CHARACTERIZATION OF FUNGI AND ITS ENZYMES FOR APPLICATION IN RETTING OF JUTE RIBBONS

A
DISSERTATION
SUBMITTED TO THE **DEPARTMENT OF MICROBIOLOGY**
UNIVERSITY OF DHAKA
IN FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY (SCIENCE)

SUBMITTED BY
FIROZA AKHTER

DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF DHAKA
DHAKA-1000, BANGLADESH

JUNE, 2011

DEDICATED

TO MY

LATE PARENTS, MY LATE GRAND MOTHER, MY UNCLE

AND

MY HUSBAND Md. Kabir Ahmed Akond

EXAMINER'S COPY

DECLARATION

I do hereby declare that the work presented in this thesis entitled **"Characterization of fungi and its enzymes for application in retting of jute ribbons"** is the original result of my own investigation. I further declare that this thesis has been composed by myself and no part of this thesis has been submitted anywhere in any form for any academic degree.

June, 2011

Firoza Akhter

CERTIFICATE

This is to certify that the research work presented in this thesis entitled **"Characterization of fungi and its enzymes for application in retting of jute ribbons"** is hereby submitted in fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Microbiology from Dhaka University, Dhaka, Bangladesh. This is a recorded of bonafide research work carried out by Firoza Akhter under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

(Prof. Dr. Md. Mozammel Hoq)
Supervisor
Dept. of Microbiology
University of Dhaka
Dhako-1000, Bangladesh

ACKNOWLEDGEMENTS

First of all I would like to thank the almighty Allah to help me continuing with the research work. Then I am happy to express heartfelt gratitude to my supervisor professor Dr. Md. Mozammal Hoq, Department of Microbiology. University of Dhaka, for his kind helps in selecting the topic of dissertation. His close and constant guidance with constructive criticisms helped and encouraged me in preparation of the study. I am indebted to him for his infinite patience with which he had carefully read and revised the written up.

I am grateful to Professor Dr. Shakila Nargis Khan, of the same Department for her care and suggestions during pursuing my research work and for her going through the manuscript and also making necessary corrections. I also take privilege to express my gratefulness to Dr. Md. Ilias, Associate professor of the Department of Microbiology, University of Dhaka, for his sincere help, advice throughout the study.

I am grateful to Dr. M. Anwar, chairman Department of Microbiology university of Dhaka and to Dr. M. Sirajul Islam Khan, Dr Chowdhury Rafiqul Islam, Dr. Md. Anisur Rahman Khan, Dr. Abdul Malek, Dr. Manzurul Karim and Dr. Mahfuzul Haque professor, Department of Microbiology. I would like to thank all of my respected teachers for their inspiration and valuable suggestions during the study.

I am grateful to Director General, Director (Agriculture) of Bangladesh Jute Research Institute for providing me assistance in the form of chemicals and laboratory facilities.

I am also gratefully acknowledging the cooperation of Bangladesh Agricultural Research Council for financing me to carry out the research work

during the study. I also thank Mr. Mozibur Rahman, Chief Scientific Officer and Md. Shamsul Haque Ex-Principle Scientific Officer and Md. Yunus Ali, Scientific Officer of Fiber quality improvement division, Bangladesh Jute Research Institute for their constant inspiration throughout the study. I would like to acknowledge the help and cooperation extended by the members of fiber quality improvement Division of BJRI (Agriculture), friends and well wishers of BJRI, Manik Mia Avenue, Dhaka.

Again I acknowledge gratefully the valuable help and co-operation of the students of 215 laboratory and laboratory staffs of Department of Microbiology, University of Dhaka.

Finally I acknowledge Dr. Md. Mohammad Jahangir Hossain, my brother in law and Afroza Begum, my elder sister and my niece Sofeea Mohammad for providing innumerable helps to complete the research work. Valuable advice, encouragement, sacrifices and good wishes of my children (Taha and Tasheen), younger brother, relatives, friends and well- wishers, can never be forgotten.

ABSTRACT

ABSTRACT

Jute is a herbaceous bast fiber crop of lignocellulosic origin. There are two types of jute cultivated in Bangladesh such as *Corchorus capsularis* (Deshi jute) and *Corchorus olitorius* (Tossa jute). Jute fiber produced from jute plants through a biological process called retting. The quality of fiber is largely depends on proper retting. Now a days there is a high shortage of retting water particularly in the northern areas of the country. Green jute ribbons, unlike jute plants which need less space as well as water for retting under both moist and submerged conditions may be an alternative. The present study was carried out to develop an innovative method for retting of green jute ribbons under moist condition using fungi and its enzymes.

Twenty one fungi were isolated from jute environments and were examined for their retting capacity of green jute ribbons on the basis of retting period, fiber softness and color. Based on these properties, 10 fungal isolates were found as good retter and were identified microscopically as *Aspergillus niger, Aspergillus flavus*, *Botryodiplodia sp.*, *Sporotrichum sp.*, *Sclerotium sp.* type 1, *Sclerotium sp.* type 2, *Fuserium sp.*, *Macrophomina sp.*, *Mucor sp.*, and *Trichoderma sp.* These fungal isolates were also examined for their retting enzymes (xylanase, pectinase and cellulase) activities in wheat bran medium used as inoculum in retting experiments of green jute ribbons. The highest and the lowest level of activity of xylanase, CMCase and pectinase by different fungi after 3 days of incubation at 30°C ranged from 398.11 (*Fuserium sp.*) to 33.92 (*Macrophomina sp.*), 10.74 (*A. flavus*) to 2.5 (*Fuserium sp.*) and 1.90 (*Mucor sp.*) to 0.72 U/g (*Botryodipdia sp.*) respectively. However, the activity of the individual enzymes was found to be increased with time accounting between 1382.4 (*A. flavus*) and 161.52 (*Trichoderma sp.*), 51.530 (*A. flavus*) and 4.510 (*Mucor sp.*) and 2.90 (*Sclerotium sp.* type 1) and 1.00 U/g (*Botryodiplodia sp.*) respectively. Among them, the best 4 fungi (*A. niger*, *A. flavus*, *Sporotrichum sp.* and *Sclerotium sp.* type 1) were finally selected on the basis of clear zone formation caused by xylan, pectin and CMC hydrolysis on the solid agar medium by the retting enzymes such as xylanase, pectinase and cellulase respectively.

The mycelial growth of the four selected fungal isolates in inoculum medium was a function of moisture contents (30 to 70%) of wheat bran medium. The activity of the enzymes (xylanase, CMCase and pectinase) was found to be increased with the increase in moisture level and reached its maximum at 50% by all these fungi, except the pectinase activity of *Sclerotium sp.* type 1 which showed its maximum activity at 40% moisture level.

Influence of natural fungal flora with jute ribbons (non-sterilized ribbons + inoculum) and pure fungal inoculums on (sterilized ribbons + inoculum) retting was evaluated at room temperature (28-30°C). It showed that non-sterilized ribbons retted earlier (8-10 days) than that of sterilized ribbons (10-12 days) by all fungi mixed or single culture. However, period of ribbons retting by single or mixed culture of fungi demonstrated no remarkable differences. On the other hand, about 22 days required for retting of the ribbons where no fungal inoculum were used. The age of inoculum (with Wheat bran medium) also affected the ribbons retting. Four days inoculum retted earlier than that of 7 days by different fungal treatments. Fungal treatment with 4 days inoculum was assessed to be beneficial so far quality of fiber is concerned.

The yield of fiber accounted from 10.6 g (*A. niger* + *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1) to 6.0 g (*Sclerotium sp.* type 1) and 10.7 to 5.2 g by fungal treatment in non-sterilized and sterilized conditions respectively. Single fungus produced lower yield of fiber (between 6.0 and 7.3 g) than that of multiple (10.78 to 10.6 g) fungal culture. Under non-sterilized condition, the yield was gradually lower in as compare to sterilized condition due to fungal treatment of jute ribbon. Fungal treatment produced fiber yield with cream to bright cream color irrespective of conditions. The lower grade fiber (control) showed brown and gray color. However, the variation in bundle strength measured among the treatments was not very much countable.

The bundle strength of retted jute fiber ranged from 8.11 to 10.23 under solid state fermentation where as relatively less strength (7.77 to 10.00 lbs/mg) demonstrated under submerged condition by fungal inoculum of 4 or 7 days age respectively. Fiber

color recorded were in the range of cream, off white and gray; and bright cream, cream and off white under solid state fermentation and bright cream, cream and off white under submerged condition. Fiber grade remained mostly B to C under solid state fermentation while under submerged condition the frequency of A grade remained same but that of B grade reduced with the concomitant increase in the frequency of C grade against D grade in the control.

Variation in components of jute fiber namely cellulose, hemicellulose, lignin and pectin upon fungal treatments under both solid state and submerged rettings were not much evident. The cellulose content increased from 58.54% to 65.87% where as hemicellulose, lignin and pectin content decreased to 21.07, 12.19 and 0.82 from 23.86, 14.69 and 1.80% respectively by the application of *A. niger*, combination of 4 fungi (*A. niger, A. flavus, Sporotrichum sp. and Sclerotium sp.* type 1*)*, combination of 3 fungi (*A. flavus, Sporotrichum sp. and Sclerotium sp.* type 1*)* and *A. niger* respectively.

As regard to characterization of the retting enzymes by four fungal isolates (namely *A. niger*, *A. flavus*, *Sporotrichum sp.* and *Sclerotium sp.* type 1), time course study (3 to 9 days) for the activity of enzymes- xylanase, CMCase and pectinase showed a steep increases with time and reached to its maximum after 6 days and then a sharp to gradual fall after 9 days of inoculation. The activity of the enzymes revealed an appreciable variation among the isolates with respect to xylanase and CMCase activity. In contrast, variation among the isolates in case of pectinase activity was very close except *A. niger* where comparatively lower activity of the enzyme was observed. The pH of xylanase, CMCase and pectinase of the fungal strains were found to be almost stable at the pH range of 3.0 to 8.0 with a variation in activity of the enzymes among the strains towards stability. The temperature optima as tested within a range of 40 to 80°C showed that the optimum temperature for xylanase activity from *Sporotrichum sp.* and *Sclerotrium sp.* type 1 and that of pectinase of all the isolates was 40°C. However, the xylanase and CMCase activity of the fungal isolates of *A. niger* and *A. flavus* and were found to optimum at 50°C.

Large scale application of fungal crude enzyme extract from *A. niger* and *A. flavus* completed retting of green jute ribbon within 11 days with bright cream color of A grade fiber. However, *Sporotrichum sp.* and *Sclerotium sp.* type 1 required 12 days for complete retting and produced B grade fiber with cream color. Contrary to this, combination of these fungi enhanced the process of retting to 9 days only with A grade fiber of bright cream color. However, the time required for retting jute ribbon (control) was 18 days with poor quality of fiber of C grade. The weight and bundle strength of fiber varied from 1570 to 1656 lbs/mg and 9.00 to 10.49 lbs/mg respectively revealing the fact that the four fungal combinations was successful to achieve the best quality of fiber among the treatment combinations used in the experiment. The present results thus may be a useful basis for green jute ribbons retting in large scale as an alternative to conventional jute plants retting in Bangladesh.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSA	: Bovine serum albumin
ca.	: Approximately
CMC	: Carboxymethylcellulose
CMCase	: Carboxymethylcellulase
°C	: Degree Celsius
pH	: Negative logarithm of hydrogen ion concentration
DNS	: Dinitrosalicylic acid
g	: Gram
cm	: Centimeter
U/g	: Unit per gram
lbs/mg	: Pounds per milligram
U/ml	: Unit per milliliter
g/l	: Gram per liter
w/v	: Weight per volume
v/v	: Volume per Volume
mg	: Milligram
min	: Minute
ml	: Milliliter
%	: Percent
L	: Liter
PDA	: Potato dextrose agar
Psi	: Pound per square inch

RS	: Reducing sugar
SP	: Soluble protein
SSF	: Solid-state fermentation
SMR	: Summery Mill Rejection
sp.	: Species
Scl type 1	: *Sclerotium sp.* type 1
rpm	: Rotation per minute
etc.	: Etcetera
i.e.	: That is
viz.	: Namely
nm	: Nanometer

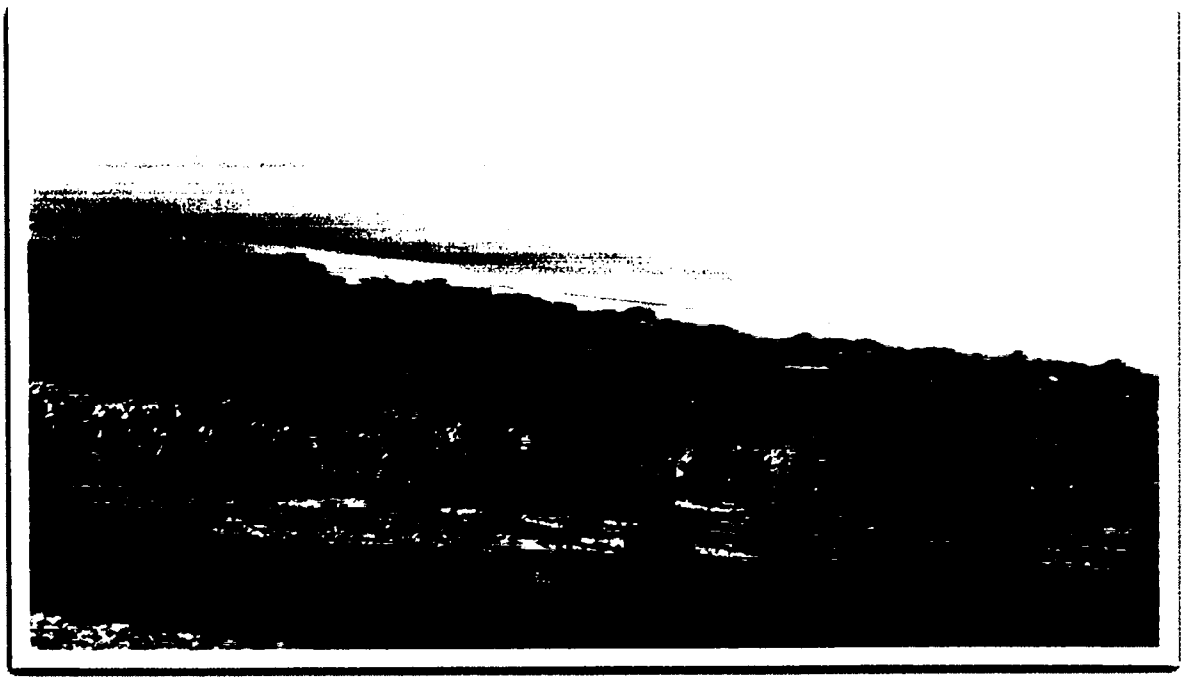
Chapter 1
INTRODUCTION

INTRODUCTION

1 Introduction

1.1 Jute plant, Jute fiber and its economic importance

Jute is a lignocellulosic bast fiber crop obtained from two cultivated species of the genus *Chorchorus* viz *Chorchorus capsularis* and *C. olitorius* of the family Tilliaceae. In Bangladesh it is commonly called pat or khosta. *C. capsularis* is called Deshi/Tita pat it is 5 to 12 feet tall and cream color fiber where as *C. olitorius* is called Bogi/Tossa pat 5 to 15 feet tall fiber golden yellow finer fiber more lustrous than deshi pat. Kenaf and Mesta/Roselle are also grown in Bangladesh in small scale. Of the total global production of about 3 million tons of jute Kenaf harvested from about 2.5 million hectares jute producing member countries of the International Jute Organization



Jute plant standing in a jute field

(IJO now it is IJSG) namely Bangladesh, China, India, Indonesia, Nepal and Thailand account for about 95% of the production. These countries except Indonesia are net exporter of raw fiber and finished products. Jute is the cheapest and the most important of all the textile fibers next to cotton and grown for commercial purpose viz. packaging materials for various agricultural and industrial and house hold products as carpet, blanket etc. Still jute plays a vital role in the national economy of Bangladesh and about 40-60 lakh bales (0.9-1.2, million tons FAO, 1994) of raw jute is being produced

annually and it is 30% of the total world production. Defoliated jute plant contain about 75% water, 12% jute stick, 6% fibers and 6.7% other non-fibrous matters composed of hemicellulose, proteins, fats, pectins and minerals. The nitrogen, phosphorus and potassium contents of leaves and top, middle and basal portion of the plants vary. The leaves contain about 2.85-3.10% nitrogen, 0.85%-1.20% phosphorus and 2.60 – 3.10% potassium. The bark contains about 0.35%-0.46% nitrogen, 0.35%-0.46% phosphorus and 0.50%-0.85% potassium. Jute fibers are composed of about 0.7% water, 60% cellulose, 24% hemicellulose, 13.5% lignin, 0.5% fat and 0.8% minerals. The jute sticks are composed of 47 to 48% cellulose, 23-24% hemicelluloses, 20-22% lignin (Sen Gupta, 1958; Bashiruzzaman, Mian and Haque, 1964; Dorgan, 1971).

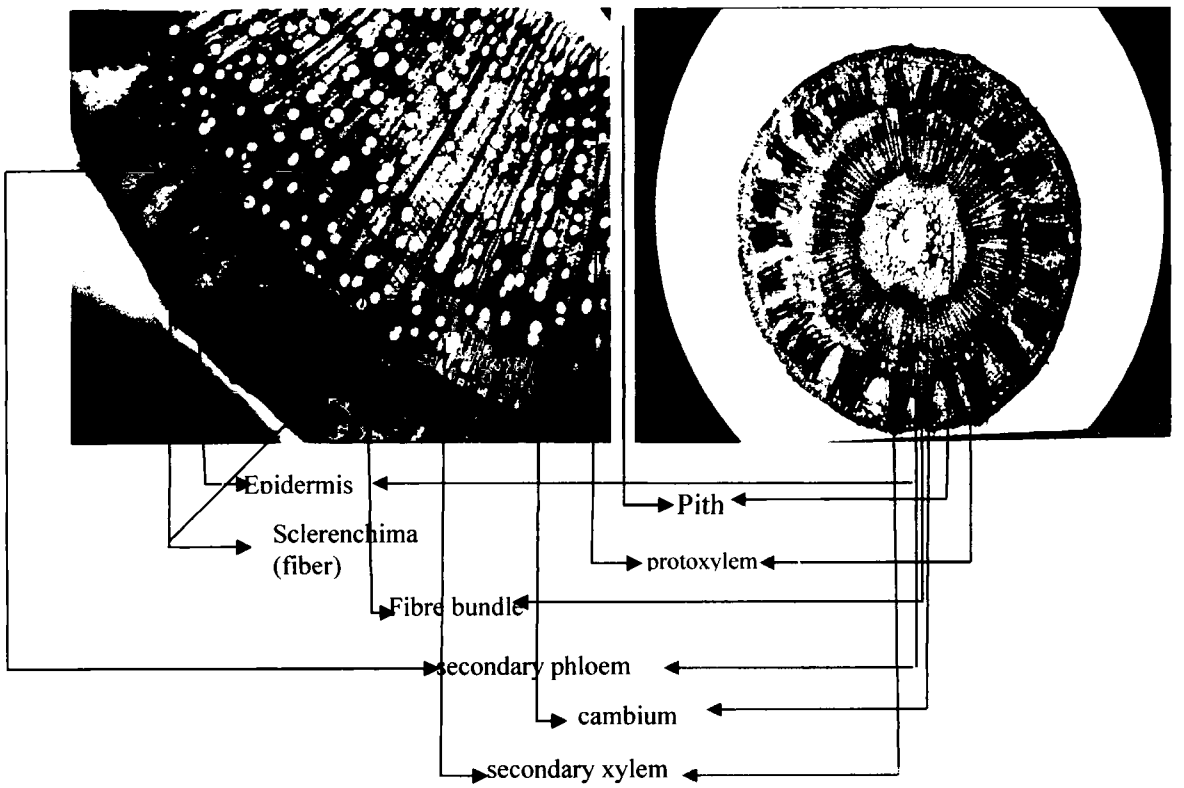
Ahmed *et al.,* 2003 studied chemical composition of different pipe line verities of jute (*Chorchorus capsularis* and *C. olitorius*). Here moisture content was found highest (12.6855%) in bottom part of C-2035 variety and lowest (8.24%) in top part of C-2005 variety. In bottom part of C-718, cellulose content was found in the top part of C-2035 variety hemicellulose was found in the top part of OM-1 and lowest (16.39%) in middle part of C-718. In case of lignin content, it was observed that 17.98% was found in bottom part of C-718 which seems to be highest and lowest (13.61%) was found in top part of C-2193 variety. In top part and bottom part of C-2005, ash content was found lowest (0.112%) and highest (0.995%) respectively. Fat content was highest (2.172%) in OM-1 variety at lowest (1.099%) in C-2193.

From the time immemorial a huge quantity of foreign currency was earned by exporting the commercial jute fiber cultivated in most of the jute growing areas of Bangladesh. About 40-60 lacks bales (0.92-1.2 million tons, FAO 1994) of raw jute are being produced annually in Bangladesh. Due to friendly environment the demand of various jute products has been increased to a great extent. Usually jute plant bears a good number of green leaves throughout the growing stages which are enriched with nitrogen, phosphorus and potassium, which is very much essential for fertility and tilth of soil. It also increased the organic matter and microbial population around the rizosphere of jute plant (Akhter *et al.,* 1999). Besides green leaves supplied free oxygen throughout the growth period, the green leaves of both *C. capsularis* and *C. olitorious*

are used as green vegetable. In Philippine, jute leaves are used as soup and salad. The calyx and epicalyx of jolly kenaf *(Hibiscus cannabinus)* are used for the preparation of jam and jellys. Jute has diversified uses as jute fiber used for making carpet, blanket, novotex blanket, fire proof Geo textile, nursery sheet, gunny bags etc. Geo textiles have variety of functions such as reinforcement, filtration, drainage, and separation. Jute is a biodegradable natural fiber. Meshy network of jute fiber along with certain structural morphological factor such as orientation of individual filament of the fiber with number of elongated ultimate cell cemented together with lignin, hemicellulose and other natural adhesive enhanced its reinforcement property. This composite nature of jute imparts certain viscoelastic property on different jute products having definite advantages to be used as Geotextile. Due to presence of lignin biodegraded products of jute is full of biomass having higher nutrition value as regard to fertility of soil (Khuda, 1980).

1.2 Formation and structure of fibers in the jute plant

The jute fiber is developed in the bark of the jute plant stem. Jute is a bast fiber of jute plant is essentially a type of Sclerenchyma cells. In the jute plant, fibers make up the greater part of the secondary phloem and occur in a variety of form and arrangement (De Bary 1984, Eames *et al.,* 1951, Esau 1953) definite bands inter spreaded by ray cells. The secondary phloem in jute plants lies in wedge shaped groups each having alternating patches of Sclerenchyma and soft tissues. The patches of fibers or the fiber bundles are arranged within these pyramidal wedges (fiber cones or fiber pyramides) in concentric arcs alternating with soft phloem parenchyma tissues, the number of which varies from 8-20 arcs depending on species, but the number is usually between 9 and 11. The number and size of the fiber bundles in the phloem wedge are variable from species to species. The oldest phloem is always towards the periphery with the youngest next to the cambium. The fiber content of the jute plant depends on the number of fiber bundles present within the pyramidal wedge (Fiber cone or fiber-pyramide in the bark region) (Kundu, 1943).



Outline of a cross section of an internode of *Corchorus capsularis*

The fiber layers which are generally four to six cells thick with constitute only 6-7 percent of whole plant. These fiber layers are made up of a varying number of fiber bundles separated by thin walled ray cells. All the fibers of an entire plant form a fiber strand or reed which is more compact and firmer towards the base and finer towards the top. The number and size of fiber bundles depend on the activity of the cambium and also on the growth of the plant. The fiber bundles vary greatly in size and shape. The oldest fiber bundles are always towards the periphery and the youngest one next to the cambium. The number of fiber cells in a bundle is variable and each fiber cell is known as ultimate fiber. These small units of ultimate fibers overlap length wise and are cemented together laterally. The ultimate cells are elongated in the direction of the stem axis with pointing ends and appear more or less polygonal in outline. Bhattacharja (1961) reported that the cementing materials are mainly hemicellulose, lignin and pectin etc. The walls of the fiber cells are thick and lignified and except for occasional transverse cracks, are relatively smooth. The lumen or cell cavity is as wide as cell walls, but it has characteristic construction at irregular intervals. Sometimes the fiber cell cavity is completely closed due to the uneven thickening of the cell wall.

1.3 Jute retting

The process of separation and extraction of fibers from non fibrous tissues and woody part of the stem through dissolution and decomposition of pectins, gums and other mucilaginous substances is called retting (Gupta *et al.*, 1976; Majumder and Day, 1977). The quality of the fibers is largely determined by the efficiency of the retting process. In other words, retting is the process by which the pectic materials which bound the fibers of remainder of the stem is broken down and the fibers are liberated (Bhattacharayya, 1973, 1974). Variation in fiber quality is dependant (Chi *et al.*, 1966) on retting in different natural conditions and duration of retting. Generally, the bottom portion of the jute plant is thick and hard which takes a longer time for retting than the upper portion. Owing to over maturity, variety and improper retting, the bottom portions of these unretted bark materials are cut down in the jute mills and are known as cuttings. In addition to these, there are some jute fibers which are naturally spicky, coarse, harsh and less pliable. These undesirable characteristics of fiber result mainly from improper retting of bark or ribbon. These fibers are known as low-grade jute and have less utility than normal jute fibers. These fibers both low-grade and cuttings, which amount to 30-40% of the total fibers, are not suitable for spinning. Hence a proper jute retting condition in order to produce better fiber quality is very important.

