



# INTERNATIONAL JUTE ORGANISATION INDUSTRIAL PROJECT

## A MANUAL FOR IMPROVED PROCESSING TECHNIQUE FOR LOW GRADE JUTE AND CUTTINGS



BY

**DR. GHULAM MOHIUDDIN**

INTERNATIONAL JUTE ORGANISATION  
DHAKA, BANGLADESH  
MAY 1992

**ARCHIV  
94303**

This report is presented as received by IDRC from project recipient(s). It has not been subjected to peer review or other review processes.

This work is used with the permission of International Jute Organisation [now IDRC-LIB International Jute Study Group].

© 1992, International Jute Organisation [now International Jute Study Group].

94503

IDRC-LIB

# INTERNATIONAL JUTE ORGANISATION

## INDUSTRIAL PROJECT

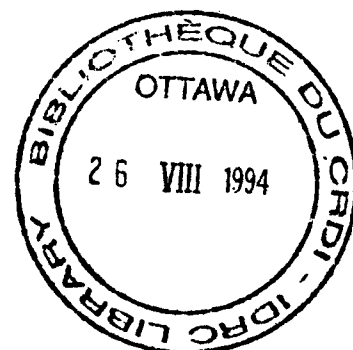
### A MANUAL FOR IMPROVED PROCESSING TECHNIQUE FOR LOW GRADE JUTE AND CUTTINGS

BY

**DR. GHULAM MOHIUDDIN**

**BIOTECHNOLOGIST  
PROJECT LEADER**

**IJO, DHAKA  
BANGLADESH  
MAY 1992**



ARCH V  
677.13 (549.3)  
M 63

## TABLE OF CONTENTS

Foreword .....	ii
Acknowledgements .....	iii
1.0 Introduction .....	1
2.0 Isolation of Microorganisms .....	3
3.0 Screening of Microorganisms .....	6
4.0 Production of Enzyme .....	12
5.0 Assays of Enzymes .....	13
6.0 Physical Charracterisic of Fibre .....	20
7.0 Application of Enzyme in the Emulsion and Piling of Low Grade Jute and Cuttings .....	23
References .....	29


## FOREWORD

Improvement of low grade jute and cuttings and their commercial utilization are one of the major problems of jute industries in the producing countries. To address the issue, a project, viz. "Improved Processing Technique for Low Grade Jute and Cuttings" was launched by the International Jute Organisation (IJO), Dhaka, Bangladesh on 1 December 1989 for a duration of 30 months with the financial support of International Development Research Centre (IDRC), Canada. Bangladesh Jute Research Institute, Dhaka, Bangladesh and the Department of Chemical Engineering, University of Laval, Quebec, Canada carried out the research activities of this project.

Biotechnological methods developed under the project in the laboratories of BJRI had undergone trials, pilot scale in BJRI and large scale in the jute mills in Bangladesh. After successful completion of these trials demonstration trials were carried out in the jute mills in Nepal.

The trials have shown promising results. Much of the credit goes to Dr. G. Mohiuddin, Project Leader, for successful implementation of the project. I appreciate his sincere effort in preparing this manual which will be of great help in the preparation of enzymes in the laboratory scale and its subsequent commercial production and use in the jute mills for the improvement of low grade jute and cuttings.

Dhaka  
May 1992

  
(Md. Shamsul Haque Chishti)  
Executive Director

## **ACKNOWLEDGEMENTS**

I am grateful to the International Jute Organisation and the Government of the People's Republic of Bangladesh for giving me the opportunity to work as the project Leader of this Industrial Project. My sincere thanks are due to the Director General of Bangladesh Jute Research Institute (BJRI) for allowing me to undertake this project activities. I am thankful to International Development Research Centre (IDRC), Ottawa, Canada, for providing financial support for this project.

I also express my thanks and gratefulness to M/s M.A. Sobhan, Khairul Kabir, S.H. Talukder, Dr. Latifa Binte Lutfar, Sk. A. Hasib, Afsana Margina and my other colleagues for their kind cooperation shown during the progress of the project work. I am particularly indebted to Professor Anh LeDuy and his associates for their assistance and cooperation during my visit to Canada for the establishment of standard procedure of enzymes as per planned programme of this project.

I wish to thank Dr. M.A. Talukder, Mr. Kazi Fatiruzzaman, Mr. A.N. Fazlul Bari, and Mr. M. Sabbir Ahamed for their cooperation in conducting the trial experiments in Karim Jute Mills.

I also express my gratitude to Mr. M. Rahman, Mr. A.K.M. Nurul Alam, and Mr. A.M. khanduker for their cooperation in performing the trial experiments in Moqbular Rahman Jute Mills in connection with the project activities.

My sincere thanks are due to Mr. D.B. Pandey and his associates for their help in conducting trial experiment in Shree Raghupati jute Mills Limited, Biratnagar, Nepal.

I am extremely grateful to Mr. Md. Shamsul Haque Chishty, Executive Director, and Mr. A.J. Brown, Director, IJO for their encouragement in performing the activities of this project. My sincere gratitude is due to Dr. S.R. Ranganathan consultant (Industry), IJO, for

*Dr. G. Mohiuddin*

---

generously giving time in my activities and editing this manual. I also thank Mr. Sk. Jamal and other colleagues of IJO for their cooperation.

Dr. G. Mohiuddin  
Project Leader

# **1. INTRODUCTION**

*A MANUAL FOR IMPROVED PROCESSING TECHNIQUE  
FOR LOW GRADE JUTE AND CUTTINGS*

---

Jute is a bast fibre obtained from the stalk of *Corchorus*. In 1991, the world production of jute and allied fibres (kenaf and mesta) was about 3.64 million tons. About 90% of the world production originates in Bangladesh, India, China, Nepal and Thailand. The crop alone provides a source of livelihood for 10 million farmers in these countries. Jute is still a source of foreign exchange earnings and employment in Bangladesh, India and Nepal though its extent has declined over the years.

