will optimize the various chemical and mechanical processes as part of their project activities.

3. **Black liquor** management would be carried out.

4. Xylanase treatment is versatile, as it is used in mills that operate with hardwood or softwood. It is used on pulp with or without AQ and surfactant

digester additive. It is used on pulp with or without oxygen delignification. It is also used in batch plants with 3, 4 or 5 stages, with chlorine, chlorine dioxide, hydrogenperoxide, oxidative extraction, or a combination of these. It is used to make fully bleached or semi bleached pulp.

Enzymes can be used to the bleaching process indirectly of directly. In the indirect method the bleachability of pulp is improved through the action of xylanase or other enzymes affecting the extractability of lignin. The most promising direct enzymatic bleaching system is the Laccase mediator system, which degrade lignin. Xylanase has been used in industrial scale for enhancing the bleachability of kraft pulp for about 10 years, where as the Laccase-mediator concept is still under development. In the Laccase mediator concept, the enzyme oxidizes and acts directly on lignin and result is efficient delignification. The common substrate of Laccase **are 2, 2′-aziono-bis [3-ethylbezothiazoline-6-sulfonate] (ABTS)** and 1 **hydroxybenzotrizole (HBT**), and **N, hydroxy-N-phenylacetamide (NHA**). Especially the later mediator results in extremely fast delignification with no significant impact on cellulose structure.

The delignification decreases after an alkaline extraction is reported to be high, up to 40%. Several studies on the mechanisms of Laccase-mediated delignification on pulp have been published.

Proper organisms will be selected for the production of xylanase enzymes in the various bleaching sequences. We have already isolated some thermophilic fungi and we are still trying to find more suitable strains to produce xylanase. Thermophilic strains are suitable to avoid contamination.

Conclusion

The progress of work is in line with the work plan. Two review meetings have taken place. One at UNIDO, Vienna with all participating Institutions, UNIDO, CFC and IJO and one at IJO Secretariat with the EC Delegation and Bangladesh Government. Both the meetings were to the full satisfaction of all concerned.

Appendix - G

APPENDIX- G

1.2 Comparative study of different microorganisms used in various pulp and paper mills in Europe, USA and Canada was made.

According to the objective a computer search was made for biopulping and biobleaching. Approximately 45 references (**Appendix-C**) were found relevant to the project. Such references provided some information about biopulping and biobleaching for selection of known culture for the studies.

Some leading Institutes of **USA, Chile, Brazil, Spain, South Africa, Portugal, Japan, Finland, etc**. are carrying out biopulping and biobleaching experiments. Necessary information which have been published in different scientific Journals have been collected and accordingly tour programme was prepared to collect suitable microorganisms, other related published literature and scientific papers to exchange ideas and to see biopulping and biobleaching operational procedures.

Biopulping

Biopulping is defined as the treatment of wood or other lignocellulosic materials with a natural lignin-degrading fungus prior to pulping. **USDA Forest Service, Forest Products Laboratory in Madison and the University of Wisconsin, USA** (Gary M. Scott. *et al.* 2000) have been working on biopulping for the last 12 years. The research established that biopulping substantially lowers the electrical energy required for mechanical pulping (or increases mill throughout), improves certain strength properties (reducing the need to augment with chemical pulps), and reduces environmental impact. Biopulping also reduces the pitch content of the pulp.

They have developed methods for decontamination of wood chips, cooling, and fungal inoculation sequentially in screw conveyers, and controlling temperature and moisture throughout the chip pile. Mill-scale refining of fungus-treated ***Ceriporiopsis subvermispora*** chips gave results similar to those obtained using the laboratory-scale bioreactors. With this information, a complete process flow sheet has been established for the commercial operation of the process. Based on the electrical energy savings and the strength improvements, the process economics looks very attractive. Several independent economic evaluations of

biopulping have now been completed by both university and industry economists and engineers and are in agreement. They have found energy saving of 33% which is a significant saving in the production of pulp and paper. It has now passed through the scientific and engineering evaluation phases, and is in the realm of business.

One of the chemical companies has already agreed to produce and supply fungal inoculum on a commercial scale. No adverse effects of lignin-degrading fungi on humans have been reported in the literature. Biopulping fungus, ***Ceriporiopsis subvermispora***, was tested by professionals for adverse effects on humans. It was concluded that the fungus is safe for use on a commercial scale.

