BIOTECHNOLOGICAL APPLICATION OF ENZYMES FOR MAKING PAPER PULP FROM GREEN JUTE/KENAF (THE WHOLE PLANT)

THIRD INTERIM REPORT

(January 2002 – June 2002)

International Jute Study Group (IJSG)
and
Bangladesh Chemical Industries Corporation (BCIC)
(Karnaphuli Paper Mills Limited)

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CONTENTS

BIOTECHNOLOGICAL APPLICATION OF ENZYMES FOR MAKING PAPER PULP FROM GREEN JUTE/KENAF (THE WHOLE PLANT)

Introduction

The project was approved by the International Jute Council (IJC) at its 25th Session held in Dhaka on 26 – 28 April 1997. The project is funded by **Common Fund for Commodities** (US$ 888,260.00) and co-funded by **Government of France** (US$ 110,000.00) and **European Commission** (EC) and **Government of the People's Republic of Bangladesh** (US$ 200,000.00) with counterpart contribution by the participating Institutes (US$ 295,000). An Agreement of the project was signed by the Common Fund for Commodities (Fund), International Jute Organisation (Supervisory Body) and United Nations Industrial Development Organisation (**UNIDO** – Project Executing Agency or the PEA) in September 1999. Consequently, Grant Agreement was also singed. After the completion of the Agreement, Memorandum of Understanding (MOU) has been singed between UNIDO and all the participating Institutes namely; Bangladesh Jute Research Institute (**BJRI**), Bangladesh Chemical Industries Corporation (**BCIC**), Agrotechnological Research Institute (**ATO** –Netherlands), Central Pulp and Paper Research Institute (**CPPRI**- India), Centre Technique du Papier (**CTP**- France), Institute of Bast Fibre Crop (**IBFC**- China). The project was formally launched by the Hon'ble Prime Minister, Government of the People's Republic of Bangladesh on 7 October 2000. The activities of the project commenced from 1st October 2000.

Jute is a bast fibre obtained from the ribbons of the plant through a process known as retting. It is traditionally used for packaging, transportation and storage of agricultural and industrial products. It has been facing severe competition from synthetics for the past decades. It has become imperative for the survival of the jute economy to make it competitive and widen the field of its application in non-traditional areas.

In view of the growing demand for pulp and paper and increasing world concern for denudation of forest, jute/kenaf, which is the annual crop, is being considered as raw materials for pulp and paper. Jute/Kenaf has already been used in Bangladesh, China, India, Thailand and USA as an alternative raw material for pulp and paper.

The interest for Biotechnological application in the manufacture of pulp and paper is a consequence of the possibilities offered by the Biotechnology in improving the products quality at reduced cost and also in protecting the environment from pollution hazard. Biotechnological process is an environment-friendly process and reduces the production cost of pulp and paper by way of reducing energy consumption in refining, cooking chemical in bleaching and improving pulp properties compared to mechanical and chemical wood pulp.

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

Objective – 1: **Identification of microorganisms and inventory of processes currently used in pulp and paper mills and selection of suitable strains for jute bio-pulping.**

Objective - 2: **To develop suitable microorganism for biopulping and enzymes for biobleaching and to apply the same at BCIC, ATO, CTP, CPPRI 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 in different establishments to determine the physical characteristics of pulp and paper and to evaluate and compare the results.

Objective - 5: Dissemination of results and completion of the project.

The following are the Sub-objectives of the project:

1.1 Identification of microorganisms and inventory of processes currently used in pulp and paper mills and selection of suitable strains for jute biopulping.

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

1.3 To collect from various laboratories, microorganisms presently being used for bio-pulping and biobleaching.

1.4 Isolation of microbial strains from local habitats suitable for biopulping and extraction of enzymes for biobleaching.

1.5 To make a comparative study of the effects of isolated and collected microorganisms.

2.1 To develop suitable microorganism for biopulping and enzyme for biobleaching and to apply the same at BCIC, ATO, CTP, CPPRI and IBFC.