In the world market, jute and jute goods used traditionally for packaging, transport and storage of agricultural products, have been exposed to severe competition from synthetic substitutes which successfully competed with jute in all its end uses. The technological innovations and production policies followed by the synthetic industries have contributed to an increased supply, at competitive prices, of synthetic products; they have also recently adopted aggressive marketing techniques to promote sales of synthetic products.

As far as prices of raw jute are concerned, no reduction has been easy as farmers are constantly faced with the increased prices of agricultural inputs including labour. In the jute industry also, due to enhanced wages of labour and inputs, production costs of jute goods are increasing. These increased costs of jute products favor easier substitution by synthetics, thus crippling the jute economy.

Further, due to over maturity and scarcity of water about 40% of total jute production is found to be of low grade. The processing problem of low grade jute and cuttings (bottom hard stiff portion of cut fibres) are common in all the jute producing countries. However, the extent of these problems varies from country to country depending on the local consumption as well as type of jute products manufactured.

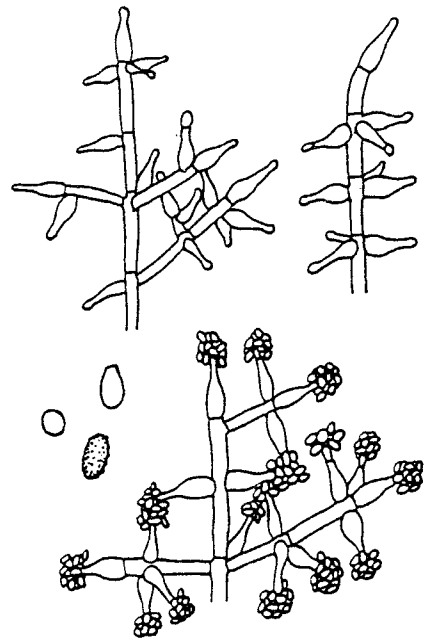
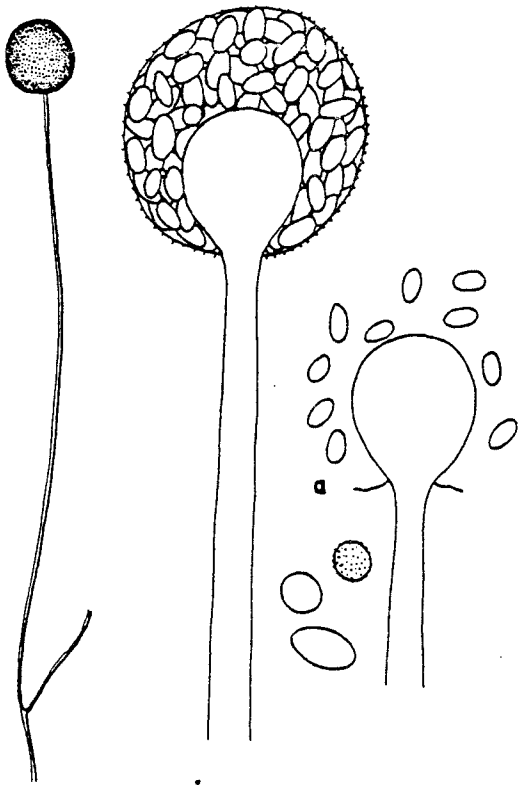
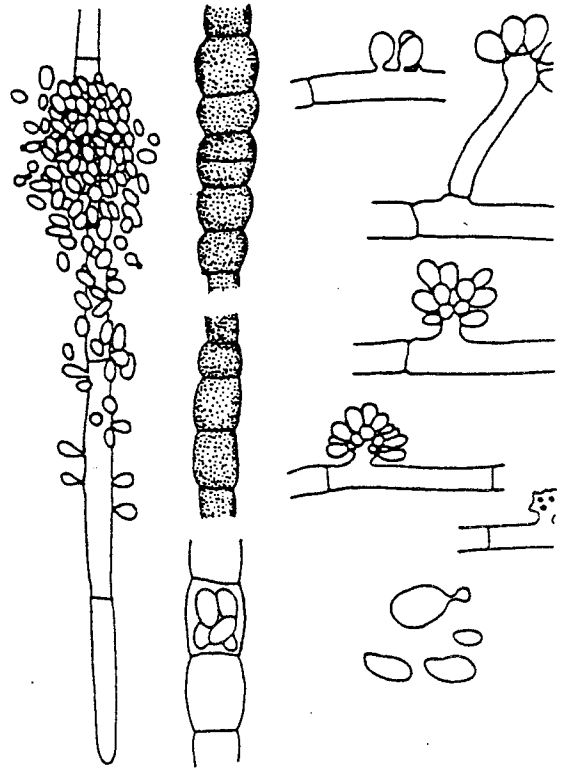
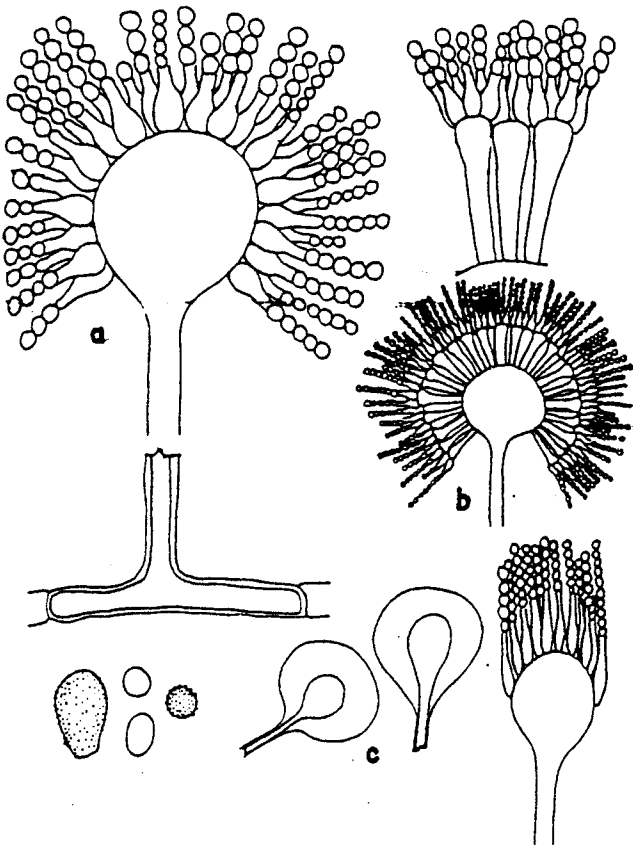
Wasteful cuttings and low grade jute have contributed to the financial woes of the farmers as well as the industrial sector. Price range of the various grades of raw jute is from US\$ 200 to 500 per ton, thus depressing severely the income of poor farmers producing lower grades of jute.