Biopulping experiments were conducted at **Wood Science and Technology at Chile** (Molina, J.G. *et al.* 2000) with typical industrial logs of *Radiata pine* using white rot fungi: *Pleurotus sp.* Based on chemical characterization, it was found that for *Radiata pine*, *Pleurotus sp.* has an effective delignification power with more residual hemicellulose. Results showed higher yields with lower Kappa No. which means that bleaching could be done with less chemicals.

Sappi Forest Products CSIR of South Africa worked on biopulping with *Pinus patula* wood chips pre-treated with a selected strain of *Stereum hirsutum* (Wolfaardt, F. *et al.* 2000). Wood was pulped on a small scale and the pulping conditions were varied. Lignin content, yield and viscosity of the pulp were evaluated and the alkali consumption determined. The relationships between these parameters were used to model a biokraft pulping process. Fungal pre-treatment reduced kappa number and yield, but not the degree of polymerization of cellulose. Alkali consumption increased when fungal pre-treated wood was pulped. This study showed that biopulping can reduce the kappa number of pulp or reduce the pulping time, but pulp yield is also reduced and chemical consumption increased. The implementation of biopulping on industrial scale would consequently be determined by the specific requirements of a mill that enables it to exploit specific economic benefits.

National Institute for Industrial Engineering and Technology at Lisbon, Portugal, conducted biopulping with fungal strains of *Pleurotus ostreatus, Ceriporiopsis subvermispora* and new isolate named B33/3 (Sena-Martins, G. *et al.* 2000).

Effectiveness of biotreatments on pine wood chips were studied at different incubation times.

Pleurotus Ostreatus has been found to posses lignolytic and lipolytic activities.

Department of Biotechnology at Lorena, Brazil conducted biopulping with *Pinus taeda* wood chips using *C. subvermispora* (André Ferraz *et al.* 2000). The compiled data suggested that lignin breakdown starts at the early biodegradation stages when the biopulping benefits are commonly observed (wood weight losses up to 2-3%). The study showed that a reduction in the alkali charge from 21.8% to 15% was possible when pulp of Kappa No. were prepared.

Department of Applied Chemistry and Microbiology, University of Helsinki, Finland (Hataka, A. *et al.* 2000), conducted impregnation of alkali into spruce wood chips prior to pulping, and the colonizing and competitive ability of the fungi growing on chips were studied by the determination of alkali uptake and consumption from 1M sodium hydroxide solution, and by fluorescein diacetate (FDA) hydrolyzing activity, respectively. *Ceriporiopsis subvermispora* CZ-3, an efficient lignin-degrading white-rot fungus, *Phlebiopsis gigantea* t55, a primary wood colonizing white-rot fungus, and a colorless commercial preparation of a blue-staining non-lignin degrading fungus, Cartapip 97®, were selected for tests on spruce wood. Sapwood and heartwood chips were studied separately. In 3 weeks the total lignin decreased was 9-10% by *C. subvermispora* treatment but other fungi did not remove lignin. Alkali soluble compounds in wood chips clearly increased by *C. subvermispora* treatment but not by the other two fungi. The decrease of FDA activity correlated with the increase of steaming times applied to chips. The results showed that about 1min. steaming decreased 50% of the FDA activity. Cartapip most rapidly started to colonize chips compared with 30 white-rot fungi tested. *C. subvermispora* and Cartapip were relatively competitive. A large amount of freshly isolated fungi were screened using these methods, to evaluate their potential for the treatment of wood chips prior to chemical pulping.

Biobleaching

Xylanase enzyme of **logen Corporation** was introduced commercially in 1991. These enzymes are now being used in 11-15 paper mills in N. America. They are producing Xylanase using the fungus ***Trichoderma sp*** (Jeffrey, S. Tolan. *et al.*

2000). It may be used in bleaching plants in 3, 4 or 5 stages with chlorine, chlorinedioxide, hydrogenperoxide, oxidation extraction or combination of these. It has been mentioned that instead of CEH sequence better results are obtained with CED, DED or DEDED sequences. Enzyme can also be used in OXDP or OXZP sequence [O = Oxygen, X = Xylanase, P = Peroxide, D = Chlorine dioxide, Z = Ozone].