3.1 To manage the black liquor produced during pulping and effluents generated during bleaching.

3.2 Collection of green jute and to identify suitable methods of green jute storage.

4.1 Large-scale trial application of enzymes.

5.1 Dissemination of Project results.

EXECUTIVE SUMMARY

(1.10.2000 to 31.12.2001)

- According to the activity 1.1(a) a computer search was made and collected information from several technical and scientific papers about biopulping and biobleaching.

- On the basis of collected information, communications were made with the various Institutes of the world working on biopulping and biobleaching

- Communications were made to collect microorganisms suitable for biopulping and biobleaching

- A comparative study was completed on the basis of information published in different Journals and reported in the **first interim report** (October 2000- March 2001)

- Project Leader and one associate went on a study tour as a part of the project activities to visit some of the Institutes of Canada, USA and Europe who are working on bio-pulping, bio-bleaching and making paper pulp using Kenaf and soft wood and also to collect micro-organism and necessary information about different processes and to observe bio-pulping and bio-bleaching operational procedure.

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

  Ceriporiopsis subvermispora, Phanerochaete chrysosporium, Ophiostoma piliferum, Phlebia radiata, Phlebiopsis giganta, Sterum hirsutum, Pleurotus eryngii, Trametes versicolor, Pleurotus ostreatus and White rot IZU 154.

- After the literature survey a list of microorganisms suitable for bio-pulping and bio-bleaching was made. Following twelve (12) microorganisms have been selected out of isolated and collected microorganisms from different Institutes:

  4 strains of *Phanerochaete chrysosporium, 3 strains of Ceriporiopsis subvermispora, 1 strain of Pleurotus eryngii*, 2 strains of CPPRI (India) and 2 locally isolated strains *(Fomes lignosus and Coryolus):*

- Screening experiments have been completed on the basis of fading of dyes and clear zone formation. 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 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. They 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.

- 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. ***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 350nm and 519nm. Spectral changes of polymeric dye (Poly-R) at different pH levels were scanned and compared with control medium. ***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.

- For biobleaching, out of 10 isolated thermophilic microorganism 3 were selected for xylanase production on the basis of zone clearance.

- On the basis of screening procedure, **ten** microorganisms have been selected for bio-pulping and **three** for bio-bleaching.

- Optimum conditions for growth of microorganism have been established.

- The optimum condition for maximum enzyme production was obtained by optimizing various parameters like carbon source, pH, temperature, moisture content and incubation time.

- Storing and transportation are two major problems for utilising whole jute as a raw material for pulp and paper. 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. **If 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 due to the growth of microorganism which will degrade the plants.

  The problem could be solved in the following ways:

  - **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 Bangladesh Jute Research Institute (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.**

- Complete chemical analysis of whole jute, bark (bast fibre), core (stick) and bamboo were carried out. From the results it was observed that holocellulose content in the bark is higher than the whole jute and stick. Fibre length of bark is higher.

- 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 using 60g materials to produce pulp with Kappa No. 20-22. Whole jute, bark, and stick have been separately used to produce pulp and to make a comparative study.

- 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. with whole jute plant. The yield and quality of pulp made with whole jute under Soda-AQ process is similar to that of bamboo.

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

- Similarly experiments were carried out to optimise alkali charge and sulfidity in Kraft process using whole jute and bark separately to produce pulp with Kappa No. 20-22. It has been found **that 17% alkali (as Na_2O) with 22% sulfidity produce pulp with required Kappa No. (20-22%) from whole jute.**

- Similarly **15.5% alkali and 22% sulfidity** is suitable for bark to produce pulp with Kappa No. 20 –22.

January 2002 – June 2002

- Eight strains have been sent to **IBFC (China)** along with optimised condition for Biopulping.

- 3 Strains found suitable were sent to **CPPRI (India)** along with optimised condition for biopulping.