1.4 Jute retting mechanism

During retting, plant absorbs water when steeped in it and swell ultimately bursting occurs at several places and the soluble constituents which include sugars, glycosides and nitrogenous compounds are liberated (Ali *et.al*, 1976, Ali 1958; Ali and Islam, 1963) creating the surrounding environment a good starter medium for the growth of micro organisms present in water as well as in the plants. These organisms gradually develop and multiply by utilizing free sugars, pectins, hemicelluloses, proteins etc. of the plants as nutrients (Majumdar and Day, 1977, Mohiuddin *et al.*, 1978; Sarkar, 1963-1964). Specific enzymes secreted by the organisms first cause degradation of the complex organic materials to simpler compounds which are then metabolized for their life processes. A series of biochemical reactions thus go on as (Chaudhury, 1951) a result of which the chemical composition and pH of the retting water changes continuously during the retting process. It is evident that the ash content is very high in

jute ribbons which are presumed to be due to the presence of polyuronides or pectins as their metallic salts. In the ash of ribbon, calcium, magnesium and iron were detected (Majumdar and Day, 1977); α-cellulose is highest in the top position and lowest at the bottom which again contains the highest cutin, water soluble and pectin matters. Decomposition (Ali *et.al.*, 1976) of the free sugars takes place at early stages of the process, followed by the breakdown of pectins. Decomposable hemicelluloses and nitrogenous compounds (chiefly proteins) are degraded at the later stage. Periodic analyses of retting water revealed the presence of several (Ali *et al.*, 1968) decomposition products such as organic acids (acetic, lactic, butyric, alpha - ketoglutarics), acetone, ethyl alcohol, butyl alcohol and various gases (Debsharma, 1976). If however, retting is allowed to continue beyond the optimum period, the enzymes by which the microbes actually carryout the decomposition have also been studied mostly of polygalacturonase and pectin methylesterase in nature (Ali, 1965; Choudhury, 1951; and Choudhury, 1962).

1.4.1 Microbial retting

It appeared that most of the retting water contain aerobic, anaerobic and microaerophilic bacteria, aquatic and other fungi, algae, protozoa, and diatoms. Of all the micro organisms, aerobic, anaerobic or microaerophilic bacteria have been found to be mostly involved in jute retting. The most predominant ones of such bacteria have been found to be *Bacillus polymixa, B. subtilis, B. macerans, B. pumilus, B. sphaerieus, Clostridium butyricum, C. tertium, C. lacunarum, Pseudomonas putida, P. aeruginosa, P. psedomollei, Micrococcus caseolyticum, M. chorchorus, M. lutens* and *M. varians*. These bacteria completed retting in sterile water under laboratory condition in 2-10 days (Table 1 & 2) where as normal retting time under growers condition is 15-20 days.

Table 1: Aerobic bacteria involved in jute retting (Pectinolytic).

Name of the bacteria	Retting time (days)
Bacillus alvei	5
B. brevis	10
B. cereus	7
B. circulans	7
B. ingramii	5
B. leutans	10
B. macerans	8
B. megaterium	7
B. manevalii	6
B. polymyxa	5
B. pumilus	4
B. spherieus	9
B. subtilis	6
Micrococcus caseolyticum	5
M. corchorus	7
M. epidermidis	5
M. lutens	10
M. ureae	2
M. varians	8
M caudidus	3
Pseudomonas aeroginosa	5
P. paranacea	5
P. pseudomollei	4
P. putida	2
Gafkya tetragena	3

Source: Ali, 1977 and Ali *et al.*, 1983.

Table 2: Anaerobic or Microaerophillic bacteria involved in jute retting (Pectinolytic).

Name of the bacteria	Retting time (days)
Closteridium allenii	5
C. aurantibutyricum	4
C. breedii	3
C. butyricum	4
C. corallinum	5
C. eneboi	5
C. felsineum	6
C. haumann	5
C. histolyticum	5
C. laniganii	4
C. lacunarum	3
C. pectirovorum	3
C. rosenum	7
C. tertium	4

Source: Alam, 1970; Ali 1983.

Among the fungi mostly non aquatic ones are *Aspergillus niger, Mucor sp., Sclerotium rolfsii, Mycelia sterilia, Macrophomina phaseoli, Mucor abundant, Chaeotomium sp., Phoma sp.* (Ahmed 1963) and several *Penicillium sp.* have been found to be good retting agent and found to ret jute under moist condition and special environment of temperature and nutrients in about 3-10 days (Ali 1958, Hussain 1963, Jalaluddin, 1965; Alam 1970; Ahmed 1963).

1.4.2 Enzymatic Maceration of Jute plant

It has been revealed by Mitscherlich (1850) for the first time that in the retting process pectic substances were decomposed by the pectinolytic enzymes secreted by the microorganisms. Kundu (1964) reported that factors like pH, temperature, volume of water, maturity of plant at harvest, fertilizers received by plants and natural activators were found to influence the retting period of jute. Kundu (1960) also reported the

presence of pectinolytic and hemicellulolytic organisms were necessary for retting to take place. Rahman (1963) reported the degradative product of pectin in the retting liquors of flax. Kerfesz and Mecolloch (1950) indicated that the retting bacteria were effective as they possessed pectinolytic enzymes which included protopectinase, pectinesterase (PE) polygalacturonase (PG) and pectin depolymerase. Protopectinase liberated the insoluble pectin from plant materials and PE demethylated pectin molecules which were then attacked by PG to form a soluble product, galacturonic acid. Ali and Islam (1965) reported that enzymes PG and PME of *Penicillium frequentans* macerated potato discs, jute bark and jute stems. (Baruah and Baruah 1944) indicated that a mixture of lamellase, pectinase and protopectinase retted jute in much shorter time without affecting the fibers. Joslyn (1962) and Wood (1960) predicted the role of hemicellulase in the maceration of plant tissue. Hancock (1964, 1965) observed the combined action of hemicellulolytic and pectinolytic enzymes on the breakdown of the cell walls. Ishii and Kiho (1976) isolated pectolytic enzymes from culture filtrates of *Aspergillus japanicus*. Bateman (1963) indicated the PGase was primarily responsible for tissue maceration, whereas cellulose was assumed to play a supporting role in the process. Hancock (1965) reported the association of high PGase activity with sunflower or tomato stems infected with *Sclerotia sclerotiorum*. Fischer (1965) reported the presence of pectinolytic enzymes, PGase, PME and CMCase in the cultural filtrates of sweet potato surface rot and wilt fusaria growth in identical cultural conditions. Bateman (1968) reported high enzymatic activities in recoverable extracts of infected plant tissues by *Sclerotium rolfsii* and the fungus appeared to be a source of enzymes for studying invitro enzymatic degradation of plant cell wall materials. Meclendon *et al.*, (1960) reported that certain plant pathogenic fungi produced a variety of hydrolytic enzymes potentially capable of destroying various components of plant cell walls. Bateman (1964) also reported that cell maceration by these enzymes were inhibited in the presence of Ba^{++} or Ca^{++} and less by Mg^{++} and monovalent ions had no influence. Jensen *et al.*, (1960) reported that increased ionic concentration influenced the release of PME in the infected host. Edgintom *et al.*, (1961) indicated that calcium deficiency increased the susceptibility to hydrolysis by pectinol 100-D, a commercial pectinase preparation.

1.4.3 Chemical retting

In chemical retting the cementing material can be removed by dissolution with certain chemicals. The fiber obtained by chemical method of retting seems to be a little coarser, rough in the feel and stiff. The gravimetric fineness values do not differ very much from that of microbially retted fiber. The fiber strands after drying needs to be softened by rubbing with hand to open up the fiber and remove the stiffness of the strand. A cationic softener may be used to the extent of 0.2% on the weight of the fiber. Ammonium oxalate and sodium sulphate was found to be suitable chemicals under standard conditions for retting of jute. As in chemical retting the fibers are extracted under controlled condition and the fiber properties are not affected by the treatment, the procedure may be adopted as a standard method of fiber extraction from jute (also mesta) ribbons (Gupta *et al.,* 1976). The process is, however, costly and is not practicable in the cultivators' field. The method has no adverse effect on the fiber properties.

1.5 Factors affecting jute retting

The efficiency of retting depends on a number of factors (Bhattacharyya, 1974, Ghose and Bose, 1973, Asaduzzaman *et al.,* 1985). Some of these factors are within the easy control of the cultivators, whereas others are dependent on nature. Some of the factors are as follows –

1.5.1 Plant age

As the jute plant grows older, the tissues become more and more matured; the structure of the decomposable matters like pectins and hemicelluloses may be modified to more resistant forms and their quantity may increase. As a result microorganisms take longer time to ret a plant. Usually, fiber loss is 17.3% if 75 days old plant is ribboned and 9.5% if a plant of 120 days is ribboned.

1.5.2 Fertilization of crop

Higher dose of nitrogenous fertilizer applied to the crop has been found to reduce the retting period. The reverse happens when phosphatic fertilizers are used.

1.5.3 Retting water

Retting is a biochemical process in which, various decomposition is carried out in stagnant water and accumulation of these products causes hindrance to the growth and activity of the causative microorganisms. Very fast moving water, on the other hand, removes these toxic substances quickly, but it also carries away the microbial population along with it resulting in uniform retting. As such, retting is best (Kundu *et al.,* 1952) carried out in slow moving clear water (Cannal, river and ditch etc.) with low content of such materials as salt, iron and calcium content is preferable for good retting. It is desirable to change water to keep the pH about 7 and temperature near about 35°c during retting.

Ali and Islam *et al., 1965* found that, the breakdown of the pectic substances cymenting the fiber cells is due to the action of bacterial enzymes called pectinase. Biswas (1968) observed that delay in the retting may be caused by slower insufficient production of bacterial enzymes mediated by a number of environmental factors. Ali and Alam, 1973 found that pH of the retting medium place a vital role for retting.

When retting water is soft, the quality of fiber is better than when hard water is used. The presence of iron, particularly ferrous iron, is not desirable as it imparts a dark color to the fiber. Good results are obtained when nearly 15cm water is left over the top layer of the charge of plant bundles (care should be taken to see that the change does not touch the bottom of the retting tank), bundles of jute stems to be retted are approximately 20cm in diameter and plant water ratio is nearly 1:15-20 normally (Haque *et al.,* 2001).

1.5.4 pH and temperature

Laboratory experiments have shown that two pH values are optimum for retting, one being in the acidic region (around pH 5.0) and the other in the neutral or slightly alkaline region and that the retting period is relatively shorter in acidic pH. Natural water from different sources has a pH between 6.0 and 8.0 and is conveniently used for retting purposes (Ali and Alam, 1973). The optimum temperature for jute retting was

found to be around 34°c. Retting takes a longer time when the temperature deviates from 34°c, either on higher or lower side.

Number of both aerobic and anaerobic bacteria, and fungi in the retting effluent decreases with increase in retting period. Incorporation of urea improved the color of the fiber too.

1.5.5 Activators

Retting is accelerated in the presence of several activators. Natural activators like Dhaincha *(Sesbania aculeata)* and sun hemp *(Crotolaria junccea)* plants are generally introduced into the jute stem bundles put for retting. These leguminous plants being rich in nitrogen content help the growth and activity of the retting microbes by supplying additional nutrients to them. The accelerating effects of some synthetic inorganic chemicals and trace elements on retting have also been studied. Indian central jute committee used tribasic calcium phosphate, dibasic potassium phosphate, ammonium sulphate, ammonium oxalate, dibasic ammonium phosphate, sodium oxalate, calcium nitrate, potassium nitrate, bone dust and gelatin with retting water as activators of jute retting. Only bone dust and ammonium sulphate, ammonium oxalate, dibasic ammonium phosphate, calcium carbonate, monobasic potassium phosphate, sodium oxalate, calcium nitrate, potassium nitrate, bone dust and gelatin with retting water as activators of jute retting. Only bone dust and ammonium sulphate when added singly or in combination reduced the retting period considerably. Stems of sunhemp or Dhanicha when put in the bundles of retting jute also shortened the retting period (Ali *et.al.*, 1972).

1.5.6 Neutralization of retting liquor

The retting speed can be accelerated and quality of fiber can be improved significantly if the retting liquors are neutralizing by chemicals like $NaHCO_3$. It was also found that during the process of flax retting, butyric acid, lactic acid and acetic acids were produced in the retting liquor.

It was observed that in the case of *C. capsularis* although the addition of sodium carbonate and sodium bicarbonate turned the retting liquor dart at the end, this color did not at all have any effect on the color of the fibers. This eliminates the usually common

contention that whenever the retting liquor is dark the fibers are likely to be dark (Ali *et al*, 1972). The dark liquor rendering dark color to fibers must be having specific characteristics which are not yet clearly established. It might be suspected that the tannin-iron-reaction may be responsible for the dark color and is possibly operative only at a particular hydrogen-ion concentration of the retting liquor having different chemical properties. These properties do not exclude the possibility of the influence of phenolic compounds of the jute plants. It was also reported that in case of *C. olitorius* only the phenolic compound is responsible for the dark color. Contradictorily enough, it has been found that the retting liquor of *C. capsularis* is far darker than that of *C. olitorius.*

1.5.7 Urea

Retting water incorporated with urea ahead higher count of bacteria (both aerobic and anaerobic) maintained higher pH favorable to the bacterial growth and provide increase supply of total nitrogen which increase retting (Ali *et al.,* 1972).

1.5.8 Acidity and total nitrogen

During retting, the retting water became acidic which impaired the proper multiplication and activity of retting bacteria of the retting water, since it is known that the optimum growth of most bacteria is towards the neutrality. If the growth of retting bacteria is not favorable the retting progress is also not likely to be optimum and thus, in turn, will affect the quality of the fibers (Ali *et al.*, 1972). Further findings indicates that in jute retting the neutralization of the acidity of the retting liquor could be useful approach for conducting retting is more favorable conditions (Ali *et al.,* 1972).

1.5.9 Harvesting time

Considering the quality of the fiber it seems advantages to harvest jute about 25 days earlier than the usual recommended stage of average flowering. The slightly lower yield obtained because of the early harvest is likely to be compensated by the improved quality of the fiber (French, 1959).

Harvesting period of Jute plant (CVL-1)

1.5.10 Fibrous matter

It seems probable that the speed of retting depends on the non-fibrous matter and soluble carbohydrates contents of the stem which provide food to the retting microbes and eventually get decomposed into organic acids. Jute plants contain much more non-fibrous matter that can ret effectively.

1.6 After effect of imperfect retting

Imperfect retting leaves one or more defects in fibers which cause processing difficulties for the industry. Some of these defects are carried even to the finished goods and devalue them to the point of even rejection. The more common defects are described below (Bhattacharyya, 1973-1974).

1.6.1 Rooty fiber, centre root, runner and hunka

In all these defects, fiber is masked by barks. In rooty fiber, barks remain at the bottom, in centre root at the central region, in runners along the entire half of the bottom and in hunka all over the stem. These defects may be due either to unusual developments in some portions of the plant or to improper retting (Haarer, 1958).

1.6.2 Creepy and gummy fiber

Fibers with top ends rough and hard are called creepy. While fibers which are held together by undissolved and undecomposed gummy substances, they are called gummy. A little care during retting may remove both the defects.

1.6.3 Sticky fiber

If not properly cleaned after extraction, fibers may contain adhering sticks; these may create trouble in the processing machinery.

1.6.4 Shaymla fiber

Presence of excessive iron in retting water or use of weighting materials rich in tannin, such as, banana stems, freshly cut mango trees, etc, may impart dark color to the fiber. This dark colored fiber is known as shaymla is jute. This improper retted fiber can be upgraded also by using some plant extract (Akhter *et al.*, 2001).

1.7 Upgradation of low grade jute

The Jute Technological Research Laboratories, Calcutta, has developed a fungal culture method for softening barky jute (Basak *et al.*, 1987), which involves treatment of barky jute with a pectinolytic fungus, *Penicillium corylophilum* Dierckx. By using this fungal culture, inferior quality barky jute may be upgraded by 1-3 grades (Bhattacharyya and Basu, 1981). It is believed that the pectin in the middle lamella of the softer parenchyma, which holds the fiber bundles together, decomposes because of fungal attack (Chawdhuri and Ahmed, 1968). This loosens the fiber strands which are then easily separated. It was observed that the α-cellulose and lignin content remain unaffected while the pectin content in the treated samples with *P. corylophilum* Dierckx decreases considerably by about 54.5% (Ahmed and Chaudhury, 1968). As the basal

medium of jute does not contain any carbon source the fungus utilizes the carbon from the barky jute for its growth and multiplication. (Basak *et al.,* 1993) suggested that the fungus utilizes the pectinous material of the barky jute as its carbon source. This help in softening the hard barks by removing a significant amount of pectin which acts as a binding material for the fiber strands. The fungus grows profusely on the surface of the barky jute. On the other side of the surface, which remains towards the stick, the fungal growth is less than that on the upper surface. Ghosh and Dutta, 1980 also stated in previous report that when barky jute is inoculated with the fungus *P. corylophilum* Dierckx, it utilizes and grows mainly on the pectin present on the surface of the barky jute and between the fiber strands. This causes softening of the barky jute and between the fiber strands on mechanical processing. The cellulose and lignin contents of the barky jute remain unaltered after the fungal treatment. The fungal cultures are the most economically viable microbes for upgradation of jute because –

(a) They can ret jute in shorter time then bacteria,

(b) They are easily available and can grow in cheapest medium which is needed for isolation,

(c) They can be preserved in pure form for longer time and

(d) They require less water than bacteria for ribbon retting.

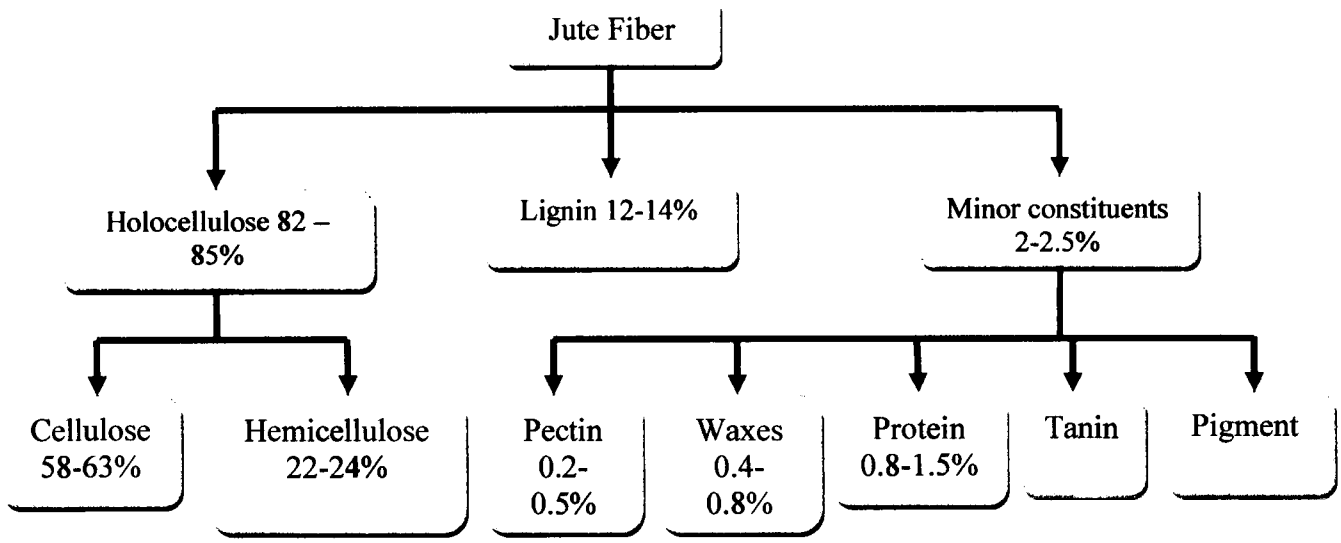
A large proportion of the total production of raw jute in Bangladesh is of very poor quality having 20-40% of cutting (hard basal parts) in the bottom portions of the fiber. Huge quantities of low grade barky fibers are also produced every year from seed plants. A good number of factors are responsible for the production of SMR and low-grade barky fibers. Over maturity of jute plants, hardness of the bottom portions of jute plants due to water logging, scarcity of good retting water facilities, faulty retting practices, fall in day temperature during retting, excessive number of rettings in the same water pool during the short retting season and quality and quantity of microorganisms present are the important factors responsible for the production of SMR (Haque *et al.,* 1989) and low grade barky fibers. The low quality barky jute fibers, so produced, are usually not suitable for spinning and need softening treatments before processing in the jute mills.

Paul and Bhattacharyya (1974) indicated that a pectinolytic fungus, *Penicillium corylophylum* Deerekx could upgrade the inferior quality fiber. It was found that the fungus *Penicillium frequentans* could macerate potato discs, jute barks and jute stems. It was also reported that the fungus *Cortcium rolfsii* could soften hard jute cuttings in much shorter time and improve the fiber quality. Ahmed and Chowdhury (1968) stated that a fungus used in the jute cuttings softened the cuttings and improved the fiber color. Basak *et al.*, (1987) indicated that the fungus *Penicillium corylophylum* could soften the barky jute without affecting the cell walls of the fiber. Haque *et al.*, (1989) reported that SMR and seed-cut barky fibers could effectively up-graded when treated with cultures of *Aspergillus niger* or *Sclerotium sp.* grown on wheat bran media. It was also found that the percentage of pectin and hemicellulose content of the fiber after treatment separately with the microorganisms such as *Sclerotium sp.* and *Aspergillus niger* reduced appreciably. In the softening of barky jute the pectinatious matters are decomposed due to fungal action. Possibly the pectinatious matters of the barky jute acted as carbon sources and enhanced the growth and multiplication of fungus on the hard basal portions resulting in the softening of the hard barks by removing a significant amount of pectin and as the fiber quality will be upgraded.

Recently, Bhattacharyya and Basu (1981) reported that kaolin powder, which is a complex aluminum silicate and nearly chemically inert and nonabrasive, may be used for prevention of the *Aspergillus sp.* for at least 90 days without any contamination with retention of its activity in softening and upgrading barky jute. The method of softening and upgrading low quality barky jute by *Aspergillus sp.* proved successful in the cultivators' field (Bhattacharyya and Basu, 1981) as by the treatment the barks are removed to a great extent and there in an average upgradation by two grades without and loss in the properties. Culture in liquid suspended from as well as in admixture with a solid carrier showed almost equal efficiency in softening barky jute and the carrier based fungus can be easily preserved and transported.

1.8 Chemical constituent of jute fiber

Jute is a bast fiber composed of α- cellulose (60-65%), lignin (12-14%), and hemicellulose (15-23%). The minor constituents are fats, waxes, pectins etc. The X-ray diffraction study revealed the crystalline and amorphous region in jute fiber. Macromolecular polymeric structure is built of cellulose, hemicellulose and lignin in a three dimensional pattern where cellulose is mainly in the crystalline part. Hemicellulose, lignin, protein, fat, waxes are present in the non-crystalline amorphous and paracrystalline part. The physical properties such as elasticity, lusture, softness etc. depends on the amount of crystalline orderly matter present in the fiber. On the other hand chemical behaviors such as swelling, dye acceptability, etc. depend on the disordered amorphous portion of the fiber.



Chemical composition of jute

Source: S. N. Kar, 1954, Mac Mikan W.A. 1957, Dass, N. N. *et al.*, 1948.

1.8.1 The chemical structure of cellulose and hemicellulose (Xylan)

Cellulose of jute

Cellulose is a major constituent of higher plants and abundant in mature. It is also a major component of jute (60-65%). In jute a number of other polysaccharides like hemicellulose, pectin and lignin are associated with the cellulose. The chemical identity of jute cellulose was established from the yielded of crystalline glucose and cellulose

acetate. In structure, cellulose is a polymer of glucose units bound together in long chain of β – linkages at carbon atoms 1 and 4 of the sugar molecule. Different cellulose chains lie between side by side and hydrogen bonds may form between –OH groups on different chains in tight bundles.

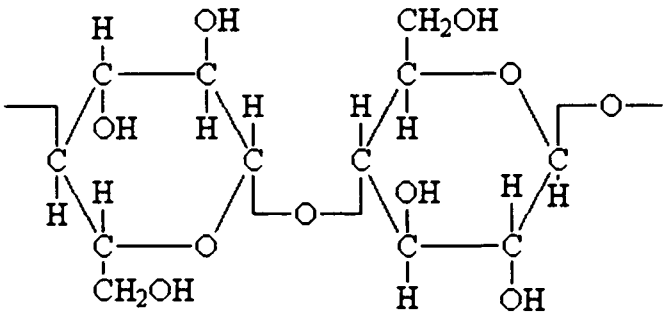
Cellulose is the world's most abundant natural polymer. It has high tensile strength and is totally insoluble in water and thus recalcitrant to degradation. Cellulose does not occur in pure form in any natural resources; the most pure form of cellulose readily available in nature contains about 10% by weight of non-cellulosic polysaccharide, protein and mineral elements. In the native state cellulose fibers are associated with other polysaccharides, i.e. hemicellulose, pectin and with lignin.

1.8.2 Structure of the cellulose

Cellulose is a linear polymer of up to 14000 anhydroglucose residues in the chain configuration. The residues are held together by β-1,4-glycosidic bonds with an average degree of polymerization in situ of 10000, yet can be as low as 15. The glucose molecules of cellulose are in the chain conformation, and because of the β-configuration all of the hydroxyl groups are in the equatorial position. This explains why cellulose has a layered structure, where the polysaccharide chains associate to form insoluble elementary fibrils with a diameter of approximately 3nm. The elementary fibrils are bonded into macrofibrils with a diameter of 10-40 nm, and these are again bonded into macrofibrils visible in a light microscope. Hydrogen bonding and van der Waals forces promote effective interchain binding with the resultant formation of the insoluble, complex, supermolecular structures with crystalline properties (Lutzen *et al.,* 1983).

Within cellulose fibers there are areas of complete order, i.e. crystalline areas, and also less well-ordered or amorphous regions. The degree of crystallinity within fibers varies with the source of the cellulose and the treatment to which it has been subjected. The susceptibility of cellulose as a substrate for enzymatic hydrolysis process is determined by its accessibility to cellulase enzymes. The resistance of cellulose to hydrolysis is, in

part, caused by the crystalline state, although crystallinity indices are not an absolute criterion and are not directly indicative of the susceptibility to enzyme hydrolysis. The crystallinity of the native material and its association with lignin are the major factors militating against the enzyme hydrolysis of cellulose. The hydrophobic character of 1,4-β- glucan chains may also contribute to the very low reaction rate observed.