University of Georgia, USA has reported that the application of xylanase as one bleaching stage yields better bleachability for both hardwood and softwood kraft pulps (Jan L. Yang 1992). Combinations of oxygen (O), xylanase treatment (X), hydrogen peroxide (P), enhanced alkaline extraction (Ep) and chlorine dioxide (D) have been tested in a variety of sequences. Hardwood kraft pulp bleached in the OXDP sequence reaches a brightness of 87.7% (ISO) with a viscosity of 17.5 mPa•s. If the xylanase treatment is not applied, a brightness of only 85.7% and a viscosity of 15.8 mPa•s. is obtained. Softwood kraft pulp bleached in the OXPDP sequence attains a brightness of 88.6% with a viscosity of 16.3 mPa•s. Without the enzyme stage, a brightness of only 84.5% and a viscosity of 14.9 mPa•s is obtained. The physical properties of our laboratory bleached pulps compare very favorably with those of commercially produced pulps.

At the **University of Georgia A25KDA** catalytic domain of Xylanase A from the anaerobic fungus *Orpinomyces* sp. *strain PC–2* was produced in *Escherichia coli* (Ashit K. Shah, 2000). The enzyme was stable for at least 120 minutes at pH 5.0 to 10.0 and 40°C. During the incubation of oxygen bleached hardwood kraft pulp for 120 min at 40°C, the xylanase released both lignin and reducing sugars at pH 6.0, 7.0 and 8.0. The enzyme reduced the kappa number and increased the brightness of the pulp at all the pH values studied. The benefits obtained with the enzymatic treatment of the pulp were most obvious at pH 8.0. The xylanase treatment reduced the kappa number of the pulp by approximately 25% and increased the brightness by 2.7%ISO at this pH. A higher brightness ceiling could be achieved when the pulp was treated with the xylanase and bleached using either an ECF bleaching (OXDP) or a TCF bleaching sequence (OXZP). With the xylanase treatment at pH 8.0, pulp was bleached with both the ECF and the TCF sequences to a higher than 90% ISO brightness, which was unattainable without xylanase treatment under the same conditions. There was no change in the strength properties of the paper at the end of both bleaching sequences.

They have also conducted Oxygen-delignified hardwood kraft pulp using a xylanase from *Thermotoga naritima* at pH 10 and 90°C and then bleached with the sequence $D_0ED_1ED_2$. A brightness of 90.5% ISO was achieved for the xylanase-treated pulp, whereas a brightness of 86.7% ISO was achieved when the untreated pulp (the xylanase stage left out) was bleached with the same $D_0ED_1ED_2$ sequence. Xylanase treatment of the pulp at pH 10 and 90°C reduced the chlorine dioxide consumption in the subsequent bleaching by 25% at a target brightness of 86.7% ISO. These results demonstrate that the *T. maritima* xylanase is effective at pH 10, 90°C in enhancing pulp bleaching.

Chemical Engineering, University of Maine, Orono, ME 04469, carried out bleaching with xylanase (Matthew S. *et al.* 2000). In order to better quantify the bleach boosting effect, the impact of the xylanase treatment on chlorine dioxide delignification was studied on a northeastern hardwood. While the enzyme treatment did result in a direct reduction in the Kappa Number, the treatment had no effect on the rate of chlorine dioxide delignification. It was also demonstrated that the enzyme treatment did not affect the reduction in Kappa Number across the extraction stage. The results indicate that the enzyme treatment has no effect on either the bleaching stage or the extraction stage, suggesting that the bleach boosting effect may be a result of the direct removal of lignin during the enzyme stage.

Nalco Chemical Company and VTT Biotechnology, Finland conducted biobleaching with a mixture of Cellulase and xylanase enzyme (Anne Kantelimen *et al.* 2000). Cellulase/hemicellulase mixtures can significantly improve beatability of chemical fibers and also result in improved handsheet properties, such as density and air resistance, which can be very important properties in many speciality papers. In order to avoid strength losses, a careful optimization of enzyme dosage and treatment conditions is important. Z-directional bonding strength can be markedly improved by cellulase/hemicellulase treatment. In mill applications, energy savings have been obtained with cellulase/hemicellulase treatments prior to refining, without any negative effects in strength properties. ESEM images gave indications of improved fibrillation when enzyme treatment preceded refining of kraft pulp.