- With optimum conditions obtained at KPM (BCIC) for bleachable grade pulp of Kappa No. 20 using Soda-AQ process, 32 biopulping experiments were conducted using 4 strains of ***P. chrysosporium***, 2 strains of ***C. subvermispora***, one strain of ***F. lignosus*** (locally isolated) and one strain of ***ST-2*** using 17% alkali (Na_2O and AQ (0.05%)

- With treated samples yield loss is about 0≅4 which has been observed by other workers also. But Kappa No. is reduced by 3 to 4 unit (15% reduction of Kappa No.). Physical Properties such as tear index and tensile index have been found to improve by 25-30% of handsheet made of chips treated with ***P. chrysosporium-1***, ***P. chrysosporium-3***, ***C. subvermispora*** and ***F. lignosus***.

  Although the yield loss is about 0 ≅ 4% but due to reduction of Kappa No. (15%) there will be less requirement of chemical in the bleaching process.

- Alkali charge was reduced from 17% to 15.5% (as Na_2O) and it has been observed that pulp of desired Kappa No. (20) was obtained with slight improvement of physical properties. Yield loss is more or less same as with 17% (Na_2O). **Desired Kappa No. can also be obtained by reducing the alkali charge (9%).**

- Cooking time (with 17% and 15.5% alkali Na_2O along with 0.05% and 0.1% A.Q) was reduced from 90 minutes to 60 minutes. **With the reduction of cooking time yield of treated and untreated samples have been found to be the same. Moreover, Kappa no. of treated samples are reduced by 15%. As a result, cooking cycles can be increased which will facilitate to have more throughput.**

- Jute chips treated with 4 strains (***P. chrysosporium-1***, ***P. chrysosporium-3***, ***C. subvermispora*** and ***F. lignosus)*** for different days (7, 14 and 21 days) were analysed and it has been found that 14 days treatment have been found to be more appropriate and suitable. Holocellulose loss is minimum with ***C. subvermispora*** followed by ***Fomes lignosus.***

- Biopulping experiments have been conducted with chips treated with 3 strains in kraft process using the optimised condition (17% alkali and 22% sulfidity, cooking at 170°C for 2 hour).

  From our result it has been observed that cooking time can be reduced by 50% (i.e. from 2 hour to 1 hr) with Kappa No. more or less same (19 to 21). Even there was no loss of yield with chips treated with ***C. subvermispora***. Yield loss of chips treated with ***Fomes lignosus*** was very negligible. But there was slight loss of yield with ***P. chrysosporium*** (4%).

- From the all the results it appears that ***C. subvermispora*** and ***Fomes lignosus*** are most suitable for biopulping in kraft process. Cooking time can be reduced significantly which will allow more throughput in the pulp and paper mill.

Work Plan for International Jute Study Group (IJSG)/ Bangladesh Chemical Industries Corporation (BCIC- Karnaphuli Paper Mills (KPM))

Proposed activities of Bangladesh Chemical Industries Corporation (BCIC) IJSG Enzyme Plant from January 2002 to December 2002.

2.1 (a – n):

- Assay of lignolytic activities (**LiP, MnP and Laccase**) of the selected microorganism (8 strains) for different periods (7 days, 14, 21 and 28 days).

- Selection of microorganism for biopulping in soda-AQ-process (using 8 microbes- 4 strains of ***Phanerochaete chrysosporium (PC)***, 2 strains of ***Ceriporiopsis subvermispora*** (CS), 1 strains of ***Fomes Lignosus*** and 1 strain of CPPRI (***ST-2***).

- Biopulping of jute chips in Kraft process (using 8 microbes)
  (8 strains x 4 treatments x 2 pulping process = 64 nos. trials)

- Black liquor analysis.

- Preliminary experiments on mechanical pulping to be carried out at BCIC with their existing facilities which will provide necessary inputs for experiments to be conducted

- Comparative study of production of xylanase in solid/liquid fermentation

- Storage stability of enzyme at two different temperatures (4°C and 30°C) for a period of 30 – 60 days. Assay of residual enzyme activity.