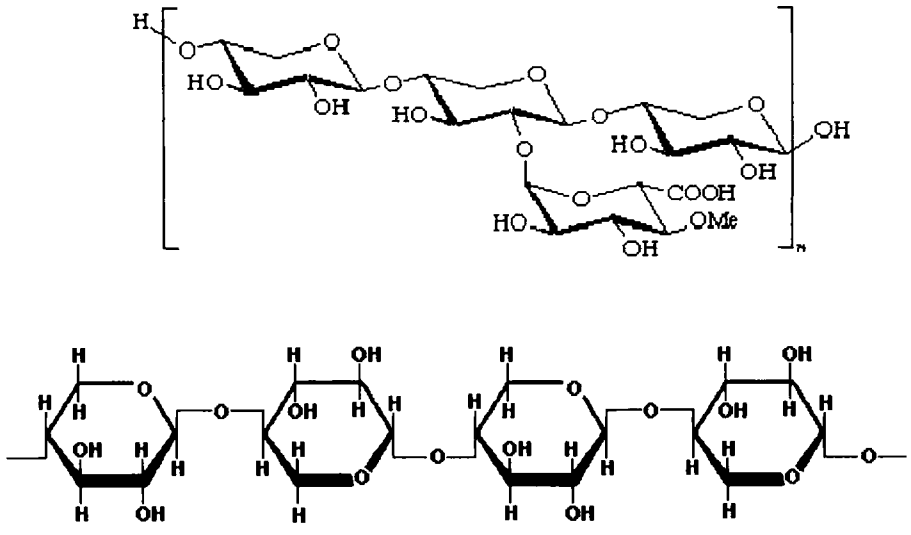


Structure of cellulose

Source:
http://dwb4.unl.edu/Chem/CHEM869E/CHEM869ELinks/qlink.queensu.ca/~6jrt/chem210/Page5.html

1.8.3 Structure of xylan

Xylan structures are variable ranging from linear homopolysaccharide to highly branched heteropolysaccharide (Wilkie, 1983). The degree of branching varies depending on the source. The main chain of xylan consists of xylan consists of homopolymeric backbone of 1,4-linked β-D-xylopyranose units. The xylans of hardwood, which may account for 10-35% of dry weight (Dekker *et al.*, 1976), are acetyl-4-O- methylglucuronoxylan with a degree of polymerization (DP) of about 200 (Plus *et al.*, 1993). Approximately one in ten of the β-D-xylopryranose backbone units is substituted at C-2 with a 1,2 linked 4-O-methyl-α-Dglucuronic acid residue, while 70% are acetylated at C-2 or C-3 or both (Lindberg *et al.*, 1973; Puls *et al.*, 1993). In addition most hardwood xylan contains small amount of rhamnose and galacturonic acid as integral components of the main chain (Johannson *et al.*, 1997).



Structure of xylan

Source: Johanson *et al.*, 1997

1.8.4 Structure of the lignin

Lignin is the most important component in jute which distinguishes jute from other textile fiber. Some of the peculiar characteristic of the jute fiber like yellowish of photo degradation is attributing to the presence of lignin in jute.

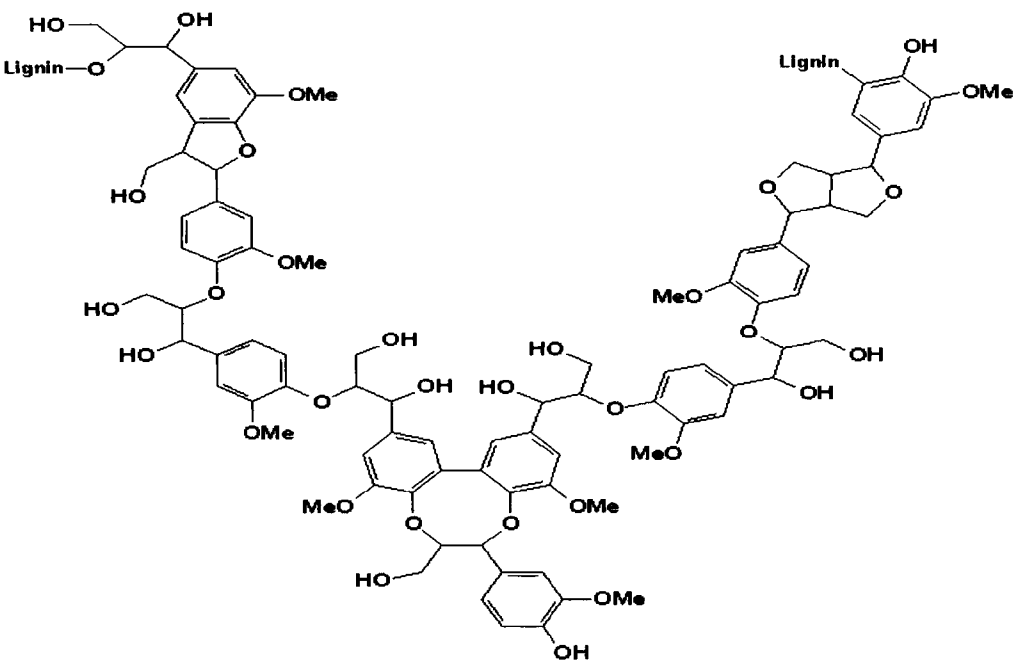
Lignin is the incrusting material of the jute plant, built up, mainly if not entirely of phenyl. Propane building unit, which then refiuxed with ethanol in the presence of hydrogen chloride gives hibberts ketones. When oxidized with nitrobenzene, lignin gives a characteristic ultraviolet absorption spectrum and color reactions with phenols and aromatic amines.

Lignin does not represent one uniform compound but rather it includes a group of higher polymerized molecules of similar chemical structure, but different size. The molecular weight varies from 300-14,000 depending on the source and method of estimation. Regardless of their source and isolation method employed, it is generally accepted that lignin contains only carbon, hydrogen and oxygen.

Although an exact structure formula of lignin cannot be given the functional groups of lignin are generally believed to include; methoxyl, phenolic hydroxyl, primary and secondary hydroxyl groups, benzyle alcohol groups, either groups, carboxyl and conjugated carboxyl and other double bonded groups. The functional groups of major influence upon the reactivity of lignin consist of phenolic hydroxyl, benzylic hydroxyl

and carbonyl groups. The methoxyl content of lignin also varies with the method of isolation and its origin. Sarkar (1931) isolated lignin from jute by Willstatter's method and found five methoxyl groups per lignin molecule of a molecular weight of 830. By the comprehensive studies of Frendenberg during the period of 1940-1960, it was conclusively agreed that lignin is polymerized form of a, β unsaturated C6C3 precursors of the coniferyl alcohol type, e.g. enzymatic dehydrogenation reactions and more than two thirds of the phenyl propane units in lignin are linked by either bonds. Again, lignin is also divided in two different classes according to the composition of precursor units.

All recognized gymnosperms formed from coniferyl alcohol whereas angiosperms (mono-cotyledons and dicotyledons such as bamboo and jute respectively) are formed from coniferyl and sinaphl alcohols, are called guaiacyl and auaiacyl-syringyl lignin respectively.



Structure of lignin

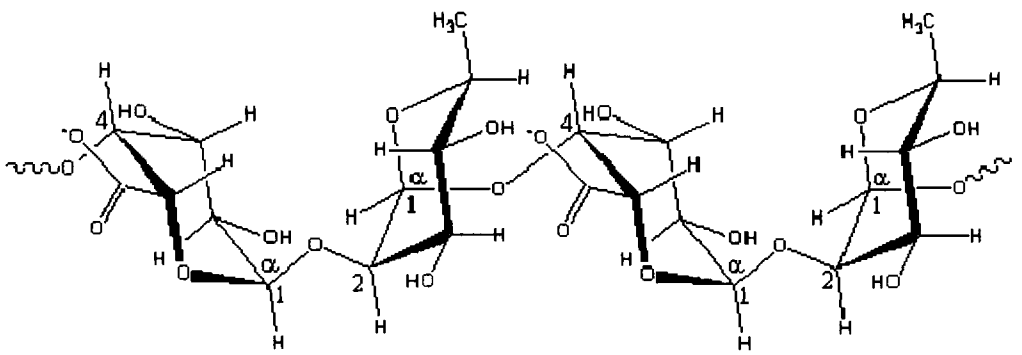
Source:http://blogs.princeton.edu/chm333/f2006/biomass/bio_oil/02_chemistryprocessing_the_basics/01_chemistry/

1.8.5 Structure of pectin

Pectin present in jute plant in very small amount (2.5%) and their importance lies to the physical structure of the plant. Fairly well retted good quality fiber contains less than 0.5% pectin. But in low grade jute the pectin substances do not decompose fully during retting, (Mohiuddin *et al.*, 1978). That is why the low grade fibers contain more pectin materials (2-4%) than good quality fibers. Thus the high content of pectic substances holds the fiber filaments together making them hard and compact. A number of workers have suggested that if these pectic materials of low grade jute can be decomposed by some methods the resultant fibers would be softer, separable and easy to spin (Shaha *et al.*, 1952).

The pectin materials are found as calcium and magnesium pectates in the middle lamella, a tissue constituent located between individual cells. The pectic carbohydrates are complex polysaccharides and composed of galacturonic acinits bound to one another in a long chain.

The pectic carbohydrates are complex polysaccharides composed of galacturonic acid units bound to one another in a long chain.



Pectin structure

Source: http://www.mdidea.com/products/proper/proper01805.html

There are three types of pectic substances:

(a) Protopectin, a water insoluble constituent of the cell wall,

(b) Pectin, a water soluble polymer of galacturonic acid which contains methyl ester linkages and

(c) Pectic acids, the water soluble galacturonic acid polymers that are devoid of methyl ester linkages. Each of these three classes represents a group of closely related pectic substances.

1.9 Xylanolytic mesophilic organism

Xylanases are produced by various kinds of microorganism including bacteria, streptomycetes and fungi. Xylanases of microorganisms reported so far are mainly from mesophilic fungi; comparatively less information is available on xylanase from thermophilic organisms (Dekker and Richards, 1976; Woodward, 1984; Biely, 1985; Poutanen, 1988; Wong *et al.*, 1988; Puls and Poutanen, 1989). A comprehensive list of microorganisms producing mesophilic xylanases is given in Table-1.

Table-3: Distribution of thermostable xylanases

Bacteria	**Fungi**
Bacillus sp.	*Aspergillus fumigatus*
Bacillus stearothermophilus	*Absidia ramosa*
Caldocellum saccharolyticum	*Acremonium alabamensis*
Clostridium stercorarium	*Chaetomium cellulyticum*
Clostridium thermocellum	*Humicola lanuginosus*
Dictyoglomus sp.	*Mucor pusillus*
Thermotoga sp.	*Melanocarpus albomyces*
Streptomycetes	*Sporotrichum thermophile*
Actinomadura	*Talaromyces byssochlamydoides*
Microbiospora	*Thielavia terrestris*
Streptomyces	*Thermoascus aurantiacus*
Thermomonospora fusca	*Thermomyces lanuginosus*
	Torula thermophila

Table 3: Source: Dekker & Richards *et al.*, 1976, 1984, 1985, 1988 and 1989.

1.10 Cellulolytic organisms

Diverse spectrums of micoorganisms are known for their ability to degrade cellulase (Mandels and Reese, 1960; Ryu and Mandels, 1980). Cellulolytic enzyme production among the mesophiles is widespread (Ryu and Mandels, 1980). There has been increasing interest during the last decade in thermostable cellulase productio by thermophilic micoorganisms (Tansey, 1971; Hagerdal *et al.*, 1978). Comprehensive list of micoorganisms producing mesophilic cellulase is given in Table- 2.

Table-4: Distribution of thermostable cellulose producing organisms

Bacteria	**Fungi**
Cellulomonas sp.	*Aspergillus fumigatus*
Cellvibrio fulvus	*Chaetomium cellulyticum*
Clostridium thermocellum	*Chaetomium thermophile*
Clostridium thermomonospora	*Humicola grisea*
Streptomyces	*Humicola inolens*
Actinmycetes	*Myceliophthora thermophila*
Thermoactinomyces	*Sporotrichum cellulophilum*
Thermomonospora fusca	*Sporotrichum thermophile*
	Talaromyces emersonnii
	Thermoascus aurantiacus
	Thermomyces lanuginosus
	Thielavia terrestris

Table 4: Source: Dekker and Richards *et al.*, 1976.

Table-5: Xylanolytic and Pectolytic organism

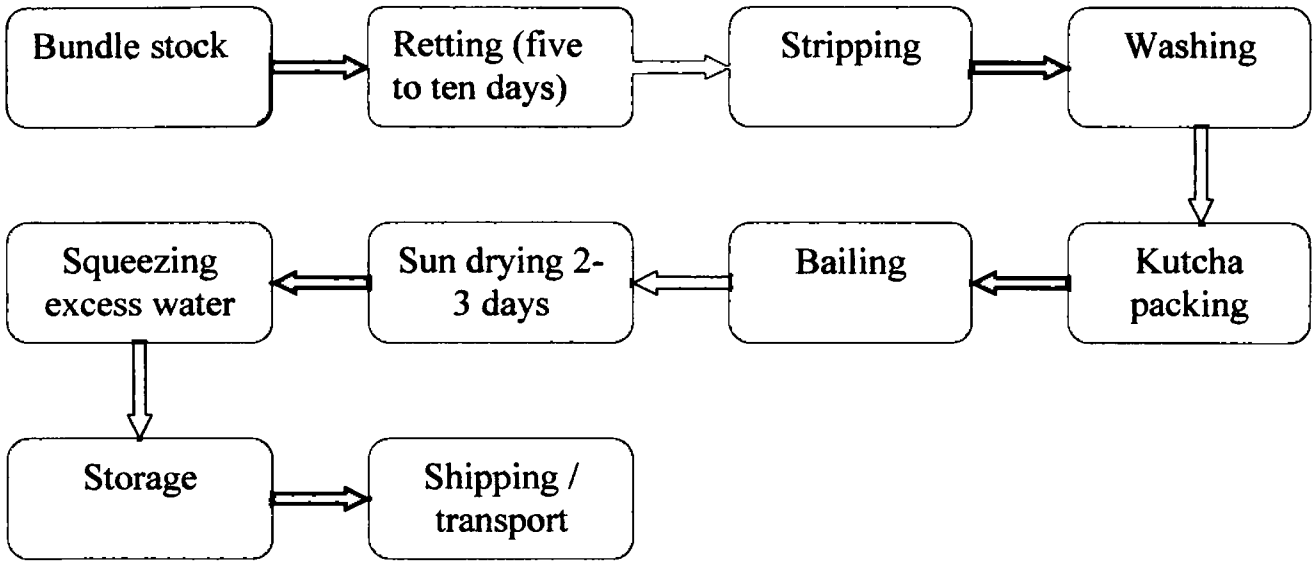
Bacteria	Fungi
Cellulomonas sp.	*Penecillum frequentance*
Bacillus polymyxa	*Sclerotium rolfsii*
Bacillus subtilis	*Aspergillus sp.*
Bacillus megaterium	
Bacillus alvei	
Clostridium thermocellum	
Clostridium thermomonospora	

Source: Ali *et al.*, 1983.

1.11 Processing of Jute

The jute is second in world's production of textile fibers. Jute is almost entirely a market oriented crop. As much as 90% of crop produced is necessarily to be sold and only 5% is retained by the farmers for domestic purposes. The growers usually market the fiber in the immediate post harvest period. The peak arrival months are September and October.

The crop is ready for harvesting when it is in small pod stage. If harvested before, the fiber is weak while if left until the seed is ripe, the fiber is stronger but is coarser and lacks the characteristic lusture. The plants are left in small heaps at different places in the field for two to four days when most of the leaves get dried up. After this period the plants are tied into bundles. At the time of bundling most of the leaves shed on the ground. The color of the fiber is darkened if the leaves are allowed to remain during the process of retting. It is also thought that drying of the plants before retting facilitates the separation of the fiber. The bundles are then taken for steeping in water. In low lands where jute plants may be standing in water, steeping is carried out immediately after harvest.



Flow chart of jute processing

1.11.1 Steeping of the bundle stalk

It is a process by which fibers in the tank get loosened and separated from the woody stalk due to the removal of pectinals and other mucilaginous substances. This is usually affected by the combined action of water and micro-organisms. The tied bundles of jute stalks are taken to the tank or ditch for retting. The bundles are steeped in water at least 60 to 90cm in depth. Jute bundles rot better when steeped out at a depth of 15cm to 23cm below the surface in slow flowing clean water. The retting process is completed in 8 to 30 days.

When the barks separate out easily from the stick or wood retting is completed. When retting is complete, fibers are ready for extraction. Fiber must be extracted as quickly as possible otherwise the quality will suffer.

1.11.2 Stripping (Fiber Extraction)

This is a process of removing the fibers from the stalk after the completion of retting. Generally the fibers are removed from the stalk by any of the three methods. In the first method single plants are taken and their fibers are taken off. This method though very slow, results in good quality of fibers as it is free from pith, etc. The second method implies taking off a handful of stalks breaking at one end and stripping off the fibers by

dashing it into and formation in water. The third method consists of washing the stalks first by standing in waist deep water and then stripping afterwards. None of the above methods is efficient as they are slow and the recovery is also low.

The fiber bundles lie beneath the bark, surrounded by gummy materials in the living plants. To obtain the fiber from the stem these entirely soft tissues must be softened, dissolved and washed away. This is done by steeping the stem in water, the bundle of stalks are laid in the ponds ditches or slow moving streams, weighted down the stones leaves, or clods of earth, and left for 5-15 days. A plentiful supply of water for retting is required. Approximately 2,800 gallons are needed to pond-set one 28 nasse of green stalks yielding 112 leaf fibers. The optimum water temperature for retting is 26.7°C . Retting is caused by micro-organisms which soften the tissues and gums, starting at the continue and extending outward so that the outer cells of the cortex are the last to disintegrate. Retting is better if the stems are uniform in thickness. The type of water which is used for retting has an influence on the value of the fiber. The best place for retting is slow running streams which are free from pollution as possible.

The fiber is taken from the water as soon as possible when the daily examination of the stems shows that the bark can be removed easily from the rest of the stem. This stage is called stripping. A bunch of stem is held in one hand and the root end tapped lightly with a mallet. This action frees the fibers at the root of the stalk. The labourers then grasps the fibers and by jerking and lashing the stems about in water, loosens the rest of the fibers, picks off odd pieces of bark, washes, the fiber and squeezes the excess water out. The fiber is then collected and laid out on bamboo racks to dry for 2-3 days.

1.11.3 Washing and drying

Before hanging for drying the extracted fibers are washed in clean water. The dark color if present can be removed to a great extent by dipping the fiber in tamarind water for 15 to 20 minutes and then washing in clean water. Five kilograms of ripe tamarind fruits in two buckets of water will give the necessary solution to treat about 5.5 to 7.5 quintals of fiber. The fibers are hung on bamboo railings for drying which takes 2-3 days. After drying the fibers are ready to be sold in the market. The fiber extraction is done during the months of August and September.

Washing of Jute in conventional retting process

The yield of the crop depends upon the quality of the soil and the attention which the fiber has received in its various stages. The fiber content of plant generally ranges between 4.5 to 7.5% of the green weight with an average of 5.5%. The normal average yield per hectare in India is estimated at about 11 to 21 quintals.

1.11.4 Grading

There are two stages of grading, one for the home trade and another for the export trade. Grading factors for jute are color, length, firmness of fiber, luster, strength, clearness, freedom from defects, and the amounts of root end which will have to be cut off. The preliminary grading is done by kutcha balers.

Kutcha Bales grading of raw jute:

Grade	Characteristics
Tops	Very strong fiber, excellent color and 30 nasse free from all defects
Middles	Strong, sound fiber, average color for the district, free from specks, runners and hard crop end
Bottoms	Sound fiber, medium strength, free from hard-centered fiber
B- Bottoms	Sound fiber, medium strength, not suitable for higher grades
C- Bottoms	Medium strength fiber, any color, free from runners and cropiness
X-Bottoms (cross bottoms)	Weak hearse Jute, free from tangle Jute and stick jute of any sort, free from dust and cutting

1.11.5 Spinning process

Type of jute yarns manufactured can be classified according to the use to which they will be put -

Fine Yarns: These yarns are of low count for making fine fabrics for tailors inter-lining and the like. The volume of trade in these is comparatively small since they are expensive and the top grades of the jute must be used to enable such yarns to tie spun.

Hessians Qualities: Medium weight yarns for weaving cloths for general packing purposes, linoleum backings carpet backings etc. come under these qualities.

Carpet yarns: These are yarns usually medium-heavy weight yarns of good quality either single or top-play for the carpet industry.

Sacking yarns: These are medium/heavy yarns of lower grade used for the manufacture of sacks and bags.

Type A, B and C can be divided into warp and weft qualities; the warp being superior to the weft as it must withstand the tensions of weaving and undergoes little strain.

1.11.6 Marketing of Jute

The important link in the chain of the movement of jute from the growers to the home mills or the exporter is collection, assembly storage and transportation at several stages. The first link is the bi-weekly village market or hat. As the top becomes ready in June or early July, dealers travel round the homes of the growers buying their jute and then taking it to the hats where they and some of the growers who bring their own jute to the market sell or merchants. The jute at the hat is sold in an 31nasserted fashion, the only distinction being between white-Tossa jutes. The fiber is transported by country boat, back animal, or cart to the larger secondary centre's where jute buying and selling goes on daily during the seasons.

The fiber is graded into Tops, middles, B, C and X-Bottoms by the Kutcha baler. A kutcha baler is one who grades the raw jute and packs it into kutcha bales weighing about 250 pound for use in the home trade. A pucca baler grades the fiber for export, cuts off the hard root end and presses the jute into pucca bales weighing 400 pounds of 49" x 18" x 20". This is done to some valuable cargo space for the material which is to be supplied overseas. The home mills buy their jute either from the primary or secondary centres being the chief sources.

1.12 Conventional method of retting in Bangladesh

In Bangladesh biological retting is more convenient and popular due to the high expense of chemicals. After harvesting the plant (bundle) is stepped in the water of river, bil, pond and roadside ditch for several days. Microbes in the water attack the cementing materials and non-fibrous matters are hydrolyzed, thus the fibers are separated.

Conventional jute retting in Bangladesh.

Jute plant

↓

Absorbs water and swell

↓

Bursting occur at several places

Soluble constituents (sugar) liberated

↓

Good medium for growth of microorganisms

Growth and multiplication of microorganism

Degradation of complex organic materials to simpler

Flow sheet diagram of Jute retting

Disadvantages of conventional retting process

- Require a lot of water for jute retting.
- Jute retting in a pond resulted in a sharp increase in the BOD (>1,000 times) and COD (>25 times) of the water, decrease in dissolved oxygen (DO). Free CO_2, total ammonia and nitrate nitrogen also increased (3-5 times) in water that has a great impact on aquatic environment.
- Over retting and under retting may affect the quality of the fiber.
- Reduces dO_2 concentration.
- Odor problem.
- Water pollution.
- Adverse affects on farmers' health.

On the basis above problems ribbon retting process was introduced.

1.13 Green ribbon retting

In some areas of Bangladesh jute grows well specially in the northern part of the country but for want of sufficient retting water solving this problem by evolving new methods of retting the ribbons (green bark of the plant) instead of retting the whole plants and by providing retting water facilities by excavating roadside canals, new retting tanks and re-excavating the available derelict tanks in the strategic jute growing areas.

Farmers carrying jute from field for ribboning

It is observed that the ribbons are retted instead of the whole plants, retting problem of the water scarce areas can be greatly solved in which the advantages are achieved viz.

(i) It requires much less water,

(ii) Retting time is reduced to almost half,

(iii) Cuttings are almost eliminated,

(iv) Bulk is reduced to about half and as such fewer labors are required,

(v) Fiber quality is greatly improved.

Preparing green ribbon from jute

Attempts were made to extract ribbons form the plants using two types of machines called decorticators or ribboners. One was Japanese type and other was an American type. The main disadvantage with these ribboners was that they broke the jute sticks of the plants into small pieces which could not be used as fence, fuels, etc. The other disadvantages are farmers will not be able to purchase those costly decorticator machine (Ali *et al.,* 1976).

In view of the disadvantages of the machine ribboners, efforts were made to produce a short of hand ribboners which would produce ribbons without breaking the stick and also be very cheap. Box method, Sandle method, Cylinder method, Roller method, Bamboo hook method etc. were tired. Finally the bamboo hook method was found to be most suitable. In this method green jute barks were separated with the help of 'U' shaped bamboo hooks practiced by hand and the ribbons thus obtained were made into

ring from and allowed to ret in earthen vats, polythene lined artificial ditches, for submerged, and moist retting using fungal inoculums. If 0.01% urea or 1% retting effluent or fungal inoculums in case of moist retting, enhances the retting activities. Good quality fibers were obtained when plants were harvested a few days earlier than the average flowering time and immediately ribboned. Although the rather younger plants gave lower fiber yield, it was likely that the quality of the fibers would compensate the loss of yield.

The hand ribboning and ribbon retting method has been taken to the growers of water scarce areas and are gaining much popularity. It has been observed that although a few more laborers are involved in the hand ribboning method of retting, its other advantages such as good quality fiber, quicker retting etc. more than compensate for these additional labors. (Anon. 1968, 1969, 1970, 1971; Ali *et al.*, 1976; Ali *et al.*, 1989).

Green jute ribbon ready for retting

1.14 Use of fungi an alternative means of retting

1.14.1 Use of microbes in jute ribbons retting

Microorganisms can be used for jute retting under controlled condition. Among the microorganisms, use of fungi is more advantageous as they need less amount of water for their metabolic function. (Haque *et al.*, 1992)

Table-6: Mesophilic microbes used in jute retting (both xylanolytic and pectinolytic).

Fungi
Sclerotium rolfsii
Aspergillus niger.
Penicillium frequentans
Mucor piriformis
Schizophyllum commune

Source: Hoq *et al.*, 2001

Conventionally several fungi are involved in jute retting by producing different enzyme (xylanase, CMCase and pectinase, being major). Some of the examples include *Aspergillus sp., Penicillium sp., Sclerotium sp. and Rhizopus sp.* etc. as mentioned above in the Table no 6.

1.14.2 Enzyme involve in jute retting Occurrence of xylanases

Xylanases are widely distributed. They are present in both bacteria and fungi (Dekker and Richards, 1976) and have been also demonstrated in other organisms including insects, snail, etc (Taiz and Honigman, 1976). Intracellular xylanases occur in rumen bacteria and protozoa, while extracellular xylanases produced by a fairly large number of bacteria and fungi in the natural ecosystem **(Dekker and Richards, 1976).**

1.14.3 Endoxylanases

The first step in the complete hydrolysis of xylan in the depolymerization is a catalyzed by a highly specific endo-type hydrolase and xylanase. The enzyme hydrolyze β-1, 4-

glycosidic linkages in xylan backbone to produce shorter xylooligosaccharides, xylobiose and even xylose.