Jaime Renewable Resources Laboratory, Universidad de Concepción-Chile, used xylanase in organic solvent to bleach Eucalyptus Kraft –Oxygen Pulp (Ruiz José, 2000). Xylanase treatment of *Eucalyptus* kraft-oxygen pulp was bleaching with the ECF sequence X_mDED. The D first stage charge of ClO_2 were varied from 0.2 to 1.0%. Non solvent effect was observed in this sequence. The selectivity, kappa number, brightness and viscosity protection were similar to those observed in XDED sequence in which the Cartazyme-treatment was carried out in aqueous medium. But, in both sequences, the improvement in bleaching was better than in the non-xylanase-treated pulps. A large reduction in ClO_2 requirements was observed. The xylanase treatment permitted bleaching to high brightness (87.6% ISO) at ClO_2 levels equivalent to 0.6% in a sequence $XD_{0.6}$ $ED_{0.6}$, similar value (87.3) was obtained in the sequence $D_{1.0}ED_{1.0}$, while in the sequence $D_{0.6}ED_{0.6}$ the brightness was 84.8%. The results suggest that mills using Cartazyme 9704E could operate under conditions equivalent to 0.6% ClO_2, employing at least 40% less ClO_2. This valuable reduction in bleaching chemical requirements would also have a major impact on AOX reduction and would help to improve the cost efficiency of mill operations.

ICI Canada Inc, Forest Product Research Group used Xylanase for treatment for the bleaching of softwood Kraft pulps (Janice Hamilton, *et al.* 1993). The effectiveness of xylanase treatment of softwood pulps in bleaching to high brightness is influenced by the degree of chlorine dioxide substitution used in the bleach sequence and by the final target brightness to be achieved. In a standard (CD) EDED sequence, xylanase treatment reduced chlorination-stage charges at all substitution levels examined, but was particularly effective at 100% substitution (DEDED sequence). Up to 40% reduction in AOX can also be achieved. Organic halide content of pulps was only marginally improved by xylanase treatment. Xylanase treatments also raise the brightness ceiling of fully bleached pulps. Therefore, the use of xylanase in pre-treatment of pulps bleached using (CD) EDED type sequences to > 91% ISO brightness becomes attractive. These brightness can be realised using lower chlorine multiples than the controls and with lower chlorine dioxide substitution.

Scott Paper Company Kraft Pulp Mill in Spain, conducted trials to evaluate enzyme prebleaching as a means of reducing or eliminating chlorine as a bleaching agent (James C.Turner, 1992) the chlorination stage of a (CD) (EO)

D(ED)D bleach sequence was temporarily eliminated and replaced with an enzymatic pre-treatment stage followed by a shortened (EOP)D(EP)D bleach sequence. A pulp of 88% ISO brightness was obtained using high dosages of chlorine dioxide and hydrogen peroxide. Without pre-treatment, the shortened bleach sequence provided a pulp of 82.5% ISO at comparable chemical dosages. The results show that high-brightness pulps can be produced without chlorination.

Department of Forest Product, Faculty of Agriculture, Kyushu University, Japan applied fungus IZU 154 to bleaching of oxygen bleached hardwood kraft pulp (Ryuichiro Kondo, *et al.* 2000). The fungus IZU-154 brightened the pulp and simultaneously decreased its kappa number. Brightness was increased by 17 and 22 points by three-day and five-day treatments, respectively, and kappa number was decreased from 10.1 to 6.4 by a five-day treatment. Yield loss was less than 1% by the five-day treatment. The combination of the three-day treatment with IZU-154(F), alkaline extraction with 2% charge of NaOH on pulp (E), and hydrogen peroxide bleaching with 5% charge of H_2O_2 (P) gave a pulp of 86.3% ISO brightness. The five day-treatment pulp was brightened to 87.3% ISO brightness by 4% H_2O_2 bleaching after alkaline extraction. Optical and strength properties of OFEP–bleached pulp were sufficiently comparable to those of conventional OCED-bleached pulp.