In view of the above a project was initiated on 1st December 1989 by the International Jute Organisation (IJO) with the financial assistance of International Development Research Centre (IDRC), Ottawa, Canada. Research activities of this project were carried out at the **Bangladesh Jute Research Institute** under the supervision of **Dr. Ghulam Mohiuddin**, and at the **Department of Chemical Engineering** under the supervision of **Professor Anh LeDuy**. Through this project, a technical knowhow in the area of Biotechnology has been developed for the enzymatic processing of the low grade jute and cuttings, and comprising the following:

- 1. Isolation of microorganisms**
- 2. Screening of microorganisms**
- 3. Production of enzymes**
- 4. Assay of enzymes**
- 5. Physical Characteristic of fibres**
- 6. Application of enzyme in the emulsion**
- 7. Processing of yarn**

## **2.0 ISOLATION OF MICROORGANISMS**



## **2.1 COLLECTION OF MICROORGANISM**

In order to isolate desired microorganism from natural habitats, samples from matured hot piling bins, caddis and jute dust, damaged jute and jute goods, retting water, rotten wood are to be collected from different areas. Samples are usually kept in sterilized container and are to be labelled properly. Before inoculation of the samples certain amount of it is to be taken in a conical flask containing definite volume of sterile water and are mixed well. For the isolation of thermophilic microorganism samples are usually boiled in a conical flask for 30 minutes to eliminate all the mesophilic microorganisms. Inoculation is to be done with 0.1 ml suspension by spread culture method and incubated 60°C for the isolation of thermophilic and 35°C for the isolation of mesophilic microorganism. The isolated colonies are to be randomly picked, purified and maintained on the following potato dextrose Agar (PDA) slants

### Potato Dextrose Agar (PDA)

Slice potato	200g
Glucose	20g
Agar	15g

finely sliced potatoes are to be cooked in 500 ml water, filtered through cheese cloth and water to be added to filtrate to make up to one liter. The agar is to be dissolved in the filtrate by heating, and glucose is to be added prior to sterilization. The medium is to be autoclaved for 20 minutes at 115°C.

### **Stock culture**

Stock cultures of fungi are generally maintained at 4°C and subcultured at one month intervals.

## **2.2 PRELIMINARY SELECTION OF MICROORGANISMS**

### **2.2.1 PRINCIPLE**

The preliminary activity is to be demonstrated by the hydrolysis of cementing materials in between the fibres in petridishes by feel and torch method.

### **2.2.2 PROCEDURE**

To study the jute softening microorganism jute cuttings are to be moistened with the following Czapek dox solution containing jute extract in the petridishes.

#### **CZAPEK'S DOX**

NaNO <sub>3</sub>	3.0g
K <sub>2</sub> HPO <sub>4</sub>	1.0g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.5g
KCL	0.5g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.01g
Sucrose	30.0g
Distilled water	1.0L

Jute cuttings (about 10 cm long) are placed in glass petridishes and autoclaved in a dry cycles for 15 minute at 121°C. It is observed that autoclaving in a dry cycle does not change the characteristics of cuttings. These cuttings are to be moistened with Czapek's solution and inoculated with the isolated microorganism. After 3/5 days of incubation at 35°C, the cuttings are to be examined by feel and touch method. A control experiment is to be performed with water only. The strains which can soften fibres are to be selected preliminary.

## **2.3 COLLECTION OF STRAIN FROM TYPE CULTURE COLLECTION**

A computer search for information in the literature may be made. For collection of microorganism emphasis should be given for those microorganisms having xylanase (since major component of hemicellulose of jute is xylan), pectinase (polygalacturonase and pectin lyase) and trace amount of cellulose activity.

## **3.0 SCREENING OF MICROORGANISMS**

### **3.1 STANDARD PROCEDURE FOR SCREENING MICROORGANISMS FOR CARBOXYMETHYL CELLULASE AND XYLANASE**

#### **3.1.1 PRINCIPLE**

Enzyme activity is to be 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.

#### **3.1.2 PROCEDURE**

**Step 1:** Petriplates of following media composition are to be prepared for their respective enzymes

	<u>CM Case</u>	<u>Xylanase</u>
Carbon source:	filter paper	larchwood xylan
	2.0 g/L	2.0 g/L
Yeast-N-base:	6.7 g/L	6.7 g/L
Oxgall:	10 g/l	10 g/l
Yeast extract:	2 g/L	2g/L
Agar:	15 g/L	15 g/L

**Step 2:** Media of above composition are to be prepared in distilled water and autoclaved at 121<sup>0</sup>C for 15 minutes. The sterile liquified medium is cooled to 45<sup>0</sup>C to 50<sup>0</sup>C and distributed into petridishes.



- Step 3:** The plates are to be inoculated with microorganisms to be screened for enzyme activity.
- Step 4:** The plates are incubated at optimum temperature for 3-10 days.
- Step 5:** Hydrolytic zones around the growing colonies are recorded for CMCase. Xylan hydrolysis can be only observed after staining the plates.
- Step 6:** To enhance the visibility to hydrolytic zones around the growing colonies, the plates are to be treated as described below.
- a) For CMCase activity: the plates are flooded with 10 ml Congo red solution. The coloration is terminated by pouring off the dye after 20 minutes and replacing with 10 ml of 5 mol/L NaCl solution. After an additional 20 minutes the salt solution is discarded and CMCase activity is revealed by a pale orange zone around the colonies producing CMCase.
  - b) For Xylanase activity: the plates are to be flooded with 96% ethanol. Although clear zones are visible within 3-4 hours of incubation but prolonged incubation enhances the contrast.