Microorganisms often produce more than one type of xylanases (Dekker, 1985). These differ in substrate binding site and consequently in the number of unsubstituted xylose units in series required in the polysaccharide backbone. Five different xylanases have been purified from culture filtrates of *Aspergillus niger* (Shei *et al.*, 1985; Frederick *et al.*, 1985). Other workers have purified at least xylanases from several organisms such as *Clostridium stercorarium* (*Barenger et al.*, 1985) *Penicillium janthinellum* (Takenashi and Tsjisaka, 1973), *Trichoderma ressi* QM 9414 (Dekker, 1985), etc. The occurrence of xylanases in multiple forms is as a result of different mRNA processing, partial proteolysis or differences in the degree of amidation and glycosylation (Biely, 1985).

1.14.4 Properties of xylanase

A very large number of reports on the production, properties and applications of endoxylanases have been published in last 25 years. In many cases multiple forms of these enzymes are found. Molecular weight value range from 11,000 to 85,000 and pH values form 3.6 to 10.3 (Frederick *et al.*, 1985; Bastawde, 1987; Kesker *et al.*, 1989). Wong *et al.* (1988) noted that many endoxylanases fall into patterns of low molecular mass/basic pH value and high molecular mass/acidic pH value. However, there are many reported exceptions to this general pattern. Carbohydrate moities are covalently linked with protein or are present as dissociable complexes with various xylanases (Wong *et al.*, 1988). Carbohydrate groups play an important role in stabilizing the enzyme protein structure as well as its activity and thermostablity (Woodward, 1984). The glycoprotein nature of xylanse from *A. niger* was reported by Fredrick (1985). The glycoprotein content of three endoxylanases form *A. fumigatus* was between 46.4 and 68.0% (Flannigan and Sellers, 1977).

1.14.5 Pectinase

Pectin matter occurs in the jute plant and it holds the fiber bundles together. Fairly well retted good quality fibers contain less than 0.5% of pectin. But in low grade jute and cuttings this pectin is not decomposed fully during retting (Mohiuddin *et al.* 1978).

That is why jute grade become low and cuttings contain more pectin materials (3-4%) than that of good quality fibers. If this can be decomposed by some methods the resultant fiber would be softer and more separable.

A number of workers have suggested that this pectin can be decomposed by microbial action promoted by the addition of ammonium phosphate with the emulsion during conventional softening process (Shaha, *et al., 1952*).

In biological retting of fiber plants relies on microbial enzymes to digest the pectic substances of the middle lamella. The results of hydrolyzing of pectic polysaccharides leads to a loosening of the cellulose fiber filaments from the residual tissues. Among the organisms that cause natural retting are *Bacillus* and *Clostridium sp.* They solubilize the pectic materials of middle lamella by means of their pectic enzymes and thereby separate individual fiber cell from one another.

Three enzymes are concerned in the degradation of pectic substances:

(a) Protopectinase, which decomposes protopectin with the formation of soluble pectin,

(b) Pectin methyl esterase (PME), which hydrolyses the methyl ester linkage of pectin to yield pectic acid and methanol,

(c) Polygalacturonase (PG), which hydrolyses the linkages between galacturonic acid units of either pectin or pectic acid with the release of smaller fragments and ultimately free galacturonic acid.

1.14.6 Ligninase

The biochemistry of lignin degradation, the mechanism of the enzymatic cleavage and intermediates in the process has still to be established. The enzymes concerned as a single catalyst termed as ligninase. Degradative products like vallinin, vanillic acid and ferulic acid etc. have been reported by Henderson *et al.,* (1952). During the fungul rotting of Spuruce sawdust. Thus enzyme system functioning converts the lignin molecule to give simple 4 aromatic substance for further growth. The enzyme system is undoubtedly extracellular. Mandels and Sternberg (1979) but only very few of these fungi are able to produce considerable level of extracellular enzyme which can degrade the cellulosic materials invitro and are found mostly in higher fungi.

Sclerotium rolfsii (Sadana, Shewale and Despande, 1980) *Sporotrichum pulvaranticulum* (Eriksson *et al.*, 1983) *Schizophyllum commune* (Paice *et al.*, 1978), *Phoma hibernica* (Urbanek *et al.*, 1978) and the members of the genus *Aspergillus*, *Penicillium* and *Fusarium* (Fahnrich *et al.*, 1981).

However, a great variation exists among the lignocellulolytic organisms in respect of production, composition and abilities to degrade lignocellulosic materials. Some of them produce all the essential components for cellulose hydrolysis, while others produce incomplete cellulose system. For instance *T. reesii* which is known to be the best of all cellulose producing fungi, has a complete cellulose system rich in C_1 and C_x activities but with a suboptimal β–glycosidase activity for rapid and complete degradation of cellulose to glucose (Sternberg *et al.*, 1977).

Schurz *et al.*, (1985) put forward a reaction mechanism for cellulose hydrolysis, calling it as "All or nothing". They reported that during the enzymatic degradation of cellulose firstly the degree of polymerization decreased very little, secondly the index of crystallinity increases quite little and thirdly volume of the hollow-surface increased slightly so that the surface enlarged a little. Based on this findings they postulated that a cellulose chain will be either totally degraded to cellobiose, especially to glucose or it will not, at all, be degraded.

1.15 Aim of the present work

Jute is the major cash crop of Bangladesh and it was known as golden fiber from time immemorial. In recent times, the cultivation of jute faces problem for retting of jute plants under conventional conditions. This is because; nowadays in Bangladesh there is a great scarcity of retting water. Million tons of jute remains unretted ever year in the field particularly in the northern part of Bangladesh. Alternatively, instead of jute plants, ribbons from the plants can be separated and retted using less amount of water. Fungal retting in the moist condition can solve the problem considerably because the process is natural and requires less space and water. Due to ribbons, instead of whole jute plants, the volume is reduced and the system can be monitored by addition or reduction of humidity under normal temperature, because the fungi are mesophilic in nature. Till now no detailed study on jute ribbons retting has been performed in our country. So the objectives of the study are given below -

1. Isolation, selection and identification of mesophilic fungi suitable for jute ribbon retting.
2. Inoculum production under solid-state fermentation using wheat bran for substrate to be used as ribbon retting.
3. Optimization of moisture level for inoculum production.
4. Retting of green ribbon under sterilized and non-sterilized conditions.
5. Retting of ribbon under solid state and submerged state conditions.
6. Chemical analysis of the fungal treated fiber.
7. Retting of whole ribbon under submerged condition in earthen vat.
8. Determination of bundle strength of the treated fiber.
9. Grading of fiber
10. Characterization of the enzymes involved in the retting
11. 1. Effect of time span on enzyme production.
11. 2. Optimization of pH, temperature and pH stability

Chapter 2

MATERIALS AND METHODS

MATERIALS AND METHODS

2. *Materials and methods*

2.1 Isolation of fungi

To isolate retter microorganisms for the improvement of jute retting practices, samples of rotted fiber, retting water, damaged jute goods, jute seed germination were collected from Bangladesh jute research institute and jute experimental station Jagir, Manikgonj. Samples were kept in sterilized containers and were labeled carefully.

The procedure consists of diluting the sample with series of sterile water. One milliliter of the solution was transferred to bottles number containing 9 ml sterile water. From bottles B measured amount of diluted microorganisms transferred on the surface of the media and spread evenly throughout. After 3 days of incubation, colonies appeared in varying numbers based on the amount of dilution from the original habitats of fungi.

2.1.1. Separation of fungi

For the separation of the fungi, Petri dish containing melted PDA medium was taken. After inoculation, new colonies appeared and each distinct colony was transferred to another petridish containing PDA medium pH 5.4. Incubation period for growth of organism was 3-7days at 27-30°C.

2.1.2. Screening of jute retting fungi

Fungal isolates were tested for various hydrolytic enzyme activities following zone clearing method (Mohiuddin, 1992). Enzyme activity was demonstrated by the hydrolysis of the substrate xylan in case of xylanase and CMC in case of CMCase and Pectin in case of pectinase incorporation in petridishes. The medium contained all ingredients necessary to support the sufficient initial growth of the inoculums. After the incubation period of 36 hours, the colonies by the appearance of zones revealed by substrate, clearance (xylanase activity) or decoloration (cellulase) and pectinase activity.

2.2. *Testing the retting ability of the isolates in small scale*

Twenty one isolates were inoculated to 10 grams of green ribbons in petriplates and incubated until retting was completed. (Fig. 1, section 3) Retting ability was determined by the time taken by the isolates to ret. Fiber quality was measured by touch and method. Ten fungal isolates were found which have better retting ability among the 21 isolates

2.3 *Identification of jute retting fungi*

Identifications of ten fungal isolates were done by microscope with high power resolution (100x). Characterization method employed for fungal isolates was by an examination of the culture and morphological features according to Barnett and Hunter (1972) and Alexopoulus and Nims (1979) and also by microscopic examination (Koneman *et al.*, 1978).

2.3.1. *Teasemount preparation*

The use of tease mounts is a rapid method of preparing fungal mycelia for microscopic examination. With a stiff bent needle, a small portion of the culture was *avulsed* from the growth on the agar surface. This small culture fragment was transferred to a drop of mounting medium on a glass microscope slide and teased apart with dissecting needles, polished to remove any barbs and covered with a cover glass for observation under the microscope.

2.3.2. *Maintenance of the culture medium*

Stock culture of fungi were maintained on Potato Dextrose Agar (PDA) at 4°C and subcultured at 15 days interval. Finely divided potato (20 g) was boiled in 500 ml tap water for one hour. This was then filtered through cheesecloth and the filtrate volume was adjusted to one liter with tap water. After addition of dextrose (20 g) the mixture

was stirred well and the pH was adjusted to 5.5 Agar (20g) was then added and the medium was autoclaved at 121°C and 15 psi for 15 minutes. The medium was then plated and allowed to solidify under aseptic condition.

2.3.2.1. Wheat bran medium

Wheat bran medium was used as a sole source of carbon and nitrogen in solid state fermentation, 12.5 g of wheat bran were taken in each of 250 ml flask and moistened with water to give a moisture content of 50%. The flasks were cotton plugged and autoclaved at 121°C for 15 minutes.

2.4. Selection of four potential fungi

For screening of fungi for carboxymethylcellulase (CMCase), xylanase and pectinase enzyme test are as follows by the method: (Described in Section 2.1.2)

2.4.1. Optimization of the moisture level of the inoculum

Four fungal species (*Aspergillus niger, Aspergillus flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1) were used for the optimization of inoculum development. Wheat Bran was used as sole source of carbon and nitrogen in solid-state fermentation. 12.5 gm of wheat bran were taken in each of 250 ml flask and moistened with water using different moisture level (30%, 40%, 50%, 60% and 70%). The flasks were cotton plugged and autoclaved at 121°C for 15 minutes. Inoculum was added to the flasks aseptically and then the flasks were incubated at 29°C up to 6 days in an incubator and enzyme activity was determined after extraction at 30°C.

2.4.1.1. Production of retting enzymes

Wheat bran was used as sole source of carbon and nitrogen for the organisms in solid state fermentation, 12.5 gm of wheat bran were taken in each of 250 ml flask and moistened with water to give a moisture content of 50%. The flasks were properly cotton plugged and autoclaved at 121°C for 15 minutes. Inoculums were added to the

flasks aseptically and then the flasks were incubated at 30°C for up to 6 days in an incubator. After appropriate incubation time, distilled water (5 times volume of wheat bran medium) and 1% NaCl were added in each flask and left for 2 hours at room temperature with occasional shaking. Then it was filtered with cheese cloth. The filtrate was then centrifuged at 4000 rpm for 15 minutes and the supernatants were stored at 4°C for determination of enzyme activities and reducing sugar. The soluble protein concentration in the culture filtrate was also estimated according to the Bradford method (Bradford, 1976).

2.4.1.2. Storage of the enzyme solution

The enzyme was stored in plastic container in a refrigerator for further studies. For long-term use, they were stored at freezing temperature.

2.5.1. Effect of normal flora on retting in presence of inoculum in small scale

2.5.1. Sterilization of green ribbon.

Cut pieces of green jute ribbons were sterilized under UV irradiation sterilizer for half an hour to both sides (surface sterilization) so that no change of color and characteristic of green ribbon were changed.

2.5.2. Preparation of retting experiment with sterilized and non-sterilized green ribbon

Four potential fungal species, *Aspergillus niger*, *Aspergillus flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1 were selected for the retting experiment. For inoculum production 50 grams of wheat bran was moistened with distilled water to give moisture content of 50% and autoclaved in each of the 8 conical flasks (500 ml) of *A. niger*, *A. flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1 in four of the flask respectively. There of the remaining flasks were inoculated with *Sporotrichum sp.* + *Sclerotium sp.* type 1, *Aspergillus flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 and *Aspergillus niger* + *Aspergillus flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 respectively. The last remaining flask was un-inoculated and used as negative control. After 6 days of incubation at 29°C the contents of the flask were added with 5 volume of water and the

total content was used as inoculum for Jute retting. Then 250 g of ribbons, both sterilized and non-sterilized, were mixed with the inculums and the retting experiment was conducted in earthen shunkies at room temperature.

2.6. Enzyme assays

2.6.1 Xylanase

Xylanase activity was determined according to Bailey *et al.* (1992) using 1% birchwood glucuronoxylan (Sigma, DNS) in 0.05 M citrate buffer pH 5.5 as substrate. The reaction mixture, containing 1.8 ml substrate and 0.2 ml of suitably diluted enzyme solution in the buffer, was incubated at 50°C for 5 minutes and the reaction was stopped by addition of 3 ml of DNS reagent and boiling the solution in a water bath for 15 minutes. The amount of reducing sugar was determined by DNS method (Miller, 1959) using xylose as standard. The enzyme activity was expressed as U/ml and U/g. U/ml corresponds to micromole of xylose equivalents released per ml per minute under the assay condition. Xylanase activity was measured by the following equation and expressed in U/ml.

$$\text{Enzyme activity (U/ml)} = \frac{\text{Concentration of reducing sugar released (mg/ml) x 1000 x dilution factor}}{\text{Molecular weight of reducing sugar x incubation (min)}}$$

2.6.2. Carboxymethylcellulase (CMCase)

CMCase activity was determined according to IUPAC instructions (Ghose *et al.*, 1987) using 1% solution of Carboxymethyl cellulose (Sigma, USA) in 0.05 M citrate buffer pH as substrate. To 1.8 ml of substrate solution, 0.2 ml of enzyme solution was added and the reaction mixture was incubated at 50°C for 30 minutes. Adding 3 ml of DNS reagent stopped the reaction and the liberated reducing sugars were estimated by the method of Miller (1959). The enzyme activity was expressed as U/ml and U/g. U/ml correspond to micromole of glucose equivalents released per ml per minute under the assay condition. CMCase activity was measured by the following equation and expressed in U/ml.

$$\text{Enzyme activity (U/ml)} = \frac{\text{Concentration of reducing sugar released (mg/ml) x 1000 x dilution factor}}{\text{Molecular weight of reducing sugar x incubation (min)}}$$

2.6.3. Pectinase Polygalacturonase assay

Polygalacturonase activity was determined according to Bailey *et al.*, (1992) using 1% Polygalacturonic acid-Na (Sigma, DNS) in 0.05 M citrate buffer pH 5.5 as substrate. The reaction mixture, containing 1.8 ml substrate and 0.2 ml of suitably diluted enzyme solution in the buffer, was incubated at 50°C for 5 minutes and the reaction was stopped by addition of 3 ml of DNS reagent and boiling the solution in a water bath for 15 minutes. The amount of reducing sugar was determined by DNS method (Miller, 1959) using D-galacturonic acid as standard. The enzyme activity was expressed as U/ml and U/g. U/ml corresponds to micromole of D-galacturonic acid equivalents released per ml per minute under the assay condition.

$$\text{Pectinase activity (μmol of galacturonic acid equivalent released/mL-min), U} = \frac{\text{Released (g/L) x 1000}}{\text{Incubation time (min) x 194(Molecular weight of pectin)}}$$

2.6.4. *Pectin lyase*

Enzyme extract was passed through a filter paper (pore size 5μm) to make it spore free. The enzyme solution was appropriately diluted with distilled water so that absorbance readings remained within the spectrophotometer's scale. Enzyme solution was diluted for other reason so as to avoid the substrate limiting factor during hydrolysis with high concentration of enzymes. Diluted enzyme solution 0.5 ml was mixed well with 4.5 ml of 0.1% pectin buffer solution into screw stopper test tube. For control 0.5 ml of distilled water was with 4.5 ml of 0.1% pectin buffer solution into another test tube. The absorbance of above sets was made at 235 nm at time zero. The tubes were then incubated at 40°C for 20 minutes. Again their absorbances were taken at 235 nm and the actual increases in absorbance of the samples (*Mohiuddin et al.*, 1992) were made as follows.

One unit of PL is defined as an increase in absorbance at 235 nm by 1.0 in the reaction mixture per minute under assay conditions.

$$\text{PL activity (units/ml)} = \frac{\text{Actual increase in absorbance x DF}}{\text{20 minutes}}$$

2.7 *Chemical analysis*

2.7.1 *Estimation of reducing sugar*

Reducing sugar was estimated by dinitrosalicylic acid (DNS) method of Miller (1959), using glucose (Oxoid, USA) and xylose (Merck, Germany) as standard. In 1 ml of sample, 3 ml of DNS reagent (composition in appendix) was added. The mixture was heated in a boiling water bath for 15 minutes, cooled down in cooling water bath and diluted with 6 ml of distilled water and mixed well. Sample blanks and standards were also prepared along with the test sample. The absorbance was read at 540 nm against reagent blank. The amount of reducing sugar was calculated from the standard curve and was expressed as mg RS/ml of the test sample.

2.7.2 *Estimation of soluble protein*

Soluble protein in the culture filtrate was analyzed by the method of Bradford (Analytical Biochemistry 72, S.248-254, 1976) using bovine serum albumin (Sigma, USA) as standard. To 5 ml of Bradford test solution (Composition in appendix) 0.1 ml sample was added and mixed well. After 5 minutes the absorbance was read at 595 nm against reagent blank. The amount of soluble protein was expressed as mg SP/ml of test sample.

2.8. Influence of natural flora on retting of green jute ribbon using the potential isolates in small scale and determination of bundle strength

The experiment was described in the section 2.5. and 2.5.2. After retting of green ribbons (sterilized, non sterilized and control) the bundle strength of the fiber was determined by the pressly index method (described in the section 2.10.

2.9. Mechanism of Jute retting

250 g of ribbons were used for direct inoculation and 250 g are used in submerged condition (Ribbons + inoculum + enzyme). The retting experiment was conducted in earthen shankies at room temperature.

After 3, 7 and 9 days enzyme are collected from the retting jute and then xylanase, CMCase and pectinase, pectinlyase, reducing sugar and soluble protein was measured.

Four potent fungal species, *Aspergillus niger, Aspergillus flavus, Sporotrichum sp., Sclerotium sp.* type 1 and there mixed culture were selected for the experiment.100 g of wheat bran was moistened with distilled water to give moisture content of 50% and autoclaved in each of the 8 conical flasks (1000 ml) at 121°C for 15 minutes. The moist sterilized wheat bran medium was then mixed with four pure fungal cultures (1 cm^2) of *A. niger, A. flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1 in 4 of the

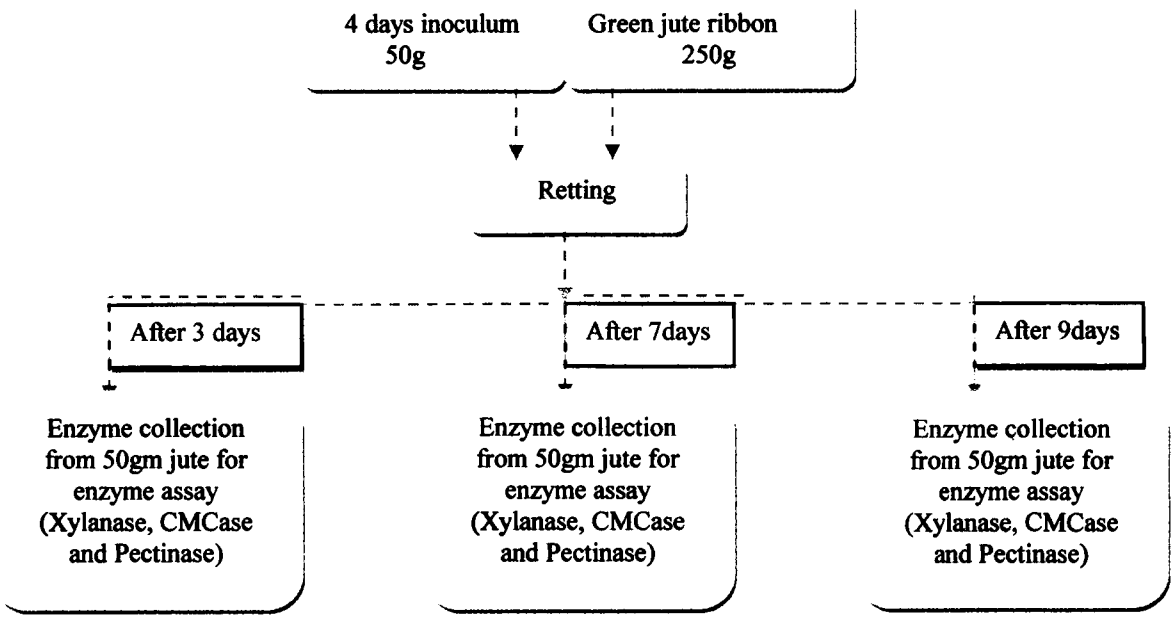
flasks respectively. Three of the remaining flasks were inoculated with *Sporotrichum sp.* + *Sclerotium sp.* type1, *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1, *A.*

flavus + *A. niger* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 respectively. The last remaining flask was uninoculated and used as negative control. After 4 days of incubation at 29°C the contents of all the flasks were used in two types of retting.

Green ribbon retting in Solid state condition : 250 gram green ribbon were taken in 8 pair earthen Shanki where *A. niger, A. flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1 were used singly and in combination of *A. niger* + *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1, *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1, *and Sporotrichum sp., Sclerotium sp.* type 1. The inoculum of 4 and 7 days were used for retting both in solid and submerged state of retting and one control were maintained where no inoculum were used. The shankies were covered with moist Hessian for aeration and time to time moisture was checked.

2.9.1. Solid state retting

In solid state condition of retting green ribbon of 10.54" pieces were mixed with 50g inoculum with 50% moist condition. Here no free water was present but a moist condition was maintained only. In solid state condition 50gm inoculum are directly used in 250g green jute ribbon.

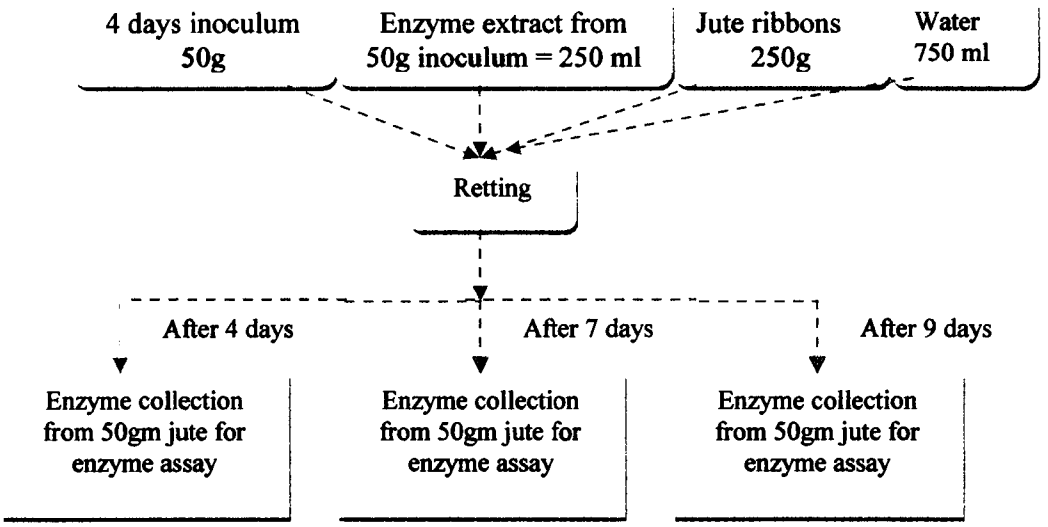


Flow sheet diagram of extraction of enzyme using 4 days inoculums.

2.9.2. Submerged condition of retting

In submerged condition of retting 250g green ribbon and 50g inoculum, 250 ml crude enzyme extract and 750 ml water were used and ribbons were kept in submerged condition using stone as weighting material.

In submerged condition, 50g inoculum + enzyme extracted from 50g inoculum + 250g jute ribbons + 750ml water were used.



Flow sheet diagram of enzyme extraction from 4 days inoculum.

In the case of 7 days inoculum, same procedures as enzyme extraction from 4 days inoculum were followed.

2.9.3. Grading of fiber

Fiber grading was determined by touch and feel method where visual observation of color and softness, percentage of cuttings and Bundle strength of fiber were considered.

2.10. Bundle strength (Pressley index)

Bundel strength of fiber was determined by pressly fiber bundle strength tester (*Brand: J.M. DOEBRICH AND CO. TUCSON, ARIZ*) using zero gauge length. The flat bundle approx 6.35mm width was held by a pair of clamps. All protruding ends were then shared off even and tension applied to separate the clamps and thereby to break the fibers. The broken bundle was then weighted in a precision balance. The resulting strength was then computed as

$$\text{Pressley index} = \frac{\text{Breaking load (Pound)}}{\text{Bundle weight (mg)}}$$

2.11 Determination of chemical constituents of fungal treated fiber

2.11.1 Cellulose

Cellulose content of enzyme treated fibers was estimated by Cross and Bevan method (C. Doree. 1950). One gm powder of enzyme treated fiber was boiled with 1% NaOH solution in round bottom flask. The content of the flask was then cooled, filtered and washed well with water in previously cleaned and weighed sintered crucible of porosity 1. Residue of each crucible was then transferred in conical flask placed in an ice bath and a slow stream of washed chlorine gas was passed through the materials for half an hour and allowed to stand for sixty minutes to ensure completion of reaction. Chlorinated fibers were then filtered, washed well with water to remove HC1, transferred to a beaker containing 50 ml of 2% Sodium sulphite solution. Content of each beaker was then heated to boiling. Later 2.5 ml of 4% NaOH solution was added and boiled for another five minutes. The resulting mixture of the beaker was then filtered, washed repeatedly with hot water and finally bleached by immersion in 1% K_2MnO_4 solution followed by 5% oxalic acid. Cellulose content was then digested for thirty minutes with hot distilled water, filtered and dried to a constant weight.