Tamil Nadu Newsprint and Papers Limited (TNPL) used enzymes to improve the bleachability of bagasse mechanical pulp (Duggirala Y. Prasad. *et al.* 1996). Two crude enzyme preparations were tested for their potential to enhance the bleachability of bagasse mechanical pulp. Enzyme preparations were obtained from fungi growing on wet bagasse in bulk storage. Pulp brightness after bleaching with hydrogen peroxide was 2-3 points higher for the enzyme-treated pulp than for an untreated control pulp. Strength properties of the enzyme-treated pulps were comparable with the control. Various chemical pretreatments were tested in an effort to enhance the enzyme treatment. Conventional chemicals had little effect, but a two-stage enzyme treatment-xylanase followed by treatment with one of the tested enzymes – boosted the bleached brightness by an additional 1.3 points.

Appendix - H

APPENDIX-H

List of the papers collected for Biopulping and Biobleaching

Biopulping

1. Agarwal, U.P., *et al.,* "Understanding Fungus – induced brightness loss of biomechanical pulps" TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

2. Akhtar, M., *et al* "Biomechanical pulping of Loblolly Pine with different strains of the white-rot fungus *Ceriporiopsis subvermispora*, TAPPI Journal, pp. 105-109, 1992.

3. Akhtar, M., *et al* "Using Simons Stain to Evaluate fibre characteristics of biochemical pulps, TAPPI Journal, pp. 121-124, 1992.

4. Blanchette, R. A, *el at., "*Biological Control of Pitch in Pulp and Paper Production by *Ophiostoma piliferum"* TAPPI Journal, pp.102-106, 1992.

5. Brush, T.S. *et al.*, "Improving Soft wood Mechanical Pulp Properties with *Ophiostoma piliferum*" TAPPI Pulping Conference Proceedings, pp. 303-308, 1995.

6. Dorado, J. *et al.*, 'Transformation of wheat straw in the course of solid-state fermentation by four *Ligninolytic basidiomycetes"*, Accepted for publication in the Journal of Enzyme and microbial technology 26 (1999)

7. Eriksson, L. Karl-Erik "A overview of biotechnology in pulp and paper industry" TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

8. Ferraz André, *et al.*, "Characterization of residual components in samples biotreated by the biopulping fungus *Ceriporiopsis subvermispora*" TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

9. Hataka, A *et al.*, "Evaluation of Fungi for Biokraft Pulping of Softwood" TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

10. Hataka, A. *et al.*, "Mineralizatin and solubilizatin of synthetic lignin by manganese peroxidases from *Nematoloma frowardii*and *Phlebia radiata",* Journal of Biotechnology, 67, pp. 217-228, 1999.

11. Hataka, A. *et al.*, "Ligning peroxidases, manganese peroxidases, and other ligninolytic enzymes produced by *Phlebia radiata* during solid-state fermentation of wheat straw", Applied and Environmental Microbiology, 61(10), pp. 3515-3520, 1995.

12. Hataka, A. *et al.*, "The effect of Quinone-reducing and Phenol-Methylating enzymes on the yellowing of mechanical pulp", Holzforschung, 48 (1) pp. 82-88, 1994.

13. Hataka, A. "Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation", FEMS Microbiology Reviews, 13, pp. 125-135, 1994.

14. Mohiuddin, G. *et al.*, "Biopulping of whole jute with different strains of white-rot fungus in soda process", TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

15. Molina J.G, *et al.*, "Yield Increase with Softwood Kraft Biopulp" TAPPI 2000 Pulping/ *et* Process and Product Quality Conference, Boston, USA.

16. Myers, C.G., *et al.,* "Biopulping small diameter trees" TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

17. Scott, G.M. *et al.*, "TAPPI 2000 Pulping/Process and Product Quality Conference", Boston, USA.

18. Sena-Martins, G., *et al.*, "Biopulping of Pine Wood Chips for Production of Kraft Paper Board" TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

19. Wolfaardt, F., *et.al.* "Modelling of parameters for the kraft pulping of fungal treated softwood" TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

Biobleaching

20. Archibald, F.S. "Lignin peroxidase activity is not important in biological bleaching and delignification of unbleaching kraft pulp by *Trametes versicolor*", Applied and Environmental Microbiology, 58(9), pp.3101-3109, 1992.

21. Archibald, F.S. "A new assay for lignin-type peroxidases employing the dye azure- B", Applied and Environmental Microbiology, 58(9), pp. 3110-3116, 1992.

22. Ehara, K *et al.*, "Biobleaching of softwood and hardwood kraft pulp with manganese peroxidase", Mokuzai Gakkaishi, 43(10), pp.861-868, 1997.