### **3.1.3 MATERIALS**

1. Yeast-N-base, oxgall, yeast extract agar, CMC can be obtained from any chemical company.

2. Congo red solution: 0.1 g Congo red is to be dissolved in 100 ml distilled water.
3. 5M NaCl solution: 29.2 g NaCl are to be dissolved in 100 ml distilled water.
4. Citrate buffer. 0.05 mol/L Na-citrate solution may be prepared dissolving 14.7 g Na-citrate in 1 L distilled water. 0.05 mol/L solution of citric acid is to be prepared by dissolving 10.5 g citric acid in 1 L distilled water. Citric acid is to be added in Na-citrate solution until the pH of the mixture reaches 5.3.

## **3.2 STANDARD PROCEDURE FOR SCREENING MICROORGANISMS FOR PECTINASE ACTIVITY**

### **3.2.1 PRINCIPLE**

Most of the pectinolytic fungi produce polygalacturonase (PG) and pectinlyase (PL). PG attacks polygalacturonic acid and PL attacks pectin. Enzyme are secreted by fungi when grown on minimal media supplemented with polygalacturonic acid for detecting PG activity and pectin for detecting PL activity. After a period of incubation, hydrolytic zones are revealed by staining the plates with cetyltrimethyl ammonium bromide which precipitates the ungraded substrate, leaving a clear zone.

### **3.2.2 PROCEDURE**

**Step 1:** The following media composition for detecting PG and PL enzyme activities are to be prepared.

	PL	PG
Pectin (from apple)	10 g/L	0 g/L
Polygalacturonic acid	0 g/L	10 g/L
Na salt		
NaNO <sub>3</sub>	2.0 g/L	2.0 g/L
KH <sub>2</sub> PO <sub>4</sub>	1.0 g/L	1.0 g/L
MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.5 g/L	0.5 g/L
Yeast extract	0.2 g/L	0.2 g/L
Agar	20 g/L	20 g/L
Buffer	HEPES buffer 0.1 mol/L (pH 8.0)	Na-citrate buffer 0.1 mol/L (pH 4.8)

(a) pH of the pectin solution in water should be 4.0 which is natural pH of pectin. This prevents unusual hydrolysis of pectin during autoclaving.

(b) HEPES: N-2 hydroxyethyl piperazine-N2-ethane sulphonic acid. It is non-metabolizable buffer, therefore, the pH of the medium does not change significantly.

**Step 2:** Dissolve polysaccharide in 500 ml water and rest other chemicals in their respective buffers (500 ml). Polysaccharide solutions and mineral solutions are to be autoclaved separately at 121°C for 15 minutes.

**Step 3:** Plates containing PG or PL medium are to be inoculated with microorganisms to be screened for enzyme activities.

**Step 4:** Plates are to be incubated at optimum temperature for 2-7 days.

**Step 5:** Plates should be flooded with 1% (W/V) cetyltrimethyl ammonium bromide solution. Zones of hydrolysis are apparent after 5-10 minutes of incubation.

### **3.2.3 MATERIALS AND REAGENTS**

- a) Polygalacturonic acid-(Na salt), pectin (from apple), HEPES, yeast and cetyltrimethyl ammonium bromide are to be purchased from any chemical co.
- b) Citrate buffer: 29.4 g Na-citrate are to be dissolved in 1 L distilled water and 21.01 g citric acid-H<sub>2</sub>O in 1 L distilled water to prepare 0.1 mol/L solutions. Citric acid solution is to be mixed in Na-citrate solution until the pH reaches 4.8 (approx 45 ml of 0.1 mol/L citric acid is required for 100 ml of 0.1 mol/L Na-citrate to give a buffer solution of 0.1 mol/L, pH 4.8).
- c) HEPES Buffer: 23.83 g of HEPES are to be dissolved in 1 L distilled water. Concentrated NaOH (saturated solution) is to be added to increase pH to 8.0.
- d) Cetyltrimethyl ammonium bromide: 1% (W/V) solution is to be prepared dissolving 1 g cetyltrimethyl ammonium bromide in 100 ml distilled water.

The following strains may be used as reference:

*Aspergillus sojae* ATCC 20235 which is known to produce pectinlyase and polygalacturonase.

*Aureobasidium pullulans* ATCC 62921 which is known to produce xylanase and some cellulase.

*Fusarium oxysporum* FORL 332 which is known to produce pectinase.

After 5 days of incubation at 25-30°C, all strains having PG may form zones. Detecting of PG and PL can vary depending on optimum conditions for specific strains. Some strains having pectinase activity may not be detected by cetyltrimethyl ammonium bromide.

## **4.0 PRODUCTION OF ENZYME**

## **4.1 PREPARATION OF INOCULUM**

Inoculum is to be prepared in yeast malt extract containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1% dextrose at pH 6-8 in a shaker at 100 RPM at 35°C for 3 days.

## **4.2 MEDIUM PREPARATION**

Wheat bran being the cheap source and available in all the jute producing countries may be used as a substrate for solid state fermentation. Wheat bran is to be taken in stainless steel or aluminium trays containing 50-100% moisture at natural pH. Wheat bran containing moisture is to be sterilized for 15 minutes. 1% of inoculum solution is to be incubated at 35°C. Incubation is to be performed for 3/5 days.

## **4.3 EXTRACTION OF ENZYME**

Enzyme is extracted by adding 10 volumes of water containing 1% (W/V) NaCl solution in a container and is to be left for 2 hours with occasional shaking. The slurry is then filtered through nylon cloth, centrifuged at 6000 rpm for 10 minutes. Filter thus obtained is to be stored at temperature around 15-25°C containing sodium azide or other suitable chemical as preservative.