2.11.2 Hemicellulose

Hemicellulose was estimated by the method of Callow (1952). Jute powder was first defatted and then depectinized by refluxing with 0.5% ammonium oxalate solution for about four hours at 80-85°C. For delignification, the residue was first heated to boiling for about 30 minutes with 0.7% sodium chloride solution. The resulting mixture was then filtered in a sintered crucible of porosity 2, washed several times with water and finally with sodium bisulphate (pH 5.5). The process was repeated thrice for each sample. The above process was again repeated thrice by using 2.8% sodium chloride solution instead of 0.7% sodium chloride solution. The residue on sintered crucible was then transferred to a beaker and extracted by mechanical stirring with 9.3% NaOH solution for 2 hours. Extract of each sample was taken in 100 ml volumetric flask and made up to the volume by using the same extractor. 25 ml of the aliquot of each sample was first neutralized with acetic acid and then saturated with absolute alcohol to precipitate hemicellulose. The resulting mixture was then centrifuged in a previously cleaned and weighed centrifuged tube, precipitate of hemicellulose was first washed with different grades of alcohol and then with acetone to remove the last trace of water. Content of each tube was then dried to a constant weight.

2.11.3 Lignin

The bast was separated mechanically (Kertesz 1957, Mohiuddin *et al.*, 1981) from the stem and cut into small pieces, then washed with water and treated with alcohol. The pieces were dried and were extracted with 0.5 percent NH_4 oxalate at 80°C for four hours and then filtered. Acidified alcohol was added to the filtrate. Pectin would be precipitated as jelly like substance. The precipitate was filtered and washed with graded alcohol. The precipitate was transferred in water and treated with 0.7 percent NaC1 and was heated on a water bath to remove the lignin. The filtered material was lignin then it was dried in an oven at 60°C and weighted and percentage was calculated.

2.11.4 Pectin

Pectin content of enzyme treated fibers was estimated by Calcium pectase method, a modified method of Carre and Hyness (Kertesz 1957, Mohiuddin *et al.*, 1981). Two gm of fibers (Wax free by petroleum ether) was extracted with 0.05 N in a distillation flask. Extractions were repeated 3-4 times with 50ml extractor each time after half an hour interval. Extract was then collected in a 250ml volumetric flask and made up to the volume by using the same extractor. 25ml of this extraction was used to estimate the pectic substances in the sample. 25ml aliquot of extraction was first neutralized in a 500ml beaker. 5ml of 10% HCI was added and the mixture was made to a volume of 100ml. 200ml of 95% ethanol was added with vigorous stirring and it was allowed to stand overnight. The content of the beaker was filtered and the residue was first washed thrice with a water-ethanol (1:2) mixture and then with 95% ethanol. The precipitate was then dissolved in hot water.

The filtrate was made up to volume of 200ml, 150ml of 0.2 N NaOH was added and the resultant mixture was allowed to stand overnight. 60ml of IN CH_3COOH was added with stirring, the mixture was allowed to stand for a few minutes. First 25ml of 0.1M then 25ml of 0.2N $CaCl_2$ were added drop wise with constant stirring. The mixture was boiled for exactly 2 minutes, filtered and washed three times with boiling water. The precipitate was washed back with 100ml of water and boiled for minutes. Finally it was then filtered through previously cleaned dried and weighed sintered crucible of porosity 4 and the results are in Chapter-3.

2.12. Characterization of enzyme

2.12.1. Effect of time span for enzyme production

Time recorded for different enzyme production was commenced within 72 hours and were reached within 6 days using standard method the xylanase, CMCase and pectinase was measured (section 2.6.1, 2.6.2 and 2.6.3.).

2.12.2. **pH** *optima of the enzymes*

The pH optima for the enzyme activities determined by measuring enzyme activities at 50°C in 0.05 M citrate buffer at pH ranging from 2.5-7.5.

2.12.3. Temperature optima of the enzymes

The optimum temperature for enzyme activity was determined by measuring the enzyme activity at various temperatures ranging from 40° to 80°C using citrate buffer (0.05 M, pH 5.5).

2.12.4. **pH** *stability of the enzymes*

For determination of pH stability of the enzymes following buffers were used citrate buffer (0.05 M, pH 3.0-6.0); tris-HC1 buffer (0.2 M pH 7.0-9.0); glycine-NaOH buffer (0.05 M, pH 10.0-11.0). Enzyme preparations were incubated at different pH values for 24 hours. Following incubation the residual enzyme activities were assayed at pH 5.5 and 50°C.

2.13 Application of fungal inoculum in whole green ribbon retting

Three thousand gram (3000 g) whole green ribbon + 300g inoculum + 1500 ml enzyme extract + 9L water were used for submerged retting. The experiment was conducted in earthen vat. Here fungi were used singly and combindly like before as submerged condition retting of shunkies. Stones were used as weighting material and water level was checked time to time.

Chapter 3
RESULTS

RESULTS

3.1 *Isolation and screening of fungi from natural jute environments for jute ribbon retting*

Twenty one fungi were isolated from different sources of jute environment such as SMR jute retting, jute seed germination, jute retting effluent, retted jute plant, dry ribbon, dry SMR etc. and were tested for their jute retting ability. Equal amount of green ribbon (10g) were used to examine the retting capacity of the fungal isolates as described in Table 1 and Fig. 1. Retting capacity was evaluated on the basis of retting time required, fiber softness and fiber color.

Table 1: Green jute retting ability of different fungal isolates.

Serial no	Name of Isolates	Sample	Retting time (days)	Retting capacity(qualitative)
1	Isolate 1	SMR jute retting	9	+++
2	Isolate 2	Jute seed germination	10	+
3	Isolate 3	Jute retting effluent	11	+
4	Isolate 4	Retted jute plant	11	+
5	Isolate 5	Dry ribbon	12	++
6	Isolate 6	Dry SMR	12	++
7	Isolate 7	Green Ribbons	9	+++
8	Isolate 8	Jute caddise	9	+++
9	Isolate 9	Jute field soil	10	+
10	Isolate 10	Retted jute gunny bag	14	-
11	Isolate 11	Rotten jute mop	-	-
12	Isolate 12	Rotten bin	-	-
13	Isolate 13	Wet jute seed	11	++
14	Isolate 14	Dry jute plant	-	-
15	Isolate 15	Decomposed Baggase	16	+
16	Isolate 16	Jute field soil	14	+
17	Isolate 17	Jute retting effluent	12	++
18	Isolate18	Decomposed Banana plant	16	+
19	Isolate19	Jute field soil (Jagir)	-	-
20	Isolate 20	Jute seed germination	-	-
21	Isolate 21	Dry ribbon retting	11	++
22	Control	Jute ribbon (green)	22	-

NB. Good retter = +++, Retter = ++, Poor Retter = +, Non Retter= -

Fig 1: Retting of green jute ribbons in petridish by different fungal isolates.

The isolates showed quite variation in their ability in retting green jute ribbons (Table 2). The retting period varied from 9 to 16 days. Three isolates namely 1, 7, and 8 required 9 days for retting while two isolates namely 2 and 9 required 10 days. However, 4 isolates comprising numbers 3, 4, 13 and 21 showed complete retting of green jute ribbon after 11 days. Isolates having the number 5, 6 and 17 completed the retting of green jute ribbon after 12 days. In contrast, isolates 10 and 16 required 2 days more i.e. 14 days to complete the retting process. Maximum time of 16 days was found to be required by isolates numbers 15 and 18 to achieve the completion of retting process. Out of 21 funguses studied during this isolation experiment, all the fungi did not have retting abilities (Table-1). Only five fungi namely isolate number 11, 12, 14, 19 and 20 showed no retting abilities but maximum fungi showed some sort of retting properties. Finally they were used for green ribbon retting experiment. On the basis of retting ability and fiber color produced in solid state fermentation process (SSF), the most potential fungi of jute retting were selected.

Table 2: Identification of 10 selected fungi with retting time, color and comments.

Isolates	Fungi	Retting time	Color	Comments
1	*Aspergillus niger*	9	Bright Cream	Good retter
2	*Aspergillus flavus*	10	Light Cream	Good retter
3	*Macrophomina sp.*	11	Cream	Good retter
4	*Sclerotium sp.* type 1	11	Cream	Retter
6	*Mucor sp.*	12	Cream	Retter
7	*Sporotrichum sp.*	9	Bright Cream	Good
8	*Trichoderma sp.*	9	Bright Cream	Good retter
9	*Botryodiplodia sp.*	10	Cream	Retter
13	*Fuserium sp.*	11	Cream	Retter
21	*Sclerotium sp.* type 2	11	Cream	Retter

3.2 Characterization of selected fungal isolates

The fungal isolates (isolate-1, isolate 2, isolate 3, isolate 4, isolate 5 isolate 6, isolate 7, isolate 8, isolate 9, isolate 10) grew well on common mycological medium like potato dextrose agar (PDA), wheat bran medium, pectin, xylan and CMC agar (Carboxymethyl cellulose etc) (Fig 2 and 3). The growth of the fungi on PDA medium was rapid and attained a diameter of 2-3 cm in 6 days at 30°C. The descriptions of colonies along with the microscopic observation are depicted in Fig 2. The isolates of present study were identified microscopically by tease mount preparation (Section 2.3.1). Fig: 3. shows the plates of 3-7 days old culture of the fungal isolates on potato dextrose agar (PDA) medium and tease mount preparation of 3-7 days old culture of the isolates showed mature mycelia and conidiospores respectively.

3.2.1 Morphological features of 10 selected fungal isolates

Identification of ten fungi from the 21 fungi showing in Table 1,10 were selected as retter fungi and were examined by microscope with high resolution (100 x) and

identified as Isolate 9, *Aspergillus niger*; Isolate 2, *Aspergillus flavus*; Isolate 3, *Macrophomina sp.*; Isolate 4, *Sclerotium sp.*, type 1; Isolate 6, *Mucor sp.*; Isolate 7, *Sporotrichum sp.;* Isolate 8, *Trichoderma sp.*; Isolate 9, *Botryodiplodia sp.;* Isolate 13, *Fuserium sp.*; and Isolate 21, *Sclerotium sp.* type 2 respectively.

***Sporotrichum sp.*:** Colony white, mycelia hyaline Conidiophores hyaline sometimes simple, irregularly branched, with spore bearing portion near the apex, conidia hyaline 1-celled, ovoid, attached epically and laterally (Fig. 2a).

***Sclerotium sp.* type 1:** Showed white cottony growth of the mycelium at young stage and after 7-9 days numerous small Sclerotia were formed. Sclerotia were small ellipsoidal formed 7 days after growth. Mycelia were white upward growth. Huge amount of Sclerotia were found brown in color at first stage of growth and was white dot like (Fig. 2a.1).

***Aspergillus niger*:** The development of the colony of the fungus on PDA plates was observed for several days. The young vegetative mycelial growth started with whitish color mat which turned to jet black within three days (Fig-2c). On the basis of the structural features the fungus was characterized as *Aspergillus niger*. Conidiophores upright, simple, terminating in a globose or clavate swelling bearing phialides at the apex or radiating from the entire surface, conidia (phialospores) 1-celled, globose, arranged in dry basipetal chains. The young vegetative whitish compact mycelial growth turned to dark along with the age of the culture after four days of growth (Fig. 2a.2).

***Aspergillus flavus*:** The 2 days old young vegetative mycelial growth started with whitish color mat which turned to light green within 3 days. The mycelial growth was mat like compact. Conidiophores upright, simple, terminating in a globose or clavate swelling bearing phialides at the apex or radiating from the entire surface, conidia (phialospores) 1-celled, globose, greenish in mass, arranged in dry basipetal chains. The young vegetative whitish compact mycelial growth turned to greenish after four days of growth (Fig. 2a.3).

***Sclerotium sp.* type 2:** The vegetative growth of the fungus was white cottony and Sclerotium formed after 6-7 days of growth. There was no change of mycelial mass but Sclerotium formed after 7-9 days of growth. At first Sclerotia was white pinhead like but later on it was formed hard and brown color. Sclerotia are hard resisting body, brown and ellipsoidal size. After few days of (7-15 days) germination it formed white color mycelium to brown colored (Fig. 2b).

Botryodiplodia sp. : Mycelial mass leather like, ash color at the young states and it was turned black at mature state, pycnidia black, ostiolate, erumpent, stromatic, confluent conidiophores simple, short, conidia dark and 2-celled (Fig. 2c).

***Macrophomina sp.*:** It is grayish color leather like compact mycelial mass at the young stage, and it turned blackish after maturation. Under microscope black color pycnidiospore were observed. After maturation hyaline ellipsoidal spore were observed where two end was narrower and sharply at the end (Fig. 2d)

***Fuserium sp.*:** Mycelium extensive and cottony and compact in culture with pinkish in the mycelium. Conidiophores and slender are simple, macro conidia borne in chains (Fig. 2e).

***Mucor sp.*:** Mycelia of the fungi were off white fluffy growth at the young stage but it was turned brownish black sporangial mass and intermingled with the sporangia. The vegetative body is a well developed mycelium. The hyphae which constitute the mycelium are long, slender and copiously branched. They are without any cross walls or septa appear at the bases of reproductive organs and in the older hyphae (Fig. 2f).

***Trichoderma sp.*:** Mycelial growth white in early state (2-3 days) with compact mat like and it was turned dark green after 5-7days of growth. Conidiophores hyaline, much branched, not verticillate, phialides single or in groups, conidia (phialospores) hyaline, 1-celled, ovoid, borne in small terminal clusters, usually easily recognized by its rapid growth and green patches or cushions of conidia (Fig. 2g).

Fig 2 a: *Sporotichum sp.*, a1. *Sclerotium sp.* type 1, a2 *Aspergillus niger*, a3 *Aspergillus flavus.*

Fig. 2 b: *Sclerotium sp.* type 2 (3 days growth)

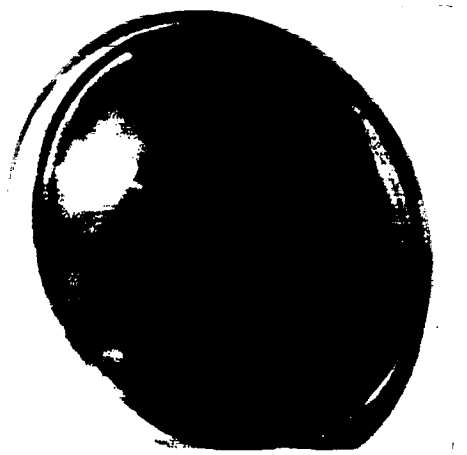


Fig-2 c: *Botryodiplodia sp.* (8 days growth)

Fig. 2 d: *Macrophomina sp.* (8 days of growth)

Fig-2e. *Fuserium sp.*

Fig-2f. *Mucor sp.*

Fig-2g. *Trichoderma sp.*

Fig. 2: Colonial Morphology of 10 selected fungi used for enzymatic and clear zone study.

3.2.1.1. Microscopic observation of ten fungal isolates

1) ***Aspergillus niger***: Link, Conidiophore upright, simple, terminating in a globose at the apex, conidia one celled, and globes, arranged in chains. (Fig 3.d)

2) ***Aspergillus flavus***: Conidiophore upright terminating in a globose conidiospore. Conidia globose. (Fig 3. c)

3) ***Sporotrichum sp.:*** Conidiophores hyaline, irregularly branched, conidia hyaline ovoid. (Fig 3.a)

4) ***Sclerotium sp.*** **type 1**: mycelia septate and flat. Sclerotia hard and brown in color.

5) ***Sclerotium sp.*** **type 2**: Sclerotia small, ellipsoidal light brown in color.

6) ***Mucor sp.***: Mycilial arrangement net like and slender. Sporangiophore tall and globose. (Fig 3.h)

7) ***Fuserium sp.***: Conidia two types' - large crescent and small crescent shaped. (Fig 3.b)

8) ***Botryodiplodia sp.***: Mycelial arrangement net like whitish in color, pycnidia black Conidiophore short, conidia dark thick and two celled.

9) ***Macrophomina sp.***: Black color pycnidia, spore thin walled sharped at two ends. (Fig 3.g)

10) ***Trichoderma sp.***: Conidiophore hyaline and branched, conidia hyaline, one celled borne in small terminal clusters. (Fig 3.i)

Spore of *A. niger*: Spore dark color globose. (Fig 3.e)
Spore of *A. flavus*: Spore contain thick wall and globose. (Fig 3.f)

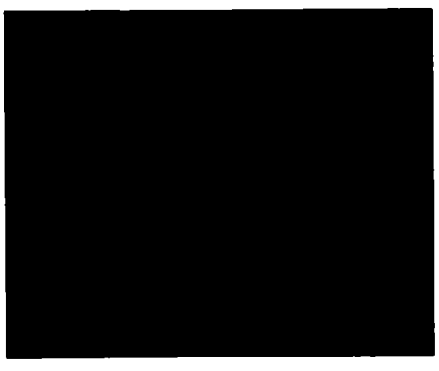


Fig. 3a : *Sporotrichum sp.*
(at 7 days of growth)

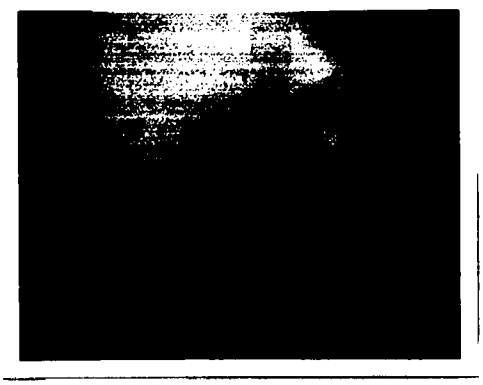


Fig. 3b: *Fuserium sp.*
(at 7 days of growth)

Fig. 3c: *A. flavus* (4 days of growth)

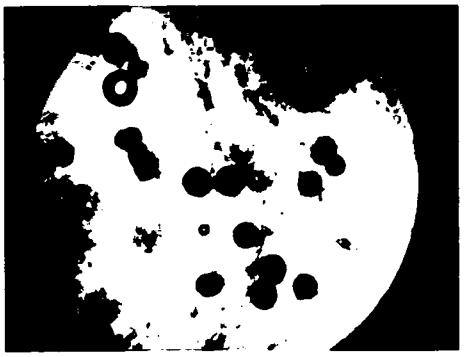


Fig. 3d: *A. niger (at 4 days of growth)*

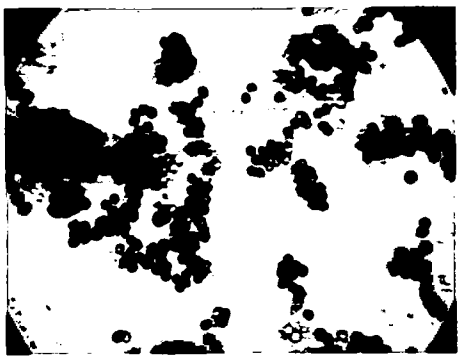


Fig. 3e: *A. niger* (at 9 days of growth)

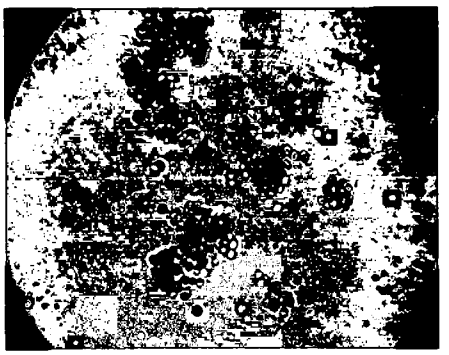


Fig. 3f: *A. flavus* (at 9 days of growth

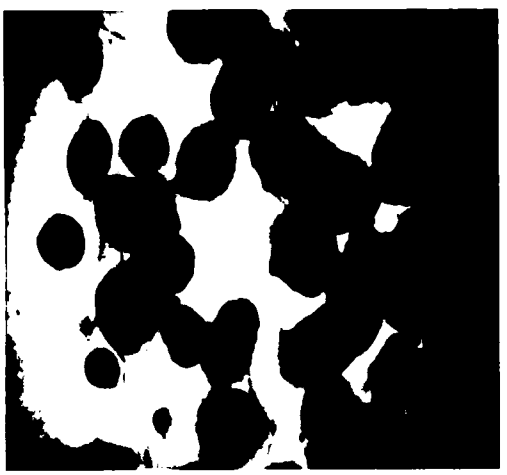


Fig. 3g. *Pycnidia* of *Macrophomina sp.* (at 6 days of growth)

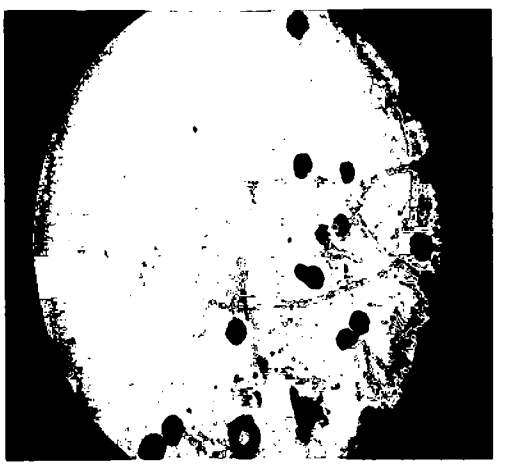


Fig. 3h: *Mucor sp. (*at 3 days of growth)

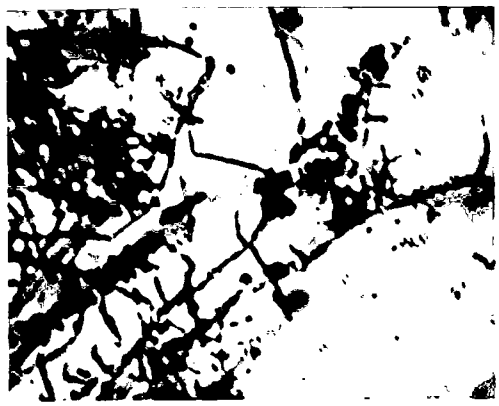


Fig. 3i: *Trichoderma sp.*(at 5 days of growth)

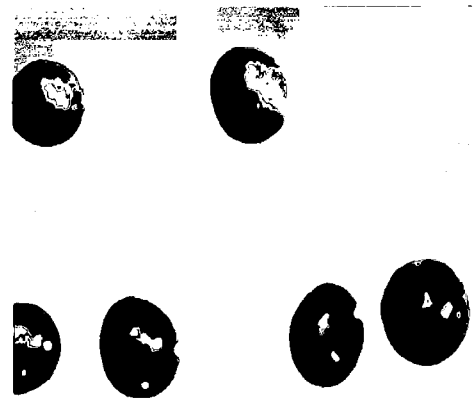


Fig. 3J: *Sclerotia of Sclerotium sp.* type 2 (at 15 days of growth)

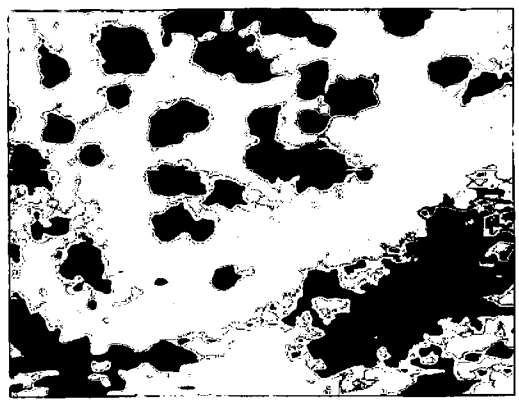


Fig. 3k: Pyicnidia of *Botryodiplodia sp.*

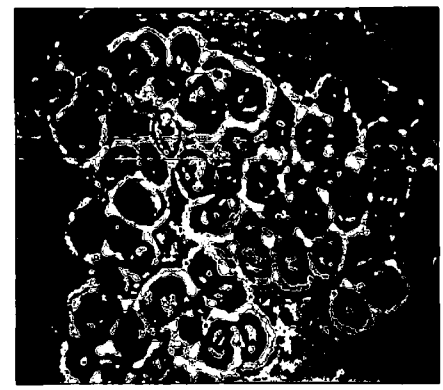


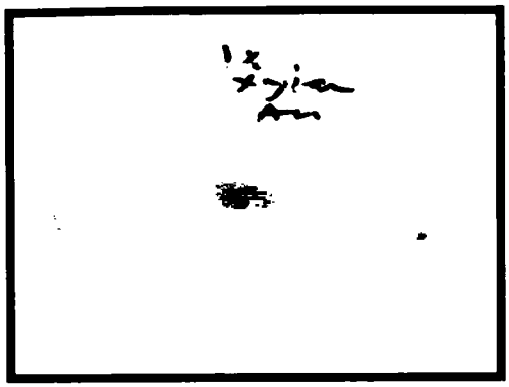
Fig. 3i: Tissue of (teasemount)) *Sclerotia* of *Sclerotium sp* type 1

Fig. 3: Microscopic observation of fungi

3.2.2 Screening of xylanolytic, celluloytic and pectinolyic fungi (Zone clearing method)

The fungal isolates were tested for xylanase, cellulase and Pectinase activities following zone clearing method (PDA) medium, (Fig 4) according to Mohiuddin (1992). Enzyme activities were demonstrated by the hydrolysis of substrate xylan in case of xylanase and CMC in the case of CMCase and pectin in the case of Pectinase.

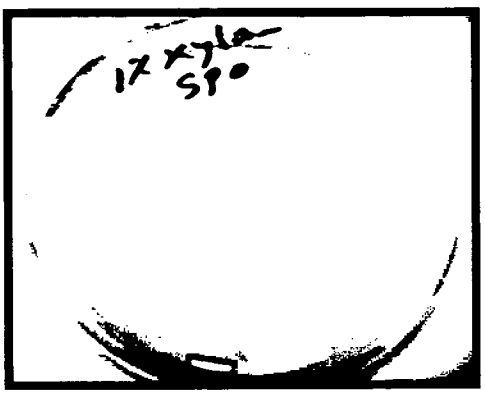
Pectinase incorporated the main carbon source, in a solid agar medium distributed in petridishes. After an appropriate incubation period (4 days) the activities were detected around the colonies by the appearance of zones revealed by substrate clearance.