- Comparative study of commercial enzyme pulpzyme HC (Novo) and developed enzyme.

4.1 (a-e) Bleaching trial on control and treated raw materials with commercial and developed enzymes using the following bleaching sequence.

C for Chlorine, **E** for Alkaline extraction, **H** for Hypochlorite, **D** for Chlorine dioxide, **O** for Oxygen and **X** for Enzymes.

C-E-H
X-C-E-H
X-E-C-E-H
D-E-D
X-D-E-D
X-E-D-E-D
O-C-E-H
X-O-C-E-H

Evaluation of physical properties of hand sheet.
Economic analysis of **Soda AQ** and **Kraft Process**.

During the period biopulping of jute chips was conducted at International Jute Study Group (IJSG) and Karnaphuli Paper Mills (KPM) in Soda-A.Q and Kraft process.

Biopulping in Soda A.Q. Process

The optimum conditions which were obtained at BCIC (Bangladesh Chemical Industries Corporation), CTP (Centre Technique du Papier) and CPPRI (Central Pulp and Paper Research Industries) for bleachable grade pulp of Kappa No. 20 using Soda-A.Q are as follows:

Institution	Alkali %	A.Q %	Sulfidity	Cooking Temperature	Cooking Time (min)	Yield %	M:L	Kappa No.
BCIC	17 (Na_2O)	0.05	0	170°C	90	48	1:5	20.5
	17 (Na_2O)	-	22	170°C	120	48	1:5	20.0
CTP	20 (NaOH)	0.10	-	170°C	120	48	1:4	18.3
CPPRI	24 (NaOH)	0.05	-	165°C	90	50	1:4	18.4
	16 (Na_2O)		19	165°C	90	47.6	1:4	20.7

1 plug of 5mm diameter of fresh culture was inoculated in 250ml conical flask containing 100 ml of sterilized inoculum media.

The composition of the inoculum media is 0.3% malt extract, 0.3% yest extract and 1% glucose.

The strain was allowed to grow in stationary condition for 7 days. Normally strains are inoculated in several conical flasks. Weight of biomass of one or two conical flask are taken which is used for the determination of required biomass for particular quantity of jute chips.

At present we are using 0.8gm of biomass per kg of jute chips.

It would be 800 gm of dry biomass per ton of jute chips. USDA FPL uses 2-3kg per ton of wood chips.

After sterilization of 1000g (OD basis) jute chips are taken in a polythene bag and we use a media which contains **100mg of KH_2PO^4, 200mg of NaH_2PO^4, 450mg of $MgSO^4$, 100μg of $FeSO^4$, 20μg of $CuSO^4$, 10μg of $ZnSO^4$, 10μg of $MnSO^4$, 100μg of $CaCl_2$, 10μg of thiamine –HC1 and glucose 20g.**

Total moisture content of jute chips including the media is 66.6%. Chips were treated with microbial strains for 15 days.

Pulps are produced with the treated chips according to our optimised condition (170°C for 1½ hours, liquor ratio 1:5, alkali charge 17% (Na_2O) and AO 0.05%.

Control experiments are conducted with sterilized jute chips in the same condition without media and fungal treatment.

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.** It may be mentioned that ***P. chrysosporium*** is the most widely used strain for biopulping. Among this ***P. chrysosporium, P. chrysosporium-3*** is known as ***B K M F 1767*** is the most widely studied strains. Considering the climatic condition of jute and kenaf growing areas mesophilic ***P. chrysosporium*** having optimum temperature of 35°-38°C was collected. Out of the locally isolated strains of ***F. lignosus*** was found suitable for biopulping.

At first 16 biopulping trial experiments were conducted at KPM using 4 strains of ***P. chrysosporium,*** two strains of ***C. subvermispora,*** one strain of ST-2 (strain of CPPRI) and a locally isolated strain ***F. lignosus.*** Biopulping experiments were conducted with all the **8** strains using optimum condition obtained at KPM (17% Na_2o and AQ from 0.1 to 0.05%).