## **5.0 ASSAYS OF ENZYMES**



## 5.1 STANDARD PROCEDURE FOR ASSAYING CARBOXYMETHYL CELLULASE, XYLANASE AND POLYGALACTURONASE ACTIVITIES

### 5.1.1 PRINCIPLE

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, with xylan for assaying xylanase activity and with polygalacturonic acid for assaying polygalacturonase (PG) activity, the reducing sugars released into solution, following hydrolysis, are measured by dinitrosalicylic (DNS) method.

### 5.1.2 PROCEDURE

**Step 1:** The following tubes are to be prepared for each sample of enzyme solution.

Tube No.:	1	2	3	4
Used for:	reading is zero in spectrophotometer	enzyme control	substrate control	test sample
Symbol:	Z	CE <sup>a</sup>	CS <sup>a</sup>	T <sup>a</sup>
Content:				
1.0 mL	Glucose buffer <sup>a</sup>	Glucose buffer <sup>a</sup>	Glucose buffer <sup>a</sup>	Glucose buffer <sup>a</sup>
0.5 mL	Acetate buffer	Acetate buffer	Substrate <sup>b</sup>	Substrate <sup>b</sup>
0.5 mL	Distilled water	Enzyme <sup>c</sup> solution	Distilled water	Enzyme solution

(a) The DNS method for detection of reducing sugars is not sensitive below 100 µg glucose. For accurate determination of low concentrations of reducing sugars, glucose should be added to each sample.

- (b) Substrate solution (1% W/V) are to be prepared in acetate buffer as follows: Na-CMC for assaying CMCase activity, xylan for assaying xylanase activity, and polygalacturonic acid-Na for assaying polygalacturonase activity.
- (c) The enzyme solution should be filtered through Type A/E Gelman fiber glass membrane filter to remove microorganisms and other particles.
- (d) The enzyme control (CE) and the substrate control (CS) measures the reducing sugars produced due to partial hydrolysis of substrate and sugars present as impurity in substrate solution or enzyme solution. A standard glucose solution along with test sample should be included to check the procedure.

**Step 2:** Tubes are to be incubated at 40°C for 20 minutes. Incubation time may vary depending the concentration of enzymes.

**Step 3:** Enzyme reaction is to be stopped for the development of colour reaction by

- a) adding 2 ml of DNS reagent to each tube
- b) then incubating the tubes at 90°C for 15 minutes
- c) then cooling the tubes to room temperature with cold water.

**Step 4:** Definite volume of distilled water may be added.

**Step 5:** Read the absorbance at 575 nm.

**Step 6:** The net quantity of reducing sugars produced by enzymatic reactions, which is measured as absorbance at 575 nm, during the enzyme assay is

*A MANUAL FOR IMPROVED PROCESSING TECHNIQUE  
FOR LOW GRADE JUTE AND CUTTINGS*

---

Actual absorbance = Absorbance of test sample (T)  
— Absorbance of enzyme control (CE)  
— Absorbance of substrate control (CS)

**Step 7:** Reducing sugar concentration (g/L) corresponding to actual absorbance from calibration curve can be determined.

Note: Standard calibration curves of glucose, D-xylose and D-galacturonic acid from their stock solutions can be prepared and the procedure is to be followed as described in step 1-5.

**Step 8:** Enzyme activity is to be calculated and expressed as **International unit (IU)**. **One IU of enzyme is the amount of enzyme which liberates 1  $\mu$ mol of reducing sugars per minute under assay conditions.**

a. CMCase activity IU =  
( $\mu$ mol of glucose equivalent released/mL-min)

$$\frac{\text{glucose concentration released (g/L)} \times 1000}{\text{incubation time (min)} \times 180}$$

b. Xylanase activity IU =  
( $\mu$ mol of xylose equivalent released/mL-min)

$$\frac{\text{Xylose concentration released (g/L)} \times 1000}{\text{incubation time (min)} \times 150}$$

c. Polygalacturonase activity IU =  
( $\mu$ mol of galacturonic acid equivalent released/mL-min)

$$\frac{\text{galacturonic acid concentration released} \times 1000}{\text{incubation time (min)} \times 194}$$

### **5.1.3 MATERIALS AND REAGENTS**

1. Carboxymethyl cellulose-Na (low viscosity), xylan (larchwood), polygalacturonic acid-Na, D-xylose, D-galacturonic acid-monohydrate, and D-glucose are to be purchased from any Chemical Co.
2. Acetate buffer at pH 4.8: 8.66 mL of glacial acetic acid is to be mixed in 1 L distilled water to obtain 0.15 mol/L acetic acid solution. 0.15 mol/L sodium acetate solution is to be added into 0.15 mol/L acetic acid solution until the pH of the mixture reaches 4.8.
3. Substrate solutions: 10 g CMC-Na or 10 g of xylan or 10 g of polygalacturonic acid-Na salt are to be dissolved in 1 L acetate buffer at pH 4.8.
4. Glucose buffer: 0.13 g of glucose is to be dissolved in 1 L acetate buffer at pH 4.8.
5. Standard solutions: 4 g of D-glucose or 4 g of D-xylose or 4 g galacturonic acid-monohydrate is to be dissolved in 1 L of distilled water.
6. Dinitrosalicylic acid reagent: The following chemicals is to be dissolved in 2 L distilled water: NaOH 40 g; dinitrosalicylic acid 20 g; sodium sulfite 1 g; phenol 4 g. This reagent should be kept in dark. It is stable for 2-3 weeks in refrigerator.

## **5.2 STANDARD PROCEDURE FOR ASSAYING PECTIN LYASE ACTIVITY**

### **5.2.1 PRINCIPLE**

The principle for assaying the pectin lyase (PL) activity is based on the rate of formation of 4,5-unsaturated reaction products. The increasing absorbance at 235 nm of the reaction products is monitored with spectrophotometer.