(a)*Aspergillus niger*

(b)*Sclerotium sp.* type 1

(c) *Aspergillus flavus*

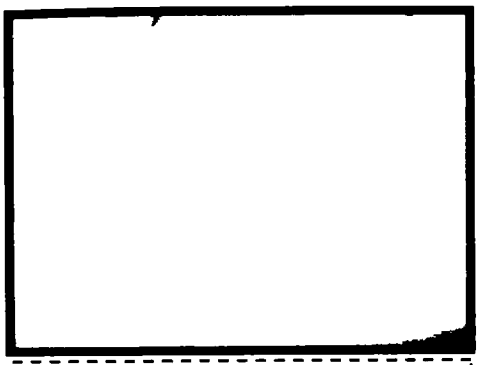


(d) *Sporotrichum sp.*

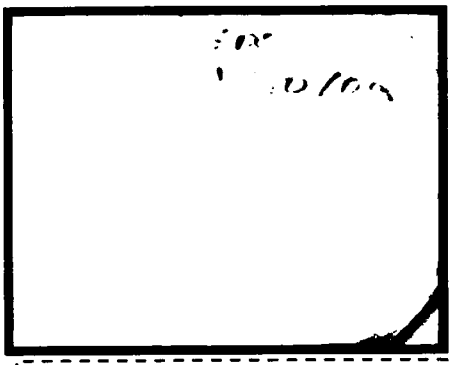
Figure 4 (a-d): Clear zone formation by 4 selected fungi in 1% xylan

(e) *Aspergillus flavus*

(f) *Aspergillus niger*

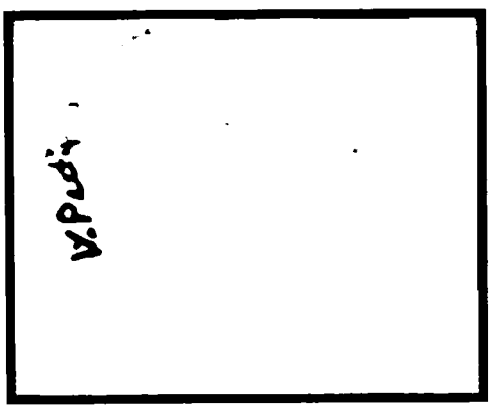


(g) *Sclerotium sp.* type 1



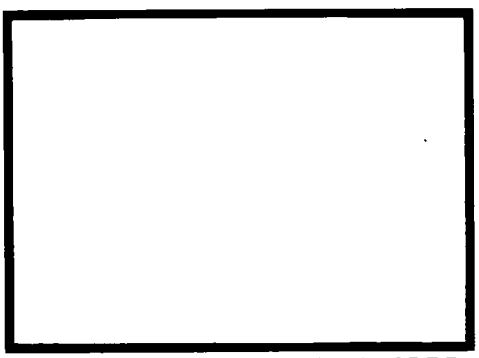
(h) *Sporotrichum sp.*

Figure 4 (e-f): Clear zone formation by 4 selected fungi in 1% CMC

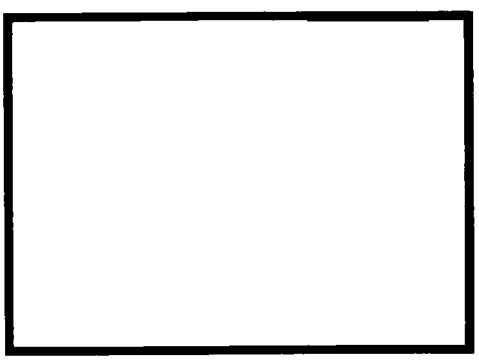


(i) Aspergillus flavus

(j) *Sporotrichum sp.*



(k) *Aspergillus niger*



(l) *Sclerotium sp.* type 1

Figure 4 (i-l): Clear zone formation by 4 selected fungi in 1% pectin

Fig 4: Clearzone foumation by 4 sclected fungal insolate in 1% xylan (Hemicellulose), 1% Carboxymethyl cellulose (CMC) and 1% pection.

3.2.3 Growth and extracellular enzyme production of the selected fungal isolates in wheat bran medium

Initial experiments were carried out in solid state fermentation process using the method described in Section 2.6. The reducing sugar, soluble protein concentration and enzyme activities (xylanase, pectinase and CMCase) were measured following the methods described in Section 2.7.

3.2.3.1 Xylanase activity after 3 and 6 days

Solid state fermentation was carried out with ten selected fungal isolates for 3 and 6 days using wheat bran as carbon and nitrogen source at 30°C following the method described in Section 2.6.1 and the results have shown in Table 3 and Fig 5. In all cases xylanase activities increased with time.

Table 3: Comparative xylanase activity of the fungal isolates after 3 and 6 days of growth

Fungi	Xylanase activity (U/g)	
	After 3 days	After 6 days
Aspergillus niger	244.51	281.61
Aspergillus flavus	396.81	1382.4
Botryodiplodia sp.	72.33	256.03
Sporotrichum sp.	264.29	499.2
Sclerotium sp. type 1	304.65	512.01
Sclerotium sp. type 2	122.25	514.56
Fuserium sp.	398.11	798.72
Macrophomina sp.	33.92	1164.81
Mucor sp.	51.21	380.54
Trichoderma sp.	124.00	161.52

Among the ten jute retting fungi, it was observed that *Fuserium sp.* and *Aspergillus flavus* produced the highest and almost equal xylanase activity amounting 398.11 U/g and 396.81 U/g after 3 days of inoculation respectively. The second highest amount of xylanase activity was produced by *Sclerotium sp.* type 1 followed by *Sporotrichum sp.*

and *A. niger* amounting 304.65 U/g, 264.29 U/g, 244.52 U/g respectively. *Botryodiplodia sp.* (72.33 U/g) and *Mucor sp.* (51.21 U/g) showed almost close activity. *Macrophomina sp.* (33.92 U/g) produced the lowest amount of the enzyme activity.

The amount of enzymatic activity was found nearly doubled after day 6 of inoculation. The highest amount of xylanase activity was found in *Aspergillus flavus* accounting 1382.4 U/g followed by *Macrophomina sp.*(1164.81 U/g.) and *Fuserium sp.* (798.72 U/g.) after 6 days of inoculation *Trichoderma sp.* produced the least quantity of the enzymatic activity after 6 days of inoculation.

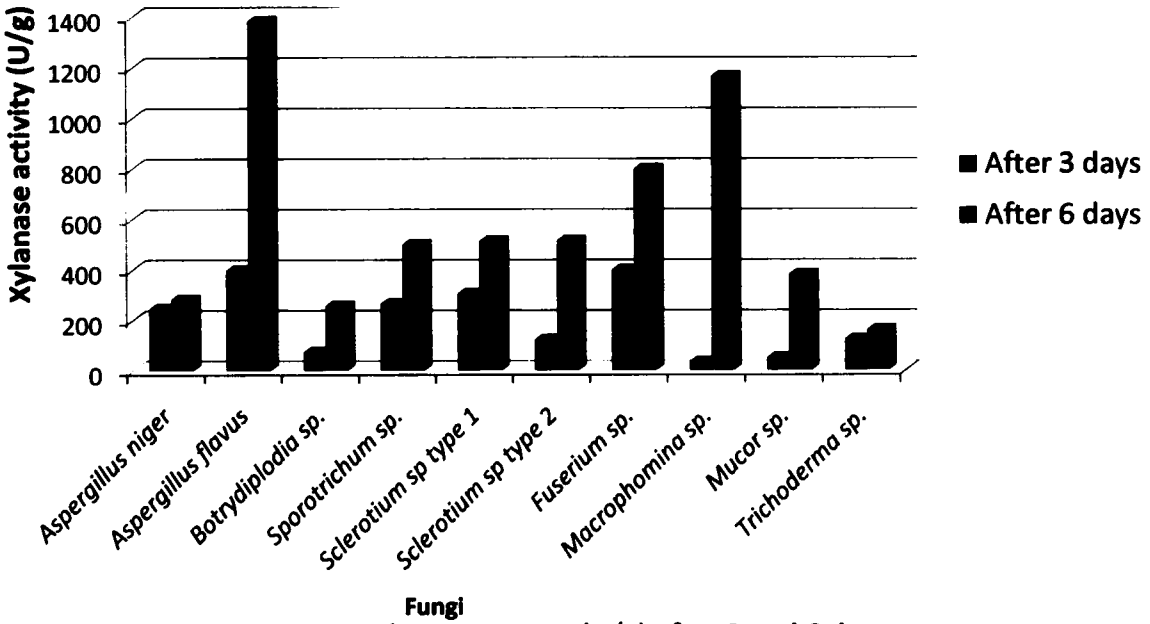


Fig. 5: Comparative xylanase activity (U/g) after 3 and 6 days.

3.2.3.2 CMCase activity after 3 and 6 days of growth

Study of CMCase activity after 3 and 6 days showed an appreciable variation among the 10 strains of fungi (Table 4, Fig. 6). After 3 days the highest CMCase activity (10.736 U/g) was rendered by *A. flavus* and that of the lowest was showed by *Mucor sp.* (0.939 U/g). Fungi like *Sporotrichum sp., Sclerotium sp.* type 1., *Trichoderma sp.* and *Sclerotium sp.* type 2 behaved almost similarly ranging between 4.031 U/g and 4.79 U/g in CMCase activity.

Similarly, *Botryodiplodia sp., Macrophomina sp.* and *Mucor sp.* also showed CMCase activity but less than the previously stated fungi ranging between 0.939 and 0.990 U/g. The CMCase activity after 3 days followed the order: *A. flavus* (10.736 U/g)>

Aspergillus niger (7.472 U/g)> *Trichoderma sp.* (4.79 U/g) > *Sclerotium* type 1 (4.564 U/g)> *Sporotrichum sp.*(4.513 U/g)> *Sclerotium* type 2(4.031 U/g) > *Fuserium sp.* (2.5 U/g)> *Macrophomina sp.*(9.99 U/g)> *Botryodiplodia sp.*(989 U/g)> *Mucor sp.*(9.939 U/g).

CMCase activity of all the fungal isolates increased with time and was about five times higher after 6 days in comparison to 3 days except the *Botryodiplodia sp. Macrophomina sp.* and *Mucor sp.* where a decline in activity to 50% was observed irrespective of the species of fungus (Table 4, Fig 6). The efficiency of the fungi in CMCase activity followed almost the similar trend as observed after 6 days. The order of CMCase activity may be arranged as follows:

Aspergillus flavus (51.53 U/g) > *Aspergillus niger (* 35.867 U/g) > *Trichoderma sp.* (22.94 U/g) > *Sclerotium sp.* type 1 (21.977 U/g) > *Sporotrichum sp.* (21.67 U/g) > *Sclerotium* type 2 (19.35 U/g) > *Fuserium sp.* (12 U/g) > *Macrophomina sp.* (4.75U/g) > *Botrydiplodia sp.* (4.74 U/g) > *Mucor sp.* (4.51 U/g).

Table 4: Comparative CMCase activity of the fungal isolates after 3 and 6 days.

Fungi	CMCase activity (U/g)	
	After 3 days	After 6 days
Aspergillus niger	7.472	35.867
Aspergillus flavus	10.736	51.530
Botroydiplodia sp.	9.890	4.740
Sporotrichum sp.	4.513	21.670
Sclerotium sp. type 1	4.564	21.977
Sclerotium sp. type 2	4.031	19.350
Fuserium sp.	2.500	12.000
Macrophomina sp.	9.990	4.750
Mucor sp.	9.939	4.510
Trichoderma sp.	4.790	22.940

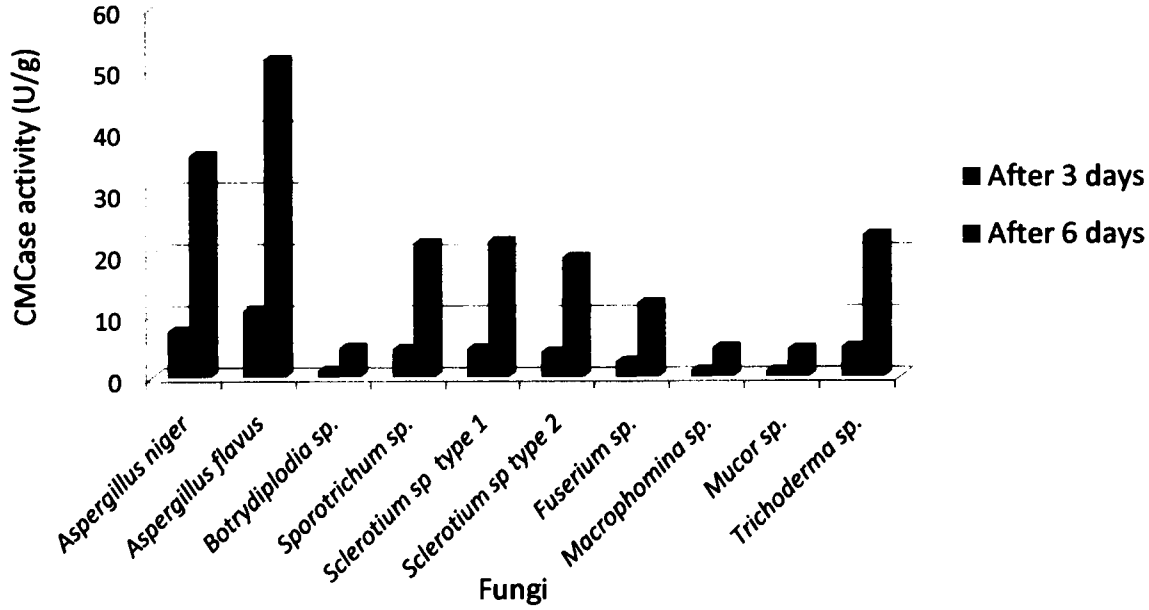


Fig. 6: CMCase activity after 3 and 6 days by 10 fungi.

3.2.3.3 Pectinase activity after 3 and 6 days of growth

Assessment of pectinase activity of the ten isolates of fungi after 3 days showed a variation among them (Table 5, Fig 7). The variation in pectinase activity ranged from 0.72 U/g (*Botryodiplodia sp.*) to 1.90 U/g (*Mucor sp.*). However, the variation in their ability to remove pectin from jute fiber was found to be very close except one species of fungus (*Botryodiplodia sp.*)., *Mucor sp., Sclerotium sp.* type 2 and *A. flavus* showed almost identical pectinase activity. The rest of the fungal species showed slightly lower but almost similar activity ranging from 1.02 to 1.26 U/g. *Aspergillus flavus* ranked to be third in efficiency recordings up to 1.60 U/g.

However, their order of efficiency in pectinase activity was found to be changed after 6 days (Table 5. Fig 7). The activity ranged between 1.00 U/gm *(Botryodiplodia sp.*) and 2.90 U/gm (*Sclerotium sp.* type 1). Fungal isolates namely *Trichoderma sp.* type 2, *Fuserium sp., Botryodiplodia sp., Macrophomina sp.* and *Mucor sp.* showed relatively less activity as compared to the rest of the fungal isolates. Isolates namely. *A. niger, A. flavus, Sporotrichum sp.* and ten *Sclerotium sp.* type 1 ranked to be more efficient in pectinase activity than the rest ones.

Table 5: Pectinase activity of the fungal isolates after 3 and 6 days of growth.

Fungi	Pectinase activity (U/g)	
	3 days	6 days
Aspergillus niger	1.11	2.18
Aspergillus flavus	1.60	2.70
Sporotrichum sp.	1.12	2.80
Sclerotium sp. type I	1.02	2.90
Fuserium sp.	1.10	1.18
Mucor sp.	1.90	1.70
Macrophomina sp.	1.19	1.15
Trichoderma sp.	1.26	1.32
Botryodiplodia sp.	0.72	1.00
Sclerotium sp. type 2	1.89	1.99
Control	0.0	0.0

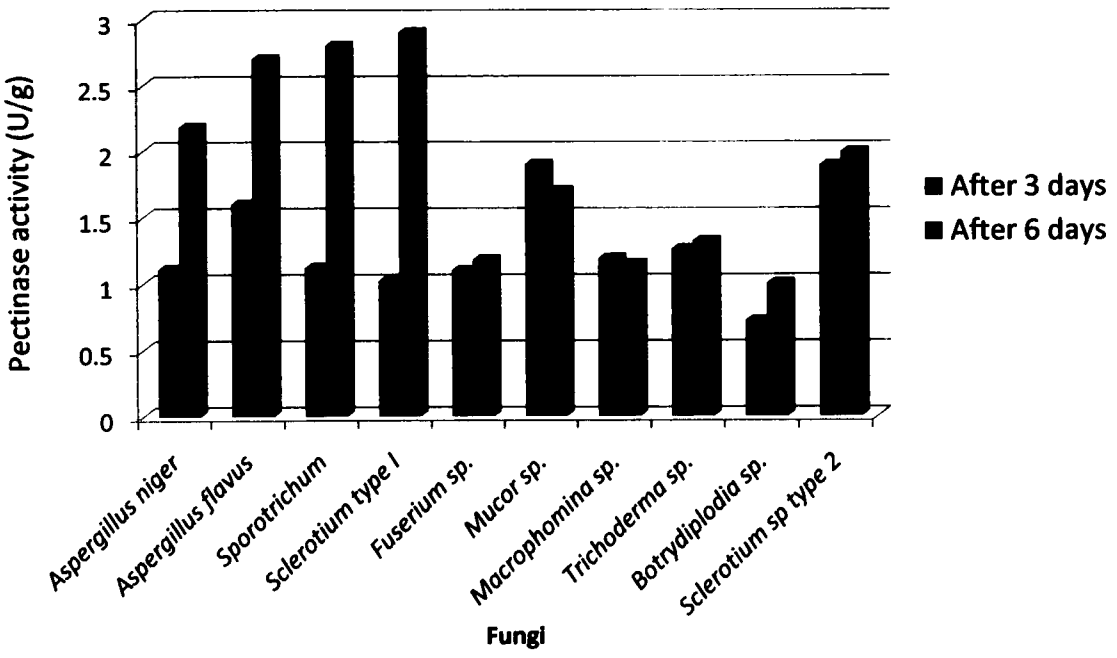


Fig. 7: Pectinase activity of the fungal isolates after 3 and 6 days.

3.3 Selection of the most potential fungal isolates

Among these ten isolates, *A. flavus, A. niger* , *Sporotrichum sp.* and *Sclerotium sp.* type 1 have good retting capability which was shown by determining the fiber quality (touch and feel method) retting period and enzymatic activity (Fig. 8).

From Tables 2, 3, 4 and 5 it is evident that both in terms of retting capacity and enzymatic activity, *A. niger, A. flavus, Sporotrichum sp.* and *Sclerotium sp. type* 1 are the most potential fungi among the ten isolates.

Fig 8: Four potential jute retter, starting from left *Aspergillus niger, Sclerotium sp. Type*1, *Aspergillus flavus, Sporotrichum sp.* in PDA medium.

3.3.1 Characterization of 4 most potential fungal isolates

Aspergillus flavus: The young colonies were white in color and after 3 days it was turned into light green color and shown light green color in the medium (Fig. 9a).

***Aspergillus niger*:** The colonies were at first white and finally turned into black and shown light black color in the medium (Fig. 9b).

***Sporotrichum sp.*:** At first the mycelia were shown white color mat like but no color in the medium was observed (Fig. 9c).

***Sclerotium sp.* type1:** The colonies were at first white and later on produced cottony mycelium and vigorous growth observed (Fig. 9d).

3.3.2 Microscopic observation

The 4 isolates of the present study were thus identified as *Aspergillus niger*, *Aspergillus flavus*, *Sclerotium sp.* type 1 and *Sporotrichum sp.* following standard microscopic method.

Fig. 9a. *A. flavus*

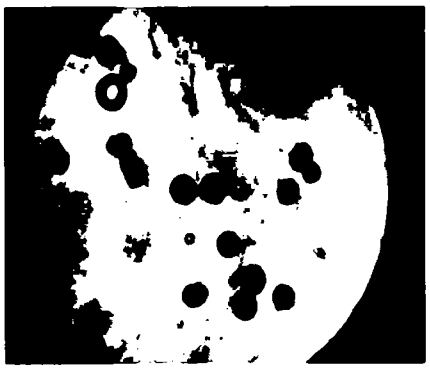


Fig. 9b: *A. niger*

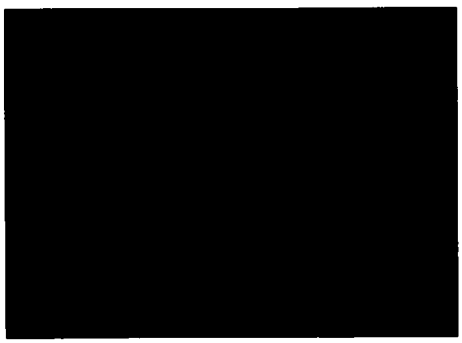


Fig. 9c: *Sporotrichum sp.*

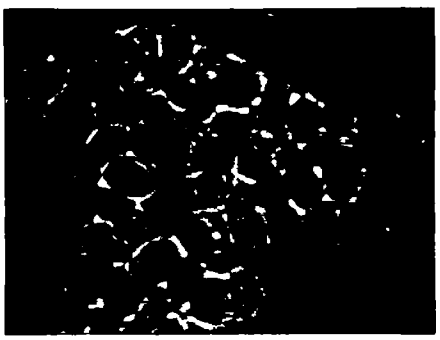


Fig. 9d: Tissue of (teasemount)) *Sclerotia* of *Sclerotium sp.* type 1

Fig. 9. Microscopic observation of selected 4 isolates by tease mount preparation.

3.4 Inoculum production

Optimization of moisture levels of the inoculum

Moisture is one of the most important factors for the growth of microorganisms and production of hydrolase enzymes required for jute retting. The success of SSF is in particular highly dependent on the optimum level of moisture.

In this study inoculum was produced on wheat bran medium at different moisture levels such as 30%, 40%, 50%, 60%, and 70% by 4 fungal isolates in SSF for green jute ribbon retting.

Solid state fermentation was carried out with four fungal cultures (*A. flavus, A. niger, Sporotrichum sp. and Sclerotium sp.* type 1) using wheat bran as sole carbon and nitrogen source following the method described in Section 2.4.1.

3.4.1 Xylanase activity at different moisture levels after 3 days of growth

Xylanase activity of all the isolates increases with increased moisture level up to certain level and then it decreases (Table 6). *A. flavus, A. niger*, *Sporotrichum* and *Sclerotium sp.* type1 have shown their highest activity at 50% moisture level attaining 199.571, 198.576 U/ml , 193.103 and 304.65 U/ml respectively (Table 6, Figure 10).

Among the four isolates highest xylanase activity was rendered by *Sclerotium sp. type* 1 accounting 304.65 U/ml at 50% moisture level. *A. flavus, A. niger* and *Sporotrichum sp.* were found to be almost equal in efficiency to xylanase activity. The order of their activity at 50% moisture levels may be arranged as follows: *Sclerotium sp.* type 1(304.65 U/ml) > *A. flavus* (199.571 U/ml)> *A. niger* (198.576 U/ml) > *Sporotrichum sp.* (193.103 U/ml).

Table 6: Xylanase activity at different moisture levels by 4 fungi after 3 days of growth

Moisture level (%)	Xylanase activity (U/ml)			
	A. flavus	***A. niger***	***Sporotrichum sp.***	***Sclerotium sp.* type 1**
30	125.932	118.468	90.179	111.00
40	143.346	148.817	118.468	122.25
50	199.571	198.576	193.103	304.65
60	176.185	147.324	89.609	180.00
70	85.131	125.981	65.228	59.00

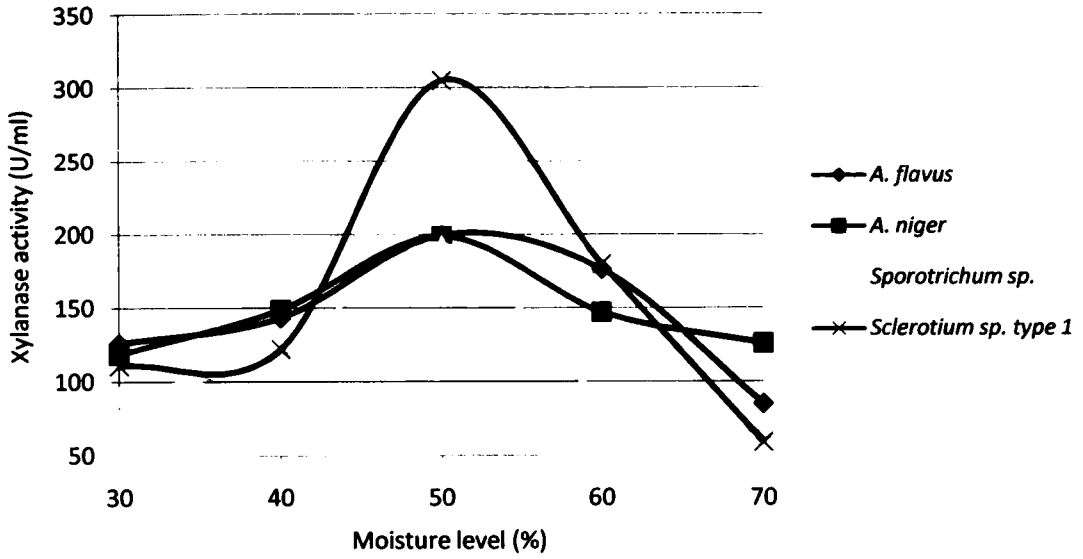


Fig. 10: Xylanase activity at different moisture level (%) after 3 days.

3.4.2 CMCase activity at different moisture level after 3 days of growth

CMCase activity of the isolates increased with the increase of moisture up to certain level and then it decreased (Table 7, Fig 11). *A. flavus, A. niger, Sporotrichum sp. Sclerotium sp.* type 1 showed their highest activity at 50% moisture level recording 4.611, 14.967 and 6.337 and 5.330 U/ml. The activity of the fungi decreased at 60 and 70% moisture level. Maximum activity was shown by *A. niger* (14.967U/ml) and that of minimum by *A. flavus* (4.611) at 50% moisture level.