Table –1: Biopulping of jute chips with 8 fungal strains using Soda- A.Q Process
(Alkali charge 17% and A.Q. 0.05 – 0.1%)

Chips treated with	Alkali % (Na_2O)	A.Q %	pH of liquor	Un-screened yield %	Screen reject %	Kappa No.
Control	17	0.05	12.1	47.50	0.50	20.50
	17	0.10	12.2	47.10	0.20	18.00
P. chrysosporium-1	17	0.05	11.5	40.00	0.80	16.98
	17	0.10	11.6	42.00	0.50	14.94
P. chrysosporium-3	17	0.05	11.5	42.29	4.30	18.70
	17	0.10	11.6	43.15	0.50	17.30
P. chrysosporium-4	17	0.05	11.2	44.00	0.60	24.10
	17	0.10	11.9	44.15	0.60	20.00
P. chrysosporium-5	17	0.05	11.9	43.02	2.35	23.30
	17	0.10	11.5	42.94	1.00	19.39
C. subvermispora-2	17	0.05	11.7	44.20	0.00	16.00
	17	0.10	11.8	44.50	0.00	15.50
C. subvermispora-3	17	0.05	11.2	46.00	3.00	28.00
	17	0.10	11.3	45.00	3.10	26.00
Fomes lignosus	17	0.05	11.6	42.12	0.00	17.50
	17	0.10	11.7	43.25	0.00	15.60
ST-2	17	0.05	11.6	42.00	0.50	22.0
	17	0.10	10.8	41.00	0.00	18.00

Rising time - 100 minutes, Cooking time - 90 minutes at 170°C, liquor ratio- 1:5

Table –2: Physical Properties of Handsheet of Treated and untreated jute chips in Soda- AQ Process (17% Alkali and AQ 0.05-0.1%)

Chips treated with	A.Q %	Burst Index $KPam^2/g$	Tear Index mNm^2/g	Tensile Index Nm/g	Freeness °SR
Control	0.05	2.78	8.55	41.04	15
	0.10	2.85	13.00	49.00	16
P. chrysosporium-1	0.05	3.28	13.01	59.43	21
	0.10	3.87	12.00	62.32	24
P. chrysosporium-3	0.05	3.35	11.56	56.00	21
	0.10	4.40	14.00	65.00	23
P. chrysosporium-4	0.05	2.85	9.60	40.30	15
	0.10	2.96	10.50	42.30	17
P. chrysosporium-5	0.05	2.78	9.50	41.10	16
	0.10	3.01	12.20	42.00	17
C.subvermispora-2	0.05	3.68	15.30	57.12	20
	0.10	3.78	15.50	58.10	20
C.subvermispora-3	0.05	2.30	15.00	42.00	15
	0.10	2.60	16.00	43.00	16
Fomes lignosus	0.05	3.56	14.80	51.00	21
	0.10	3.25	15.60	58.52	21
ST-2	0.05	3.12	8.88	40.00	17
	0.10	3.45	8.62	40.00	18

Rising time - 90 minutes, Cooking time - 90 minutes at 170°C, liquor ratio- 1:5

Results of our experiments are given in **Table –1** and **Table –2**. Analysis and comparison of result shows that among the 8 strains ***P. chrysosporium-1, P. chrysosporium-3, C. subvermispora-2*** and ***Fomes lignosus*** have been found to be more effective in reducing Kappa No. by 3-4 units.

Our objective was to produce pulp of Kappa No. around 20. Using 17% alkali (Na_20) and AQ (0.05%) on treated samples yield loss is about 3-4% which has been observed by other workers also. But in case of ***P. chrysosporium-1, P. chrysosporium-3*** and ***F. lignosus*** the physical properties such as tear index and tensile index have been found to improve by 25 – 30% (Table 2).

Although the yield loss is about 3 - 4% but due to the reduction of Kappa No.(15%) there will be less requirement of chemicals in the bleaching process.