### **5.2.2 PROCEDURE**

**Step 1:** Enzyme extract is to be filtered through type A/E Gelman fiber glass membrane filter.

**Step 2:** Appropriate dilution of the enzyme extract with distilled water.

**Note:** The dilution factor may vary depending upon (a) the concentration of enzyme, and (b) the absorbance of enzyme mixture without substrate. In solid state fermentation, the enzyme extract contains significant level of coloring material of solid substrate which may increase the absorbance of enzyme extract up to such a level that may make it impossible to read absorbance of the enzyme reaction. Also, in case of very high concentration of enzyme, the substrate may be limiting.

**Step 3:** 0.5 ml of the diluted enzyme mixture is to be added with 4.5 ml of 0.1% pectin buffer solution.

**Step 4:** For control, 0.5 ml of distilled water is mixed with 4.5 ml of 0.1% pectin buffer solution.

**Step 5:** Absorbance is to be measured at 235 nm at time zero.

**Step 6:** Tubes are to be incubated at 40°C for appropriate time t (10-20 minutes).

Note: In case of low concentration of enzyme, the incubation time should be longer.

**Step 7:** Absorbance at 235 nm at time t (min) and calculate actual increase in absorbance of the sample as follows.

$$\begin{aligned} \text{Actual increase in absorbance at time } t(\text{min}) = & \\ & (\text{Absorbance of test sample at time } t \\ & \quad - \text{Initial absorbance of the test sample}) \\ & - (\text{Absorbance of control at time } t \\ & \quad - \text{Initial absorbance of control}) \end{aligned}$$

**Step 8:** Units: one unit of PL is defined as an increase in absorbance at 235 nm by 1.0 in the reaction mixture per minute under the assay conditions.

$$\text{PL activity (units/ml)} =$$

$$\frac{\text{Actual increase in absorbance} \times \text{dilution factor} \times 10}{t \text{ (min)}}$$

Note: (a) dilution factor is determined as explained in step 2.

(b) multiplication with factor 10 is necessary because the enzyme is diluted 10 times in the reaction mixture as outlined in step 3.

### 5.2.3 MATERIALS

1. Pectin (from apple)
2. Tris-HCl buffer pH 8.5: dissolve 15.76 g Tris- HCl in 1L distilled water to prepare 0.1 mol/L solution. Add saturated NaOH solution to increase pH to 8.5.
3. Phosphate-citric acid buffer pH 5.5: dissolve 17.41 g  $K_2HPO_4$  in 1L distilled water and 21.01 g citric acid in 1L distilled water to prepare 0.1 mol/L solutions. Mix citric acid solution in phosphate solution until it reaches 5.5.
4. Pectin solution pH 8.5: prepare 0.1% (w/v) pectin solution at pH 8.5 by dissolving 0.1 g pectin in 100 ml Tris-HCl buffer solution (0.1 mol/L, pH 8.5).
5. Pectin solution pH 5.5: prepare 0.1% (w/v) pectin solution at pH 5.5 by dissolving 0.1 g pectin in 100 ml phosphate-citric acid buffer (0.1 mol/L, pH 5.5).

Table I Enzyme activities of various enzyme from isolated (BJRI) and collected strains (ATCC)

Strain	International Unit/ml				
	CMCase	Xylanase	Polygalacturonase	Pectin Lyase	
				at pH 8.5	at pH 5.5
<i>Aspergillus niger</i>	0.81	1.59	1.53	0.55	0.12
<i>Aspergillus terreus</i>	0.55	2.02	2.19	0.44	0.08
<i>Aspergillus sojae</i>	0.29	1.01	0.57	0.62	0.21

100 gm wheat bran + 50 ml water (for fermentation) + 100 ml of 1% w/v Nacl solution (for enzyme extraction).

## **6. PHYSICAL CHARACTERISTICS OF FIBRE**



In order to select appropriate microorganisms for the production of enzyme in large scale, it is necessary to apply enzyme in a small quantity to see the effectiveness in softening the fibres. The enzyme which on being applied on cuttings produce fibres showing softness, raises values of separation index, lower stiffness and fibre diameter will be finally selected for large scale production to improve cuttings.

## **6.1 TOUCH AND FEEL METHOD**

The principle of this method refers to the highly subjectivity of the softness which can be detected by touching and feeling.

In this method, the samples (control and enzyme treated) are to be tested by an expert who has the experience to evaluate the softness of the samples.

The expert should grade the samples into several categories such as soft, moderately soft, slightly soft, etc. These categories give directly the quality of softness of the fibres. This method has the advantage to be rapid and to give a qualitative approximation of the softness of the samples.

## **6.2 SEPARATION INDEX**

This index indicates the ability of the fibres to be separated from barks and specks. Higher the values of the separation index more separable the fibres are.

In measuring this index, iron combs were used for separating fibres from the bark. The number of combing is to be kept the same for each samples (20 times). The fibre bundle left after combing were weighed ( $w_s$ ) and are to be sorted manually to separate the specky fibres from the rest. The clean fibres (without specks) were weighed again ( $w_c$ ), Separation index (SI) is computed as:

$$S.I. = \frac{Wc \times 100}{Ws}$$

Ultimate objective of any softening treatment is to improve this attribute of fibres. From the results (Table II) of S.I., it is conclusive that all different micro-biological treatments increased this quality of fibre to different degrees. Interfibre cementing substances that remain after retting and subsequent drying as expected to be removed to different degrees by the treatments has been directly reflected by the plittability of the fibres within the bundle of jute (Table II).

### **6.3 STIFFNESS**

The distance between the two hanging legs of a fibre bundle of a given weight give a measure of stiffness.

A fibre bundle of 20 cm length (0.55 -0.88) is to be hung over a fixed glass rod (3.8 mm diameter) and a constant weight of 1 g is to be attached at each end of cuttings. Lower the values of stiffness more suitable the fibres are for spinning.

### **6.4 FINENESS**

The fibre fineness of jute cuttings is to be determined by measuring the diameter ( $\mu\text{m}$ ) of individual fibres under a light microscope.