Table 7: CMCase activity at different moisture level by 4 fungi after 3 days of growth

Moisture level (%)	CMCase activity (U/ml)			
	A. flavus	***A. niger***	***Sporotrichum sp.***	***Sclerotium sp.* type 1**
30	3.461	10.939	5.329	4.031
40	3.543	13.569	6.172	4.564
50	4.611	14.967	6.337	5.330
60	4.118	12.666	4.529	3.000
70	3.789	9.378	4.307	2.000

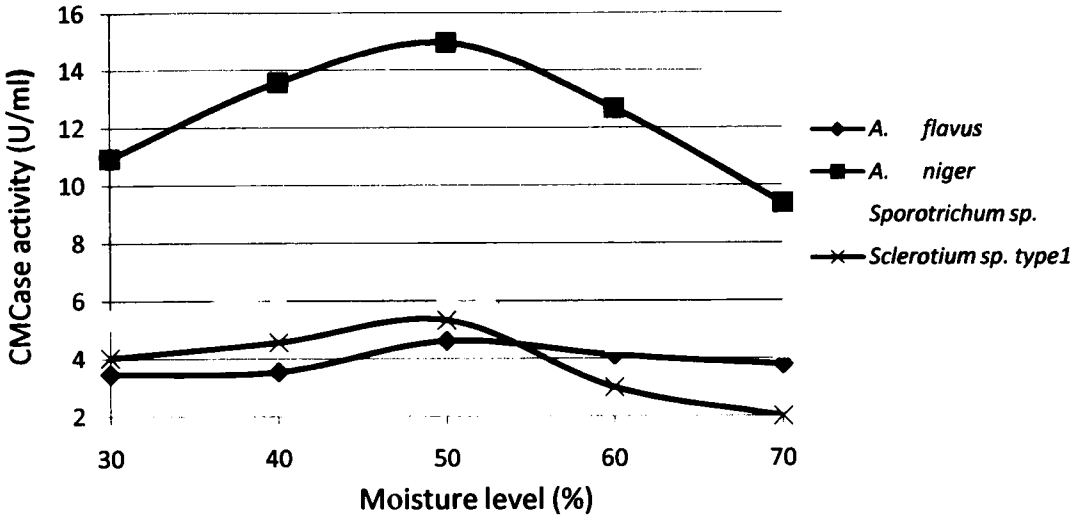


Fig. 11: CMCase activity at different moisture level after 3 days.

3.4.3 Pectinase activity at different moisture levels after 3 days of growth

Pectinase activity in all the isolates increased with increased moisture level up to 50% but a downward result was obtained when moisture level was found to increase by 70%. Among the four fungi the maximum pectinase activity was obtained in *Sclerotium sp.* type 1 in comparison to *A. niger, A. flavus and Sporotrichum sp.* (Table 8, Fig 12). The order of their activity at 50% moisture levels may be arranged as follows: *Sclerotium sp.* type 1(1.23 U/ml)> *A. niger (1.29 U/ml)> A. flavus (1.19 U/ml)> Sporotrichum sp. (1.01 U/ml).*

Table 8: Pectinase activity at different moisture levels by 4 fungi after 3 days of growth

Moisture level (%)	Pectinase activity (U/ml)			
	A. niger	***A. flavus***	***Sporotrichum sp.***	***Sclerotium sp.* type 1**
30	0.90	0.69	0.72	0.89
40	1.17	1.10	1.30	1.01
50	1.29	1.19	1.10	1.23
60	1.17	1.02	1.01	1.05
70	0.84	0.87	0.90	0.98

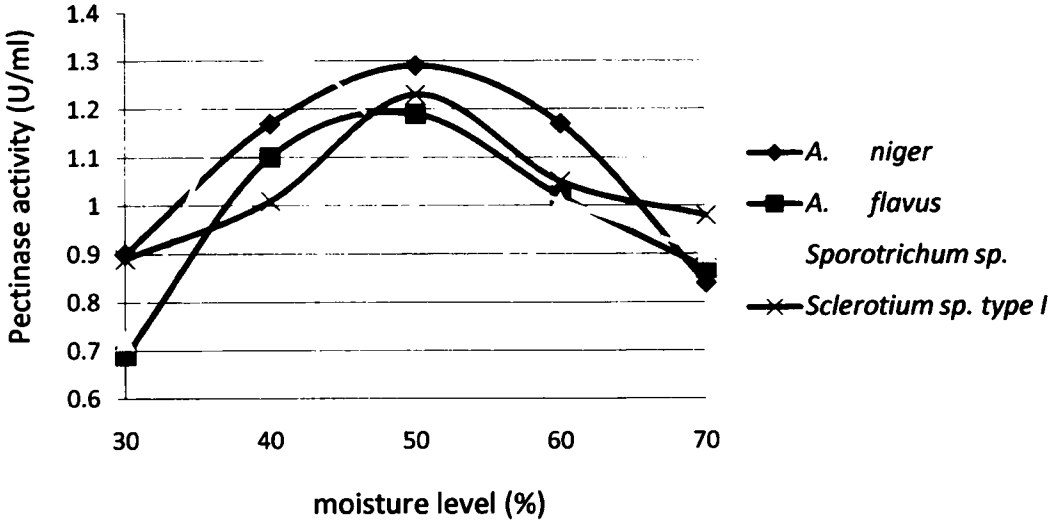


Fig. 12: Pectinase activity at different moisture level .

3.4.4 Reducing sugar content at different moisture levels after 3 days of growth

The efficiency of the fungal isolates in reducing sugar varied quite appreciably with percentage of moisture content (Table 9, Fig 13). *A. flavus* showed a decrease in reducing sugar content from 5.690 to 3.915mg/ ml due to increase in moisture level from 30 to 40% and then increased to 5.246 mg/ml at 50% moisture level. This was followed by a decrease in the content of the reducing sugar with the increasing moisture level up to 70%.

In contrast, *A. niger* revealed a sharp decline in reducing sugar from 14.123 to 7.022 mg/ml due to increase in moisture level from 30 to 40% (Table 9, Fig 13). Thereafter, the decrease in sugar content was gradual with increasing level of moisture up to 70%.

However, *Sporotrichum sp.* showed a rapid increase in reducing sugar content from 15.343 to 26.993 mg/ml indicating a favorable impact of increasing level of moisture from 30 to 40% (Table 9). The further increase in moisture level caused a rapid decrease in reducing sugar form 26.933to 4.359 mg/ml.

However, *Sclerotium sp. type1* revealed an extending trend i.e. it caused an increase in reducing sugar content from 5.02 to 11.23 mg/ml due to raise in moisture level from 30

to 50 %.(Table 9). This was followed by a sharp decline in the content of the same from 11.23 to 2.81 mg/ml due to increase in moisture level form 50 to 70%.

Table 9. Reducing sugar content at different moisture level by 4 fungi after 3 days of growth.

Moisture level (%)	Reducing sugar (mg/ml)			
	A. flavus	*A. niger*	*Sporotrichum sp.*	*Sclerotium sp.* type 1
30	5.690	14.123	15.343	5.000
40	3.915	7.022	26.993	8.100
50	5.246	5.246	14.567	11.230
60	4.359	4.359	7.466	6.710
70	3.915	3.915	4.359	2.810

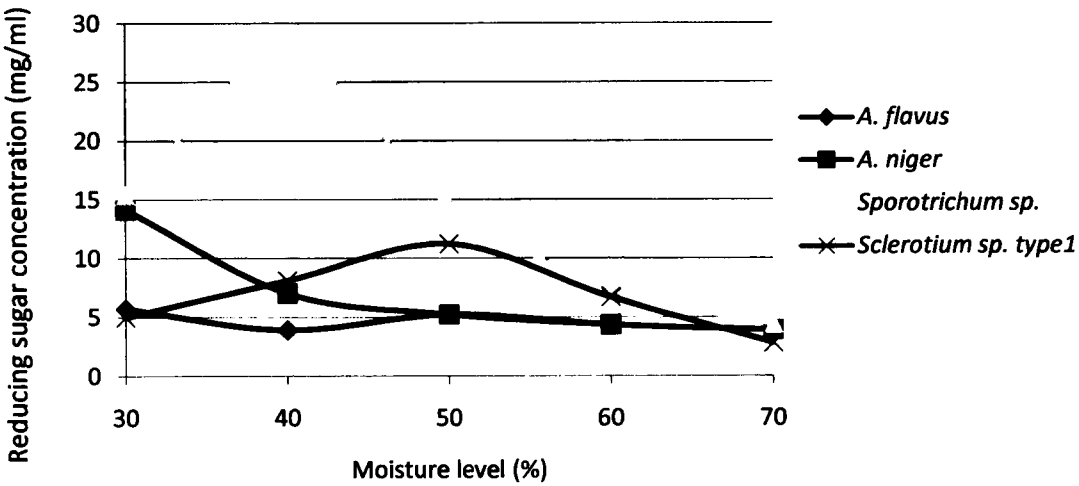


Fig. 13: Reducing sugar content at different moisture levels after 3 days.

3.4.5 Soluble protein content at different moisture level after 3 days of growth

The efficiency of the isolates to soluble protein have been observed at different moisture levels and the result thus obtained showed an increase in soluble protein content in case of *A. flavus* , *Sporotrichum sp.* and *Sclerotium sp. type*1 up to 50% moisture level and upto 40% moisture level in the case of *A. niger* (Table 10, Fig 14).This was followed by a slow decrease in the content of the same in case of all the isolates used with increasing moisture level except *A. niger* which virtually showed an increase in soluble protein content at the highest moisture level i.e., at 70% moisture level.

Table 10. Soluble protein content at different moisture level by 4 fungi after 3 days of growth

Moisture level (%)	Soluble protein (mg/ml)			
	A. flavus	*A. niger*	*Sporotrichum sp.*	*Sclerotium sp.* type 1
30	0.113	0.126	0.0030	0.020
40	0.139	0.139	0.0047	0.074
50	0.180	0.126	0.0587	0.075
60	0.113	0.0452	0.032	0.070
70	0.110	0.0722	0.031	0.020

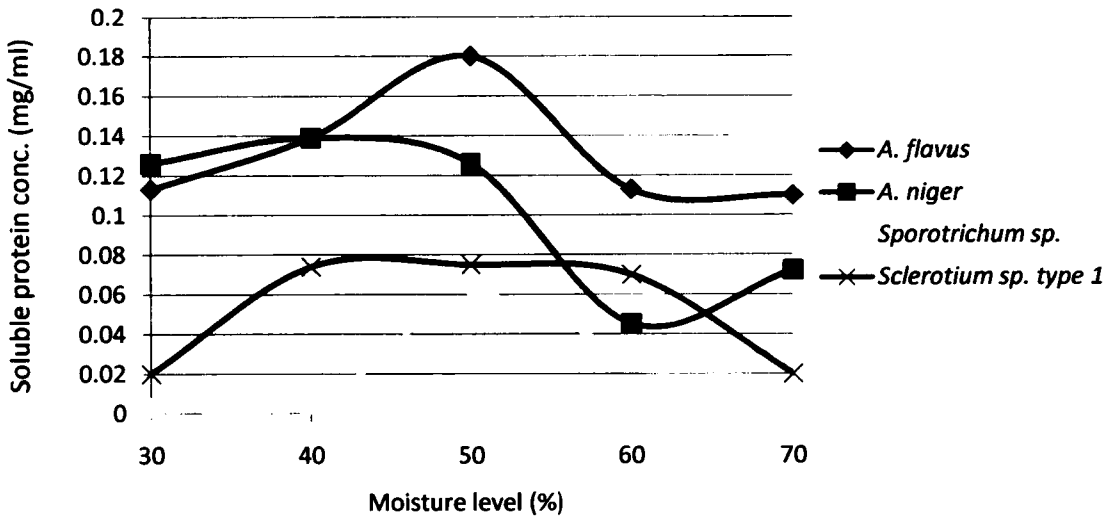


Fig. 14: Soluble protein concentration at different moisture levels after 3 days.

3.5 Effect of normal flora on retting of green jute ribbon in presence of fungal inoculum

The 4 potential isolates were grown in wheat bran medium in single and mixed culture. Then they were inoculated to sterilized and non-sterilized green ribbons to check their retting period and ability to produce improved quality fiber.

Retting period and quality of fiber have been studied under non-sterilized and sterilized conditions following fungal treatments of green ribbon of jute and the results were assessed (Table 11). Fungal treatments enhanced the retting period of jute in both the conditions over the control.

The results showed that retting in non-sterilized condition varied between 8 to 10 days due to fungal treatments. However, in control the retting process required 20 days. *A. flavus* alone, combination of *Sporotrichum sp.* and *Sclerotium sp.* type 1 and three fungal combination of *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 completed retting within 8 days while in all other cases it took 10 days.

In contrast, under sterilized condition, the retting period ranged from 9 to 12 days and was 1 to 2 days more than that of non-sterilized condition. Combination of three fungi required shortest period that is 9 days among the fungal treatments used. The variation among the rest of the fungal treatments was found to be 1-2 days only. However, in control under sterilized condition, retting completed at 22 days. Weight of fiber ranged from 10.6 to 6.0 and 10.7 to 5.2 g in non-sterilized and sterilized conditions respectively (Table 11). Fungi inoculated individually resulted comparatively lower yields of fiber than those of their combinations varying from 6.0 to 7.3 and 7.8 to 10.6g under non-sterilized condition and 5.2 to 7.9 and 8.0 to 10.7g under sterilized condition respectively. It could be noted that yield of fiber was generally lower in sterilized condition as compared to non-sterilized condition due to treatments of individual fungi. However, an opposite trend was observed in case of fungi inoculated in combinations i.e. fiber yields were found to be higher in sterilized condition than those of non-sterilized conditions. The yield of fiber due to various fungal treatments and conditions of retting may be ordered as follows:

Table 11. Retting of non-sterilized and sterilized green ribbons by the potential fungal inocula.

Inoculum	Condition of retting	Retting period (Days)	Weight of fiber (gm)	Fiber color	Bundle strength (lbs/mg)	Fiber grade
A. niger	Non-Sterilized	10	7.3	Cream	9.62	B
	Sterilized	10	6.8	Off-white	8.85	C
A. flavus	Non-Sterilized	8	6.1	Bright Cream	7.32	C
	Sterilized	10	7.9	Cream	8.63	C
Sporotrichum sp.	Non-Sterilized	10	6.8	Cream	9.14	B
	Sterilized	12	6.2	Light Cream	9.03	B
Sclerotium sp. type 1	Non-Sterilized	9	6.0	Off white	9.03	B
	Sterilized	11	5.2	Bright Cream	9.14	B
Sporotrichum sp.+ *Sclerotium sp.* type 1	Non-Sterilized	8	7.8	Bright Cream	9.66	B
	Sterilized	11	8.0	Cream	8.65	C
A. flavus +*Sporotrichum sp.* +*Sclerotium sp.* type 1	Non-Sterilized	8	8.0	Bright Cream	6.74	C
	Sterilized	9	8.7	Bright Cream	8.65	C
A. niger+ *A. flavus*+ *Sporotrichum sp.*+ *Sclerotium sp.* type 1	Non-Sterilized	10	10.6	Cream	10.49	A
	Sterilized	10	10.7	Cream	8.87	C
Control (without inoculum)	Non Sterilized	20	20	Brown	6.80	D
	Sterilized	22	18.1	Gray	6.86	D

Non-sterilized condition: *Sclerotium sp.* type 1 *(6.0)* < *A. flavus (6.1)* < *Sporotrichum sp. (6.8)* < *A. niger (7.3)* < *Sporotrichum sp.* + *Sclerotium sp.* type 1*(7.8)* < *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 *(8.0)* < *A. flavus* + *A. niger* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 *(10.6)* < *control (20.0).*
Sterilized condition: *Sclerotium sp.* type 1 *(5.2)* < *Sporotrichum sp. (6.2)* < *A. niger (6.8)* < *A. flavus (7.9)* < *Sporotrichum sp.* + *Sclerotium sp.* type 1 *(8.0)* < *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 *(8.7)* < *A. niger* + *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 *(10.7)* < control *(18.1).*

The efficiency of *Sclerotium sp.* type 1 alone was evidenced to the best (6.0, 5.2) among the fungal treatments used. While that of *A. niger* + *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 showed almost equal efficacy (10.6, 10.7) in both the non-sterilized and sterilized conditions of retting so far yield of jute fiber is concerned.

Green ribbons treated with *A. niger, A. flavus* and *Sporotrichum sp.* alone produced fiber with cream to bright cream color under non-sterilized condition and off white to light cream color under sterilized retting condition (Table 11). In contrast, *Sclerotium sp.* type 1 caused an opposite trend in color of fiber producing off white in non-sterilized condition and bright cream color of fiber under sterilized conditions of retting. However, combination of fungi produced cream to bright cream color of fiber under either conditions of retting of green ribbons of jute. Contrary to this, green jute ribbons retted without any fungi produced brown and gray color of fiber under non-sterilized and sterilized conditions respectively.

Bundle strength of fiber varied from 6.74 to 10.47 and 8.41 to 9.14 lbs/mg to inoculation of fungi alone and in combination and was generally higher than the control (6.80 and 6.86 lbs/mg) under non-sterilized and sterilized conditions respectively (Table 11). However, the variation observed in bundle strength of fiber among the fungal treatments was not very much appreciable in most of the treatments irrespective of the conditions of retting. It could be noted that strength of fiber recorded under sterilized condition was found to be relatively slightly lower than that of non-sterilized condition

Assessment of fiber grade showed that most of the treatments produced B grade fiber under non-sterilized condition (Table 11). Inoculation of green jute ribbon with *A. flavus* alone and a combination of *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 produced C grade fiber while only the combination of all the fungi used in the experiment produced 'A' grade fiber. Under sterilized condition of retting, jute fiber obtained was found to C grade in most of the fungal treatments. Only *Sporotrichum sp.* and *Sclerotium sp.* produced B grade fiber. Retting of green ribbon of jute under controlled condition produced D grade fiber in both non-sterilized and sterilized conditions.

3.6 Mechanism of jute retting

As retting is a biochemical process by which is the pectic matter bind the fiber to the remained, of the jute stem are broken down and the fibers are liberated. When jute plants are immersed under water, the stem becomes softened and the soluble constituents which include carbohydrates, glucosides and nitrogenous compounds are secreted. These provide food for the growth of the microorganisms naturally present on the jute plants and water and a rapid development of mixed flora is then accompanied by characteristic chemical changes in the liquid as well as in the stem. The retting is effected by different enzymes secreted by microorganisms which break down the pectic complex of the middle lamella of the jute plants, the fiber bundles being first separated from each other. After submergence of green jute under water, the retting or loosening of the fibers is brought about by microbial fermentation. The fibers are located in the phloem tissues of the plants which are surrounded by cortex, epidermis, phloem ray, cambium xylem and pitch tissues. The cortical tissues contain a lot of mucilaginous

substances and the epidermis contains cutins and suberins. After steeping the green jute under water, the cutins and suberins of the plants get dissolved in water and the mucilaginous and pectic substances of the middle lamellae of the cells of the cortex, epidermis. Phloem ray and cambium are decomposed by microbes as a result of the action water and enzymes, the fiber bundles of the fiber strands of the plants are separated from the hand woody core and freed from the structural elements. The microbes enter the plants through the stomata and the cell ends of the ribbon. They first invade the top and then the middle and finally the basal portion. The cambium is first attacked then the ray cells and phloem and finally the cortex when the fibers become ready for separation, pectins, hemicelluloses, reducing sugar, tannins and proteins are the major substances removed during the retting. In the submerged condition retting proceeds earlier because of the age of both inoculum and enzyme (50ml crude enzyme were used) (Ali, Sayem and Eshaque, 1970).

3.6.1 Xylanase activity

Xylanase activity during retting of jute in solid state condition measured after 3,7 and 9 days of inoculation generally decreased very sharply with time in all combination of the fungi used (Table 12, Figs. 15 and 16). Xylanase activity was comparatively better where fungi were applied singly in comparison to their mixture combination. The xylanase activity was the best in the presence of *Sclerotium sp.* type 1 up to 7 days of inoculation among the fungi under study. Generally, the xylanase activity was at its maximum after 3 days of inoculation irrespective of the fungal combination. Xylanase activity was relatively lower where the mixture of fungi was applied at all stages of inoculation. Among the mixture, the combination of *Sporotrichum sp.* and *Sclerotium sp.* type 1 was the best in xylanase activity.

Xylanase activity under submerged condition inoculated for 3, 7 and 9 days also followed the same trend as in case of solid state condition (Table12, Fig.15). Here also the activity of xylanase enzyme decreased with time attenuing the maximum after 3 days of inoculation. The activity of xylanase was comparatively higher where individual fungus was applied than there mixture combination. The efficiency of the enzyme, xylanase decreased recordedly in the presence of one another. Comparatively xylanase

activity was in generally better in the presence of fungi than the control. The best performance (59.34 U/ml) was recorded where *Sclerotium sp.* type 1 was applied and was followed by *Sporotrichum sp.* (52.007 U/ml). The combination of all the fungi played the best role among all other mixture combination.

Table 12: Xylanase activity of the fungi in solid state and submerged conditions after 3, 7 and 9 days.

Fungi	Condition of Retting	3 days (U/ml)	7 days (U/ml)	9 days (U/ml)
A. niger	Solid	35.710	12.690	4.180
	Submerge	21.95	11.400	4.430
A. flavus	Solid	14.060	10.460	5.130
	Submerge	32.720	11.080	3.810
Sporotrichum sp.	Solid	19.160	9.830	3.650
	Submerge	52.000	9.340	4.600
Sclerotium sp. type 1	Solid	48.773	8.7190	6.619
	Submerge	59.347	13.322	4.306
Sporotrichum sp. + *Sclerotium sp.* type 1	Solid	48.770	8.710	6.610
	Submerge	14.440	12.320	3.560
A. flavus + *Sporotrichum sp.* + *Sclerotium sp.* type 1	Solid	16.050	11.820	4.130
	Submerge	20.660	9.210	4.140
A. flavus + *A. niger* + *Sporotrichum sp.* + *Sclerotium sp.* type 1	Solid	18.670	10.580	3.680
	Submerge	32.600	15.310	3.360
Control	Solid	15.800	10.460	3.680
	Submerged	15.21	14.69	5.20

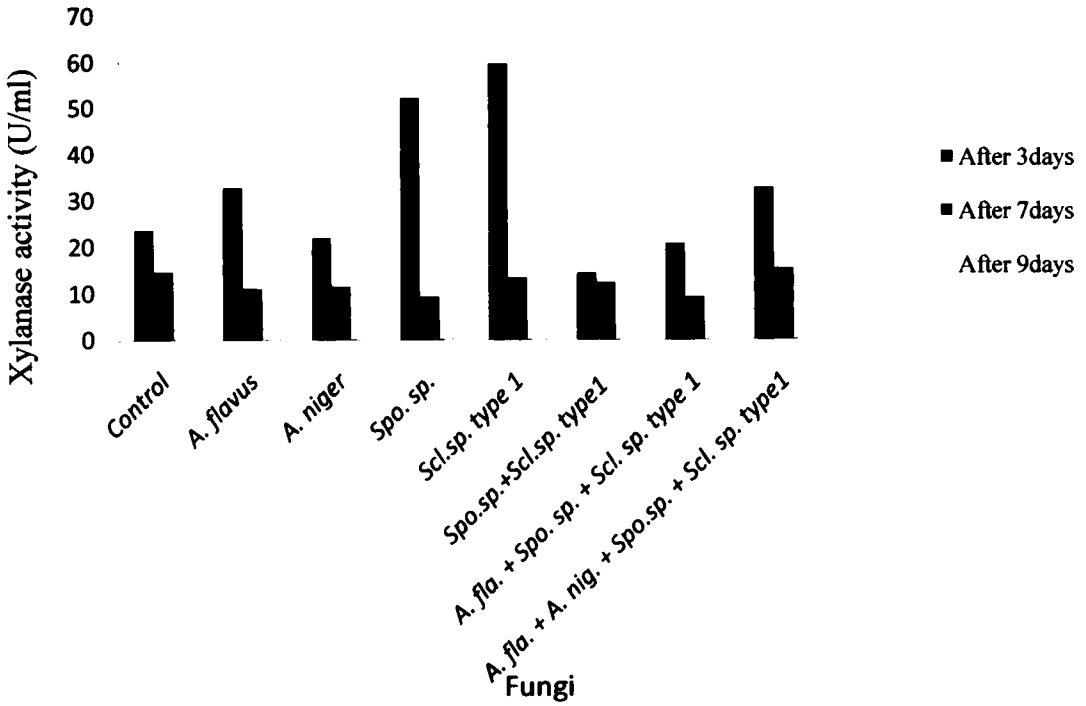


Fig.15: Xylanase activity in solid state condition after 3, 7 and 9days.

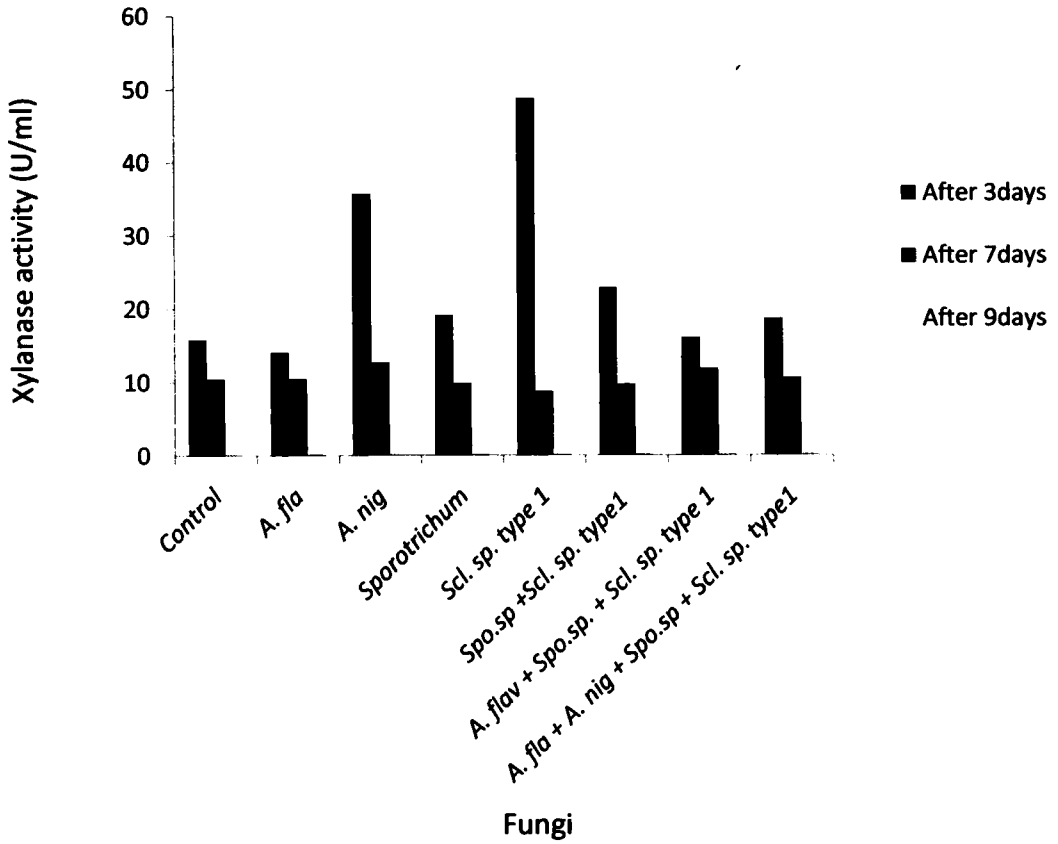


Figure 16: Xylanase activity after 3, 7 and 9days submerged condition.