Biopulping experiments with variation of Alkali charge

16 biopulping trial experiments were conducted with these eight strains varying the alkali charge 17 to 15.5% (as Na_2O) and A.Q. from 0.1 to 0.05%. Other parameters were kept same as before. Objective of conducting these experiments was to assess the effectiveness at different percentage of alkali and A.Q.

Table –3: Biopulping of Jute Chips with 8 fungal strains using in Soda A.Q Process.
(15.5% Alkali and AQ 0.05-0.1%)

Chips treated with	Alkali % (Na_2O)	AQ %	pH of liquor	Unscreened yield %	Screen reject %	Kappa No.
Control	15.5	0.05	12.00	48.00	0.85	24.20
	15.5	0.10	12.10	47.60	0.49	22.86
P.chrysosporium-1	15.5	0.05	11.40	43.50	0.58	22.60
	15.5	0.10	11.25	41.20	0.22	20.50
P.chrysosporium-3	15.5	0.05	11.80	41.20	0.52	24.24
	15.5	0.10	11.60	42.50	0.21	19.80
P.chrysosporium-4	15.5	0.05	11.90	43.55	0.60	21.00
	15.5	0.10	11.00	43.00	0.00	21.00
P.chrysosporium-5	15.5	0.05	11.00	43.02	2.30	23.00
	15.5	0.10	11.50	43.00	1.00	19.00
C.subvermispora-2	15.5	0.05	11.50	44.25	0.00	18.73
	15.5	0.10	11.40	44.83	0.00	17.26
C.subvermispora-3	15.5	0.05	11.10	46.00	3.50	28.00
	15.5	0.10	11.20	46.00	3.50	27.00
Fomes Lignosus	15.5	0.05	11.60	42.40	0.65	17.50
	15.5	0.10	11.70	43.20	0.42	17.38

Rising time - 90 minutes, Cooking time - 90 minutes at 170°C, liquor ratio - 1:5

Table –4: Physical properties of treated and untreated handsheet in Soda - AQ process (15.5% Alkali and AQ 0.05-0.1%)

Chips treated with	A.Q %	Burst Index $KPam^2/g$	Tear Index mNm^2/g	Tensile Index Nm/g	Freeness °SR
Control	0.05	2.07	13.41	44.68	14
	0.10	2.18	14.94	40.66	15
P. chrysosporium-1	0.05	3.37	14.50	61.00	19
	0.10	3.61	13.78	67.67	21
P. chrysosporium-3	0.05	3.26	11.82	45.15	21
	0.10	3.26	13.07	48.58	22
P. chrysosporium-4	0.05	1.60	13.00	36.00	15
	0.10	2.60	14.00	38.00	16
P. chrysosporium-5	0.05	2.20	10.00	33.00	14
	0.10	2.40	11.00	37.00	14
C. subvermispora-2	0.05	3.58	14.33	58.76	20
	0.10	3.60	14.25	59.28	21
C. subvermispora3	0.05	2.10	13.00	40.00	15
	0.10	2.40	14.00	42.00	15
Fomes Lignosus	0.05	2.87	12.20	51.52	21
	0.10	3.11	11.94	55.10	23

After the reduction of alkali charge from 17% to 15.5% (as Na_2O) it has been observed that we can get the Kappa No. of desired value (20) with slight improvement of physical properties. Yield loss is more or less same as with 17% (Na_20) alkali charge.

The important point to be mentioned here is that due to the reduction of Kappa No. with the treated samples less chemicals will be required in the bleaching process.

In case of bio-mechanical pulping yield loss has been found to be 4-5% by various workers of other Institutes compared to the control experiment. This is also associated with reduction of brightness. But with treated samples there is a reduction of energy around 30-35% and improvement of physical properties compared to the control mechanical pulping. But in case of biochemical pulping there is a scope in improvement of brightness.