*A MANUAL FOR IMPROVED PROCESSING TECHNIQUE  
FOR LOW GRADE JUTE AND CUTTINGS*

---

Table II Physico-mechanical properties of jute cuttings with and without treatments of enzyme

---

Sample	Fineness $\mu$	Separation Index	Stiffness (Cm between hanging legs)
Control (untreated)	70.6	48.08	18.8
Fibres treated (with water)	69.6	28.44	16.8
Enzyme ( <u>A. niger</u> )	68.4	67.89	15.0
Enzyme ( <u>A. terreus</u> )	56.4	80.67	15.0
Enzyme ( <u>A. sojæ</u> )	59.2	66.25	17.0

---

**7. APPLICATION OF ENZYME IN THE  
EMULSION AND PILING OF LOW  
GRADE JUTE AND CUTTINGS**

## **7.1 PREPARATION OF BATCHING OIL EMULSION CONTAINING ENZYME EXTRACT**

To prepare 350 litre emulsion, mix up batching oil (56 Kg), soap (nonident 1.05 kg), and water and prepare the emulsion preferably in rapisomic emulsifier. Add the water to the emulsion to make up to a total volume of 350 litres, including 25 litres of enzyme equivalent to 150,000 International Unit of enzymes (this volume of 25 litres of enzyme may vary depending upon the enzyme activity.)

The enzyme mixed emulsion thus obtained is to be applied to the fibre in the jute softner using the normal processing of jute mills, the application percentage of emulsion being about 35% for the jute cuttings and 30% to the low grade jute.

## **7.2 PILING OF JUTE CUTTINGS AND LOW GRADE JUTE**

The treated low grade jute is generally piled for 48-72 hours, and the cuttings for 72 hours to 86 hours. The bin is considered to be matured (for processing) when the temperature of the bin attains a level of 65°C (149°F).

## **7.3 PROCESSING OF FIBRE (A PATENT APPLIED FOR)**

The enzyme treated low grade jute and cuttings are to be given a total of two and three carding passages respectively. The treated cuttings and low grade should be processed according to the availability of carding facilities; where there is a facility of tandem, cuttings should be processed in it since other wise, breaker card should be used. In the finisher card the low grade jute, cuttings, and other grades should be processed. The spinning performance and percentage composition of different batches at different jute mills are shown in tables (III, IV, V, VI and VII):

Table III Spinning Performance of Sacking Weft (M/s. Karim Jute Mills, a public sector jute mill, Dhaka, Bangladesh)

	Control	Enzyme <i>A. terreus</i>
1. Average grist (lb/spy) Linear density (tex)	31.70 (1092)	34.40 (1186)
2. Average tensile strength (lb) (kg)	20.76 (9.44)	19.96 (9.07)
3. C.V%	19.93	20.38
4. Quality ratio %	60.96	68.05
5. Production efficiency %	50.00	55.00
6. Ends down/100 spy/1 hr.	180	110
7. Twist/inch	2.20	2.20

Spinning done in slip draft with flyer speed 1700 R.P.M. where two results are shown for a parameter, the results in the second of these are given in parenthesis.

Batch composition

<u>Fibre</u>	<u>Control</u>	<u>Experimental</u>
Cuttings	52.88%	72%
Low grade jute	18.18%	28%
Miscellaneous	15.71%	-
Thread	13.22%	-

**A MANUAL FOR IMPROVED PROCESSING TECHNIQUE  
FOR LOW GRADE JUTE AND CUTTINGS**

**Table IV Spinning Performance of hessian weft/sacking warp  
(M/s. Karim Jute Mills, a public sector Jute mills,  
Dhaka, Bangladesh)**

	Control	Enzyme <u>A.</u> <u>terreus</u>	Enzyme <u>A.</u> <u>niger</u>
1. Average grist (lb/spy)	10.17	10.58	12.60
Linear density (tex)	(350)	(365)	(433)
2. Average tensile strength (lb) (Kg)	7.39 (3.36)	7.79 (3.54)	7.95 (3.61)
3. C.V%	18.27	16.30	18.56
4. Quality ratio %	70.66	74.00	69.61
5. Production efficiency %	60.00	69.00	64.00
6. Ends down/100 spy/1hr.	235	163	186
7. Twist/inch	4.00	4.00	4.00

Spinning done in slip draft with flyer speed 3600 R.P.M. where two results are shown for a parameter, the results in the second of these are given in parenthesis.

**Batch composition**

<u>Fibre</u>	<u>Control</u>	<u>Experimental</u> <u>(enzyme treatment)</u>
Bangladesh White D	18.18%	-
Tossa Cross Bottom	-	27.27%
White Cross Bottom	37.19%	27.27%
Low grade jute	37.19%	-
Cuttings	-	36.36%
Thread waste	7.44%	9.09%

Table V Spinning Performance of yarn (M/s Moqbular Rahman jute Mills, a private sector Jute Mills, Chittagong, Bangladesh)

---

	Control	Enzyme <u>A.</u> <u>terreus</u>	Enzyme <u>A.</u> <u>niger</u>
1. Average grist (Ib/spy) Linear density (tex)	10.25 (353)	10.25 (353)	10.00 (353)
2. Average tensile strength (Ib) (Kg)	7.30 (3.32)	7.43 (3.38)	6.81 (3.03)
3. C.V%	19.18	18.84	21.68
4. Quality ratio %	71.22	72.49	68.10
5. Production efficiency %	60.00	65.00	64.00
6. Ends down/100 spy/1hr.	253	200	235
7. Twist/inch	4.00	4.00	4.00

---

Spinning done in slip draft with flyer speed 3600 R.P.M. where two results are shown for a parameter, the results in the second of these are given in parenthesis.