23. Eriksson, L. Karl-Erik, *at al.,* "Pulp bleaching and drinking pilot plants use chlorine-free process". TAPPI Journal pp. 80-81, 1997.

24. Hamilton, J. *at al.*, "Xylanase treatment for the bleaching of softwood kraft pulps: the effect of chlorine dioxide substitution" TAPPI Journal, pp. 200-206, 1993.

25. Hofrichter, M. *et al.*, "Production of manganese peroxidase and organic acids and mineralization of ^{14}C-labelled lagnin (^{14}C-DHP) during Solid-State Fermentation of Wheat Straw with the While Rot Fungus *Nematoloma frowardii",* Applied and Environmental Microbiology, 65(5), pp. 1864-1870, 1999.

26. Iimori, T. *et al.*, "Effects of treatment conditions on treatment times for biobleaching by SKB-1152", Mokuzai Gakkaishi, 42(3), pp.313-317, 1996.

27. Jean, P. "Mill trial experiences with xylanase AOX and chemical reductions" Pulp and Paper Canada 95:12, pp. 517-519, (1994).

28. Jose, R. *et al.*, "Eucalyptus Kraft-oxygen pulp bleaching with xylanase in organic solvent", TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

29. Kantelinen, A. *et al.,* "Application of enzyme in production of release and high density papers", TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

30. Katagiri, N., *et al.*, "Bleaching of softwood kraft pulp by white-rot fungi and its related enzymes", Mokuzai Gakkaishi, 43(8), pp.678-685, 1997.

31. Katagiri, N., *et al.*, "Correlation of brightening with cumulative enzyme activity related to lignin biodegradation during biobleaching of kraft pulp by white rot fungi in the solid-state fermentation system", Applied and Environmental Microbiology, 61(2), pp.617-622, 1995.

32. Kondo, R. *et al.*, Biobleaching of hardwood kraft pulp by a marine fungus and its enzyme", TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

33. Mehta, V. *at al.*, "Biobleaching of eucalyptus kraft pulp with *Phanerochaete chrysosporium* and its effect on paper properties", TAPPI Journal, 75(8), pp.151-152, 1992.

34. Moreira, M.T., *et al.*, "Manganese is not required for biobleaching of oxygen-delignified kraft pulp by the white rot fungus ***Bjerkandera sp.*** Strain BOS55, Applied and Environmental Microbiology, 63(5), pp. 1749-1755, 1997.

35. Nezamoleslami, A. *et al.*, "Biobleaching of kenaf bast fiber, Soda-AQ pulp using white-rot fungus"", TAPPI Journal 81(6), pp. 179-183, 1998.

36. Petit-Conil, M. *et al.*, "Screening of Basidiomycetes and Ascomycetes for the production of laccases and the potential of these enzymes in pulp and paper making", TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

37. Shah, A. K *et al.*, "Xylanase treatment of Oxygen-bleached hardwood kraft pulp at high temperature and alkaline pH levels given substantial savings in bleaching chemicals" Journal of Pulp and Paper Science, 26(1), pp. 08-11, 2000.

38. Shah, A. K. *et al.,* "Use of an extremely high specific activity xylanase in ECF and TCF pulp bleaching", 2000 TAPPI Journal Peer Reviewed Paper, pp. 01-12.

39. Stafford, M., *et al.*, "Effect of xylanase treatment of Chlorine dioxide Kinetics and Alkaline extraction efficiency during pulp bleaching" TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

40. Tolan S.J and Thibault, L. "Mill Scale Implementation of Enzyme in Pulp Bleaching" TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

41. Tolan S.J., *et al.*, "The use of a novel enzyme treatment to improve the efficiency of shive removal by bleaching", Pulp and Paper Canada 95:12, pp. 488-493, (1994)

42. Tuner, J.C. *et al.*, "Bleaching with enzymes instead of chlorine – mill trials", TAPPI Journal, pp. 83-89, 1992.

43. Viikari, L. "Enzymes in the pulp and paper industry", TAPPI 2000 Pulping/Process and Product Quality Conference, Boston, USA.

44. Viikari, L, *et al.*, "Potential of enzymes for wood debarking", TAPPI Journal, 76(2) pp. 125-128, 1993.

45. Yang, J.L, *et al.*, "The impact of xylanase on bleaching of kraft pulps", TAPPI Journal, pp. 95-101, 1992.