Biopulping in reduced cooking time

After conducting biopulping with optimised pulping condition (17% alkali charge and AQ charge 0.05 to 0.1) and also reducing the alkali charge (15.5% Na_2O and AQ charge 0.05 to 0.1%) we have also conducted trial with ***P. chrysosporium-1, P. chrysosporium-3, C. subvermispora*** and ***F. lignosus*** in reducing cooking time (from 90 minutes to 60 minutes). These four strains were selected for their better performance in respect of reduction of Kappa No. and improvement of physical properties.

Table 5. Biopulping of Jute Chips Treated with 4 fungal strains in Soda- AQ process
(Cooking time 60 minutes)

Chips treated with	Alkali %	AQ %	pH of liquor	Unscreened Yield %	Screen reject %	Kappa No,
Control	17.0	0.05	12.25	46.80	1.38	21.00
	17.0	0.10	12.38	46.00	1.26	20.00
	15.5	0.05	12.04	48.00	5.00	41.00
	15.5	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
	17.0	0.10	11.60	42.00	0.00	15.60
	15.5	0.05	11.50	42.00	1.00	22.20
	15.5	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
	17.0	0.10	11.60	41.00	0.00	18.50
	15.5	0.05	11.60	41.00	0.50	24.00
	15.5	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
	17.0	0.10	11.80	43.36	0.00	16.92
	15.5	0.05	11.60	46.66	0.00	18.18
	15.5	0.10	11.60	45.70	0.00	17.40
Fomes lignosus	17.0	0.05	11.70	43.60	0.00	19.35
	17.0	0.10	11.80	42.42	0.00	15.60
	15.5	0.05	11.60	43.00	0.00	24.00
	15.5	0.10	11.80	42.00	0.00	20.00

Rising time - 90 minutes, Cooking at 170°C, liquor ratio - 1:5

Table 6. Physical properties of hand sheet of treated and untreated chips in soda- AQ process (cooking time - 60 minutes)

Chips treated with	Alkali %	AQ %	Burst Index $KPam^2/g$	Tear Index mNm^2/g	Tensile Index Nm/g	Freeness °SR
Control	17.0	0.05	2.60	12.14	40.00	16
	17.0	0.10	2.80	11.66	41.20	17
	15.5	0.05	1.95	16.25	35.15	14
	15.5	0.10	2.42	15.70	40.20	16
P. chrysosporium-1	17.0	0.05	3.41	13.62	52.51	19
	17.0	0.10	3.67	13.48	57.40	22
	15.5	0.05	3.14	13.20	52.51	20
	15.5	0.10	3.71	13.30	53.00	23
P. chrysosporium-3	17.0	0.05	3.28	14.00	53.97	21
	17.0	0.10	3.80	14.35	64.28	23
	15.5	0.05	3.40	15.17	63.36	23
	15.5	0.10	3.54	14.70	65.00	20
C. subvermispora-2	17.0	0.05	3.98	15.33	58.78	20
	17.0	0.10	3.99	15.58	58.98	21
	15.5	0.05	3.40	14.18	56.82	20
	15.5	0.10	4.65	15.00	59.88	21
Fomes lignosus	17.0	0.05	3.30	14.50	55.56	21
	17.0	0.10	3.56	15.60	58.52	21
	15.5	0.05	3.35	13.83	54.12	17
	15.5	0.10	3.40	13.90	56.21	17

Rising time - 90 minutes, Cooking at 170°C, liquor ratio - 1:5

Observation from the above mentioned Table 5 and Table 6:

Cooking time with (17% and 15.5% alkali Na_2O along with 0.05% and 0.1% A.Q) was reduced from 90 minutes to 60 minutes. **With the reduction of cooking time yield of treated and untreated samples are the same. Moreover, Kappa no. of treated samples are reduced by 15%. As a result, cooking cycles can be increased which will facilitate to have more throughput.**

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- 7.