Batch composition (for both control enzyme treatment)

Cuttings	36.36%
Low grade jute	18.18%
Bangladesh Tossa D	18.18%
Bangladesh White D	27.27%



**A MANUAL FOR IMPROVED PROCESSING TECHNIQUE  
FOR LOW GRADE JUTE AND CUTTINGS**

**Table VI Spinning Performance of sacking weft (M/s. Shree Raghupati Jute Mills Ltd., Biratnagar, Nepal)**

	Control	Enzyme in (liquid form)	Enzyme in (solid carrier)
1. Average grist (lb/spy)	30.63	31.74	30.47
Linear density (tex)	(1056)	(1094)	(1050)
2. Average tensile strength (lb) (Kg)	24.48 (11.13)	23.84 (10.83)	19.73 (8.97)
3. C.V%	16.42	21.40	27.87
4. Quality ratio %	79.92	75.11	64.75
5. Production efficiency %	50.00	52.00	50.00
6. Ends down/100 spy/1hr.	190	190	195
7. Twist/inch	2.20	1.90	1.85

Spinning done in slip draft with flyer speed 1800 R.P.M. where two results are shown for a parameter, the results in the second of these are given in parenthesis.

**Batch composition**

<u>Fibre</u>	<u>Control</u>	<u>Experimental (enzyme treatment)</u>
Tossa middle	45%	27%
Low grade	18%	36%
Jute waste	37%	37%

Table VII Spinning Performance of hessain warp (M/s. Shree Raghupati Jute Mills Ltd., Biratnagar, Nepal)

	Control	Enzyme in (liquid form)	Enzyme in (solid carrier)
1. Average grist (Ib/spy) Linear density (tex)	10.31 (335)	10 (345)	9.84 (339)
2. Average tensile strength (Ib) (Kg)	7.35 (3.34)	7.5 (3.4)	7.12 (3.23)
3. C.V%	24.83	22.80	24.15
4. Quality ratio %	71.29	75.0	72.35
5. Production efficiency %	50	51	50
6. Ends down/100 spy/1hr.	160	155	165
7. Twist/inch	4.5	4.4	4.5

Spinning done in slip draft with flyer speed 3200 R.P.M. where two results are shown for a parameter, the results in the second of these are given in parenthesis.

Batch composition

<u>Fibre</u>	<u>Control</u>	<u>Experimental (enzyme treatment)</u>
Tossa hard	82%	45%
White hard grade	18%	18%
Jute waste	-	37%

## REFERENCES

1. Albersheim, P. 1966. Pectin lyase from fungi. *Meth. Enzymol.* 8 : 628-631
2. Collmer, A, J.L. Ried, and M.S. Mount. 1988. Assay methods for pectin enzymes. *Meth. Enzymol.* 161 :329-335
3. Daigneault-Sylvestre, N, and D. Kluepfel. 1979. Method for the rapid screening of cellulolytic streptomycetes and their mutants. *Can. J. Micro biol. Biotechnol.* 28 : 463-467
4. Durrands, P.K. and R.M. Cooper. 1988. Development and analysis of pectic screening media for use in the detection of pectinase mutants. *Appl. Microbiol. Biotechnol.* 28 : 463-467
5. Flannigan, B, and J.E.M. Gilmour. 1980. A simple plate test for xylanolytic activity in wood-rotting basidiomycetes. *Mycologia* 72 :1219-1221.
6. Ghosh, B.L., and Dutta, A.K. 1980. The enzymatic softening and upgrading of lignocellulosic fibres. *J. Text. Inst.* 71 : 108-116
7. Ghosh, B.L., and Dutta, A.K. 1983. The enzymatic softening and upgrading of lignocellulosic fibres. part II. *J. Text. Inst.* 74 : 83-91
8. Ishii, S. and T. Yokotsuka. 1972. Purification and properties of pectin trans-eliminase from *Aspergillus sojae* Agr. Biol. chem 36 : 146-153.
9. Leathers, T.D., C.P. Kurtzman, and R.W. Dectroy. 1984. Over production and regulation of xylanase of xylanase in *Aureobasidium pullulans* and *Cryptococcus albidus*. *Biotechnol. Bioeng. Symp.* 14 : 225-240.

10. Khan, A.W., D. Wall, and L. van den Berg. 1981. Fermentative conversion of cellulose to acetic acid and cellulolytic enzyme production by a bacterial mixed culture obtained from sewage sludge. *Appl. Environ. Microbiol.* 41 : 1214-1218.
11. Kluepfel, D. 1988. Screening of peocaryotes for cellulose and hemicellulose degrading enzymes. *Meth. Enzymol.* 160 : 180-186.
12. Leathers, T.D., C.P. Kurtzman, and R.W. Detry. 1984. Over production and regulation of xylanase in *Aureobasidium pullulans* and *Cryptococcus albidus*. *Biotechnol. Bioeng. Symp.* 14 : 225-240.
13. Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. *Anal. Chem.* 31 : 426-428.
14. Mohiuddin, G. S. Akhter, and M.M. Mia. 1978. softening of jute cuttings using *Bacillus subtilis* - *Bang. J. Jute Fib Res.* 3 : 21-26.
15. Mohiuddin, G., Kashem, A., and Green, J. 1987. A simple modified method for detecting cellulose activity in microorganism 12 : 83-84.
16. Mohiuddin, G. Upgrading of low grade jute and cuttings, Part I. 1992. *J. Text. Inst.* (in press)
17. Mohiuddin, G. et al, Upgrading of low grade jute and cuttings, Part II. 1992. *J. Text. Inst.* (in press)
18. Mohiuddin, G. et al, Upgrading of low grade jute and cuttings, Part III. 1992. *J. Text. Inst.* (in press)
19. Ramamurthy, V., S.N. Sinha, and R.M. Kothari. 1987. Effective and economical enzymatic upgradation of jute. *Biotechnol. letters.* 9 : 593-596.

*A MANUAL FOR IMPROVED PROCESSING TECHNIQUE  
FOR LOW GRADE JUTE AND CUTTINGS*

---

20. Wick, R.L. and D. B. Schroeder. 1982. In vitro production of pectolytic and cellulolytic enzymes by *Fusarium tricinctum*. *Mycologia* 74 : 460-466.
21. Wood, T.M., and K.M. Bhat. 1988. Methods for measuring cellulase activities. *Meth. Enzymol.* 160 : 87-112.