Table 7. 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

Table 8. Weight and component loss of jute chips treated with different fungal strains

Jute chips treated with	Incubation week	Holocellulose loss %	cellulose loss %	Hemicellulose loss %	Lignin loss %
Water	0	0.00	0.00	0.00	0.00
	1	0.92	0.00	0.00	0.67
	2	1.30	0.00	0.44	0.89
	3	1.58	0.53	0.44	2.22
P. chrysosporium-1	1	15.28	10.61	14.00	2.22
	2	19.77	12.06	32.52	17.86
	3	26.29	15.90	54.44	38.53
P. chrysosporium-3	1	13.55	9.43	19.11	2.67
	2	19.98	12.91	36.44	15.42
	3	27.83	14.04	49.86	34.31
C. subvermispora-2	1	0.71	0.73	13.91	2.35
	2	2.54	2.54	13.60	11.11
	3	3.49	2.17	17.17	15.28
F. lignosus	1	6.24	3.36	9.46	3.33
	2	15.96	9.81	12.62	11.02
	3	21.45	14.46	32.88	22.53

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 7 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 % of **cellulose** and 32% **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.

Biopulping in Kraft Process

Experiments were conducted with 8 strains for biopulping in Soda-AQ Process. From the results it appears that 4 strains (***P. chrysosporium-1, P. chrysosporium-3, C. subvermispora-2*** and ***F. lignosus)*** have been found to be more suitable than the other 4 strains. Moreover experiments were also conducted by varying the no. of days of incubation (7, 14 and 21 days). In this case also satisfactory results were obtained after 14 days of incubation.

Biopulping experiments were conducted with all these 4 strains under optimised condition (17% as Na_2O, sulfidity 22%, cooking time 2 hours at 170°C). In addition we also conducted experiments by reducing the cooking time from 120 minutes to 90 minutes, 60 minutes and 30 minutes)

Table 9. Biopulping of Jute Chips treated with 3 fungal strains in Kraft Process (17% alkali as Na_2O and 22% sulphidity).

Chips treated with	Cooking time (min)	Screened yield %	Kappa No.	Burst Index $kPam^2/g$	Tear index mNm^2/g	Tensile index Nm/g	Freeness °SR
Control	120	44.20	19.25	2.74	8.76	47.07	15
	90	45.55	21.70	2.37	12.76	46.00	16
	60	46.00	27.58	2.85	12.32	49.24	15
P. chrysosporium-1	120	40.12	16.40	3.15	10.13	51.85	21
	90	42.03	18.25	3.70	12.21	55.70	21
	60	42.75	22.50	2.96	14.17	53.82	19
P. chrysosporium-3	120	41.37	17.10	2.87	12.02	53.20	20
	90	42.56	19.75	3.11	13.17	50.55	20
	60	44.00	23.06	2.63	14.25	50.45	19
C. subvermispora-2	120	42.12	16.12	3.25	11.30	55.36	21
	90	44.25	18.65	3.18	14.38	56.63	23
	60	46.92	21.22	3.14	13.87	55.30	20
Fomes lignosus	120	41.07	15.81	3.02	11.51	59.51	24
	90	43.54	17.57	3.50	13.91	58.39	21
	60	43.00	19.33	3.26	14.53	54.65	20
	30	45.29	27.80	2.85	16.38	47.18	18

Rising time - 90 minutes, Cooking at 170°C, liquor ratio - 1:5

From our result it appears that practically there is no yield loss of chips treated with ***C. subvermispora-2*** if the cooking time is reduced from 120 minutes to 60 minutes. Physical properties have been found to improve significantly than the untreated chips. In case of cooking for 90 minutes with ***C. subvermispora-2*** yield loss is negligible.

In case of ***Fomes lignosus*** we can reduce the time from 120 – 90 and 60 minutes but the yield loss is about 3- 4%. We have also reduced the cooking time from 120 to 30 minutes with chips treated with ***Fomes lignosus***, but the Kappa no. is a bit higher. From all these result it appears that with ***C. subvermispora-2*** and ***Fomes lignosus*** are more suitable for biopulping in Kraft process. Cooking time can be reduced significantly which will allow having more throughputs.