3.6.2. CMCase activity

CMCase activity measured by the described method showed a decrease with time that is activity after 9days is lower than 7days activity and 7days activity is lower than 3 days activity. It can be seen in both direct inoculation into the green ribbons and submerged condition (Table13, Figs. 17 and 18).

CMCase activity in both the conditions was generally higher where fungus was added singly in comparison to that of mixture combination after 3, 7 and 9 days of inoculation. Activity of CMCase was maximum after 3 days and then decreased very sharply irrespective of conditions of inoculations i.e. green ribbon in solid state and submerged conditions.

Highest activity (2.030 U/ ml) was shown by *A. niger* and this was followed by *Sclerotium sp.,* type 1 (0.4484 U/ml), *Sporotrichum sp.* (0.431 U /ml) and *A. flavus* (0.387 U/ml) after 3 days of inoculation of green ribbon of jute among the singly applied fungus treatment. Similarly, under submerged condition, the maximum activity was recorded for *Sclerotium sp.* type I (0.572 U/ ml) and this was followed by *A. niger* (0.51 U/ml), *A. flavus* (0.428 U/ ml) and *Sporotrichum sp.* (0.366 U/ml).

However, combination of all the fungi together showed the best performance of CMCase (3.859 U/ml) under solid state condition and a combination of *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 resulted maximum CMCase activity (2.6263 U/ml) under submerged condition after 3 days of inoculation. A combination of all the fungi together ranked to the second position (2.6263 U/ml) so for CMCase activity was concerned irrespective conditions after 3 days of retting.

Table 13. CMCase activity of the fungi in both solid state and submerged conditions after 3, 7 and 9 days of retting.

Fungi	Condition	After 3days (U/ml)	After 7days (U/ml)	After 9days (U/ml)
A. niger	solid state	2.030	0.150	0.0201
	submerged	0.51	0.1767	0.0203
A. flavus	solid state	0.387	0.0884	0.030
	submerged	0.428	0.0863	0.0279
Sporotrichum sp.	solid state	0.431	0.2314	0.0284
	submerged	0.366	0.0251	0.0185
Sclerotium sp. type 1	solid state	0.448	0.2404	0.4003
	submerged	0.572	0.1948	0.1862
Sporotrichum sp. + *Sclerotium sp.* type 1	solid state	0.489	0.0479	0.0579
	submerged	0.613	0.1936	0.0181
A. flavus + *Sporotrichum sp.* + *Sclerotium sp.* type 1	solid state	0.243	0.2868	0.0210
	submerged	3.237	0.0173	0.0206
A. flavus + *A. niger* + *Sporotrichum sp.* + *Sclerotium sp.* type 1	solid state	3.859	0.1615	0.0194
	submerged	2.6263	0.0748	0.0189
Control	solid state	0.222	0.0835	0.0699
	submerged	0.1813	0.0309	0.0173

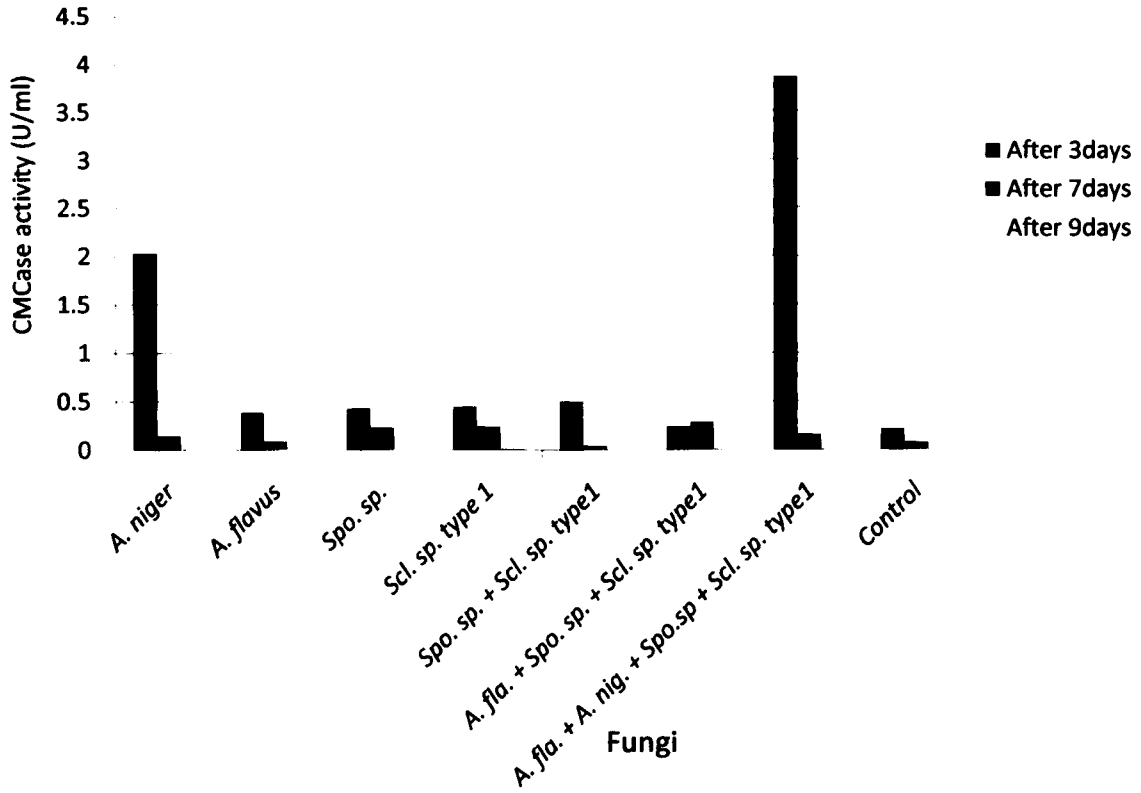


Fig. 17: CMCase activity of the fungi in solid-state after 3, 7 and 9 days of retting.

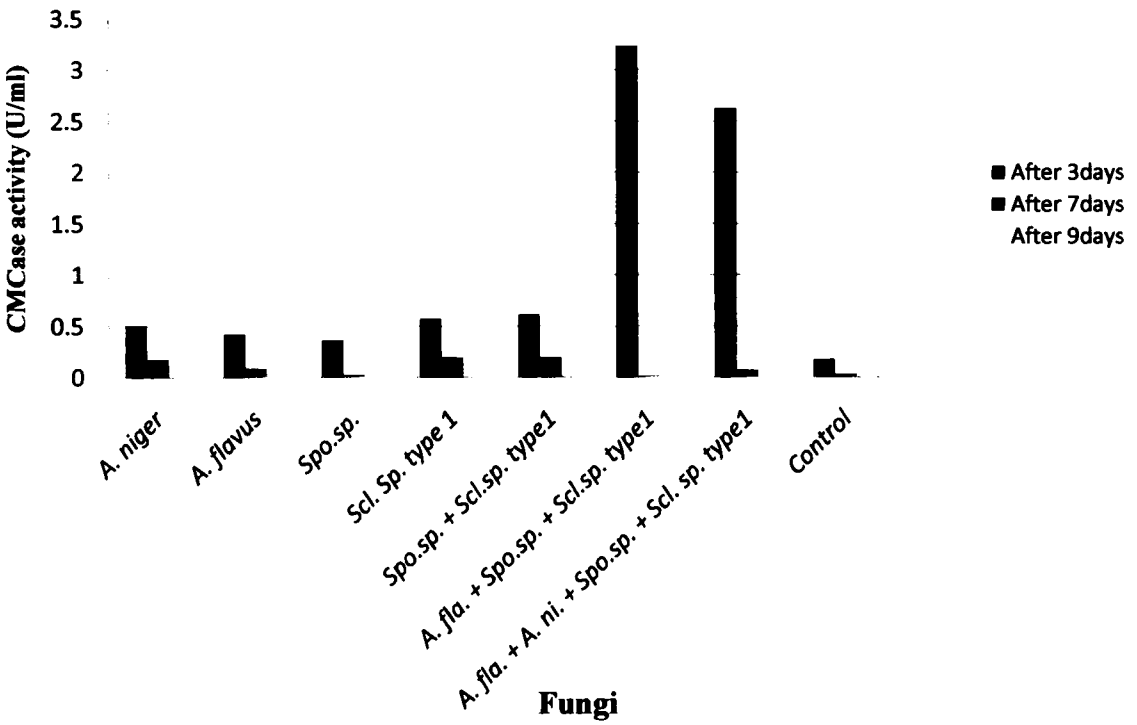


Fig. 18: CMCase activity of the fungi in submerged state after 3,7 and 9 days of retting.

3.6.3 Pectinase activity

Pectinase activity of the fungi measured after 3, 7 and 9 days during retting of green jute ribbon in solid state condition showed a gradual decrease with time attaining the maximum value after 3 days of inoculation (Table 14, Figs 19 and 20). The order of pectinase activity recorded after 3 days of inoculation was found to be all most equal in value where the fungus was inoculated singly and the order of their efficiency may be arranged as follows: *Sclerotium sp. type. 1* (1.8 U/g)> *Sporotrichum sp.* (1.7 U/g)>, *A. flavus,* (1.6 U/g) > *A. niger* (1.1 U/g).

However, a slight increase in pectinase activity was observed from 1.8 to 2.0 U/g due to combined effect of fungi during retting of jute under solid state condition. Highest activity (2.0 U/g) was measured where all the 4 fungi were inoculated together. The lowest activity was encountered to the combination of two fungi (1.8 U/g) among the combined effect of fungi. Activity of pectinase ranked to be intermediate where three fungi were inoculated together under solid state condition. After 9 days of inoculation, the pectinase actively decreased recordedly and in some cases the decrease was more than 50%.

However, the pectinase activity continued to increase up to 7 days with a subsequent fall after 9 days of inoculation when the green jute ribbon was allowed to ret under submerged condition irrespective of the combination of the fungi included (Table 14, Figs 19 and 20). The pectinase activity of the fungus inoculated singly was found to be almost same accounting 1.7 to 1.8 U/g after 7 days of inoculation. It can be noted that the enzymatic activity increased markedly while fungi were inoculated in combination from 2 strains to 4 strains together accounting 2.10 to 2.13 U/g after 7 days of inoculation and the variation in combined impact of fungi was not appreciable at all. A similar trend in pectinase actively was observed after 9 days of inoculation as in the case of solid state condition.

Table. 14. Pectinase activity of the fungi in solid state and submerged conditions after 3, 7, 9 days of retting.

Fungi	Condition	After 3 days (U/ml)	After 7days (U/ml)	After 9days (U/ml)
A. niger	solid state	1.100	1.200	0.750
	submerged	1.400	1.700	0.900
A. flavus	solid state	1.600	1.200	0.910
	submerged	1.100	1.800	0.700
Sporotrichum sp.	solid state	1.700	1.500	0.710
	submerged	1.200	1.700	0.500
Sclerotium sp. type 1	solid state	1.800	1.200	0.610
	submerged	1.700	1.700	0.300
Sporotrichum sp. + *Sclerotium sp.* type 1	solid state	1.800	1.300	0.800
	submerged	1.800	2.100	0.400
A. flavus + *Sporotrichum sp.* + *Sclerotium sp.* type 1	solid state	1.900	1.40	0.700
	submerged	1.700	2.110	0.500
A. flavus + *A. niger* + *Sporotrichum sp.* + *Sclerotium sp.* type 1	solid state	2.000	1.900	1.00
	submerged	1.800	2.130	0.600
Control	solid state	0.010	0.020	0.001
	submerged	0.030	0.004	0.002

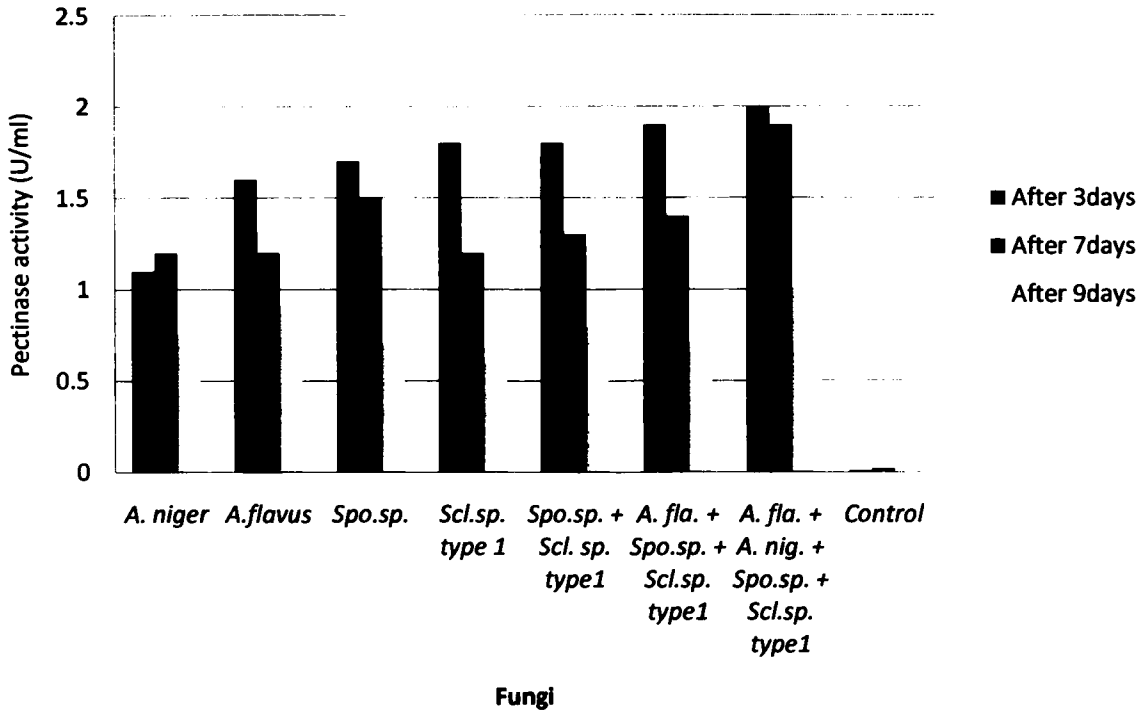


Fig. 19: Pectinase activity of the fungi in solid-state condition after 3,7 and 9 days.

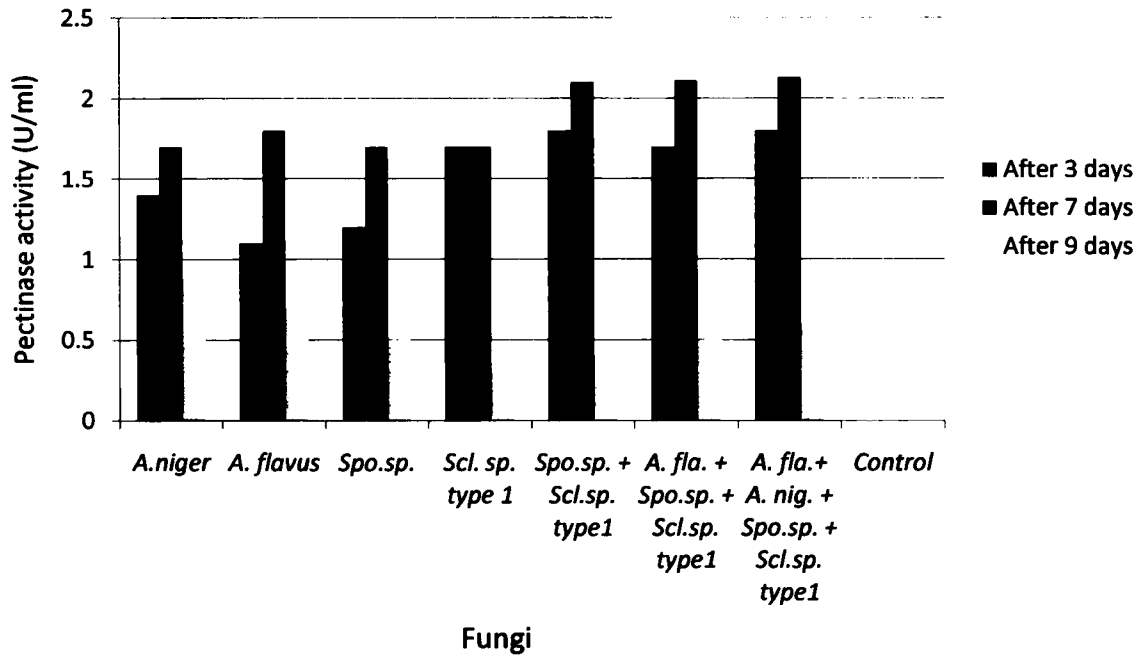


Fig. 20: Pectinase activity of the fungi summerged condition after 3, 7 and 9 days

3.6.4 Pectinlyase activity during retting of green jute ribbon

Pectinlyase activity in green jute ribbon in solid sate condition of retting at pH 5.5 showed a general decrease with time attaining the maximum at 3 days of inoculation (Table 15). This was followed by a gradual decrease up to the end of the inoculation (Table 15). Here also the signal inoculation of fungus showed better role of pectinlyase activity in case of *A. niger* and *Sporotrichum sp.* Pectinlyase activity of *A. flavus* showed all most equal activity to the combined activity of *A. flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1 at 3 days of inoculation. Similarly, *Sclerotium sp.* type 1 exerted equal pectinlyase activity to that of *Sporotrichum sp.* + *Sclerotium sp.* type 1 after 3 days of inoculation at pH 5.5. However, after 6 days of inoculation, the activity of pectinlyase in both the combination of fungi became almost three times higher than that of 3 days. The pectinlyase activity in combination of four fungi was better than di and tri combination of the fungi used.

Pectinlyase activity measured at pH 8.5 increased up to 6 days of inoculation and thereafter a decrease was observed in case of all fungi (Table 15). A comparison of pectinlyase activity showed that fungi inoculated singly were more effective than their combined inoculation. The performances of the fungi remained almost same as was observed at pH 5.5.

Pectinlyase activity measured under submerged condition at pH 5.5 showed similar trend as was observed in green ribbon retting at solid state condition (Table 15). The pectinlyase activity generally decreased with time in most of the cases except *Sclerotium sp.* type 1 where the activity increased up to 6 days of inoculation and then a fall was recorded. Single inoculation of the fungus achieved better pectinlyase activity than their combined inoculations accept *Sclerotium sp.* type 1. Pectinlyase activity achieved by *Sclerotium sp.* type 1 was found to be inferior to those of fungi inoculated in combination.

Pectinlyase activity of the fungus at pH 8.5 under submerged condition also revealed a decrease with time showing their maximum activity after 3 days of incubation (Table 15) except *A. niger*, *Sclerotium sp.* type 1 and combination of *A. flavus, Sporotrichum*

sp. and *Sclerotium sp.* type 1 where the activity increased up to 6 days with a concomitant fall (Table 15). However, an increase in pectinlyase activity after 9 days of incubation was recorded where *A. flavus*, *Sporotrichum sp.* and a combination of four fungi were inoculated.

Table. 15 Pectinlyase activitie of the fungi in solid and submerged state of retting at different pH levels.

Fungi	Pectinlyase activity at pH 5.5				Pectinlyase activity at pH 8.5		
	Condition	3 days	6days	9days	3days	6days	9days
Aspergillus niger	solid state	0.110	0.071	0.041	0.059	0.621	0.031
	submerged	0.120	0.060	0.050	0.260	0.420	0.010
Aspergillus flavus	solid state	0.117	0.014	0.067	0.108	0.018	0.289
	submerged	0.050	0.040	0.030	0.020	0.170	0.070
Sporotrichum sp.	solid state	0.061	0.0171	0.0121	0.061	0.0131	0.043
	submerged	0.050	0.018	0.110	0.040	0.012	0.034
Sclerotium sp. type 1	solid state	0.053	0.136	0.046	0.059	0.254	0.043
	submerged	0.050	0.110	0.030	0.040	0.210	0.020
Sporotrichum sp. + *Sclerotium sp.* type 1	solid state	0.054	0.176	0.010	0.020	0.009	0.275
	submerged	0.420	0.160	0.020	0.200	0.120	0.170
A. flavus + *Sporotrichum sp.* + *Sclerotium sp.* type 1	solid state	0.057	0.053	0.100	0.046	0.107	0.058
	submerged	0.041	0.032	0.120	0.032	0.110	0.420
A. niger + *A. flavus*+ *Sporotrichum sp.* + *Sclerotium sp.* type 1	solid state	0.600	0.080	0.200	0.020	0.120	0.040
	submerged	0.047	0.048	0.101	0.051	0.100	0.040
Control	solid state	0.000	0.000	0.100	0.001	0.006	0.008
	submerged	0.010	0.012	0.060	0.100	0.008	0.010

3.6.5 Reducing Sugar Content during retting of green jute ribbon

Reducing sugar content measured during retting of green jute ribbon in solid state and submerged conations after 3,7 and 9 days due to influence of inoculated fungus either singly or in combination decrease with time of incubation in most of the cases (Table 16, Figs 21 and 22). The highest content of reducing sugar was accounted after 3 days of incubation except *Sclerotium sp.* type 1 which continued to produce reducing sugar (0.6793 to 0.7813 mg/ml) up to 9 days with a slight decrease in the same (0.3753 mg/ml) after 7 days of incubation. *A. flavus* alone and the combination of four fungi also showed the similar trend i.e. production of reducing sugar increased up to 7 days and then fall again after 9 days of incubation. *Sclerotium sp.* type 1 and *Sporotrichum sp.* alone played the best role (0.7813 mg/ml; 4.0258 mg/ml) in producing reducing sugar among the fungal treatments under solid state and submerged conditions respectively. However, inoculation of combination of fungus was found to be relatively better than single inoculation of the same under either condition. A combination of *A. flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1 under solid state condition was observed to be the best (0.5151 mg/ml) so far reducing sugar production is concerned among the fungal combination. However, the situation was different under submerged condition where the combination of *Sporotrichum sp.* and *Sclerotium sp.* type 1 was inoculated together (0.555 mg/ml).

Fiber weight of jute varied from 1570 to 1656g in the fungal treated green ribbon (Table 34) However, the weight remains to 1800g where no fungal inoculums was added. The weight of fiber ranged between 1600 and 1656g where individual fungus was added. The order of weight of fiber due to fungal treatment was as follows: *A, flavus* (1656) > *Sporotrichum sp.* (1615) > *Sclerotium sp.* type 1> *A. niger* (1600).

Table. 16 Reducing sugar content in solid state and submerged condition of retting of green jute ribbon after 3, 7 and 9 days.

Fungi		3days (mg/ml)	7days (mg/ml)	9days (mg/ml)
A. niger	Solid	0.3775	0.262	0.444
	Submerged	3.835	0.1134	0.0979
A. flavus	Solid	0.109	0.1977	0.1533
	Submerged	0.178	0.364	0.1001
Sporotrichum sp.	Solid	0.1644	0.466	0.2842
	Submerged	4.0258	0.111	0.0934
Sclerotium sp. type 1	Solid	0.6723	0.3753	0.7813
	Submerged	1.95.	0.1267	0.0957
Sporotrichum sp. + *Sclerotium sp.* type 1	Solid	0,3508	0.2266	0.4729
	Submerged	0.555	0.3264	0.0957
A. flavus + *Sporotrichum sp.* + *Sclerotium sp.* type 1	Solid	0.5151	0.2709	0.2554
	Submerged	0.435	0.1245	0.1067
A. flavus + *A. niger* + *Sporotrichum sp.* + *Sclerotium sp.* type 1	Solid	0.4063	0.4640	0.4119
	Submerged	0.351	0.3086	0.1644
Control	Solid	0.2456	0.1799	0.6060
	Submerged	0.182	0.1245	0.1156

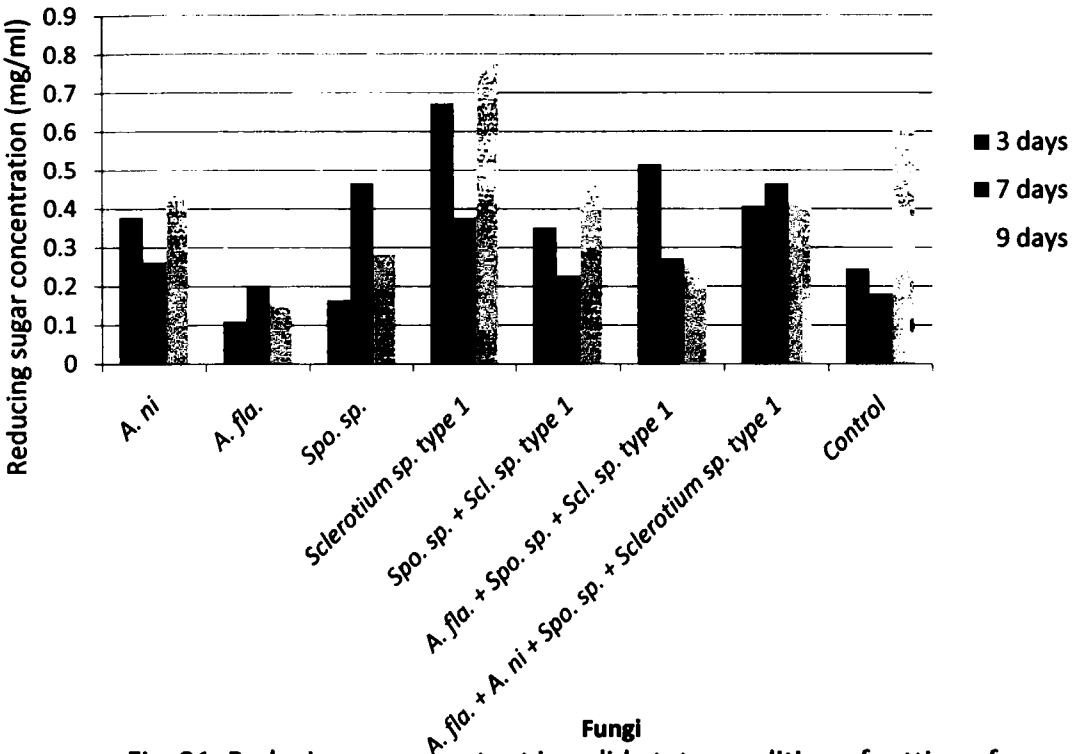


Fig. 21: Reducing sugar content in solid-state condition of retting of green jute ribbons after 3, 7 and 9 days.

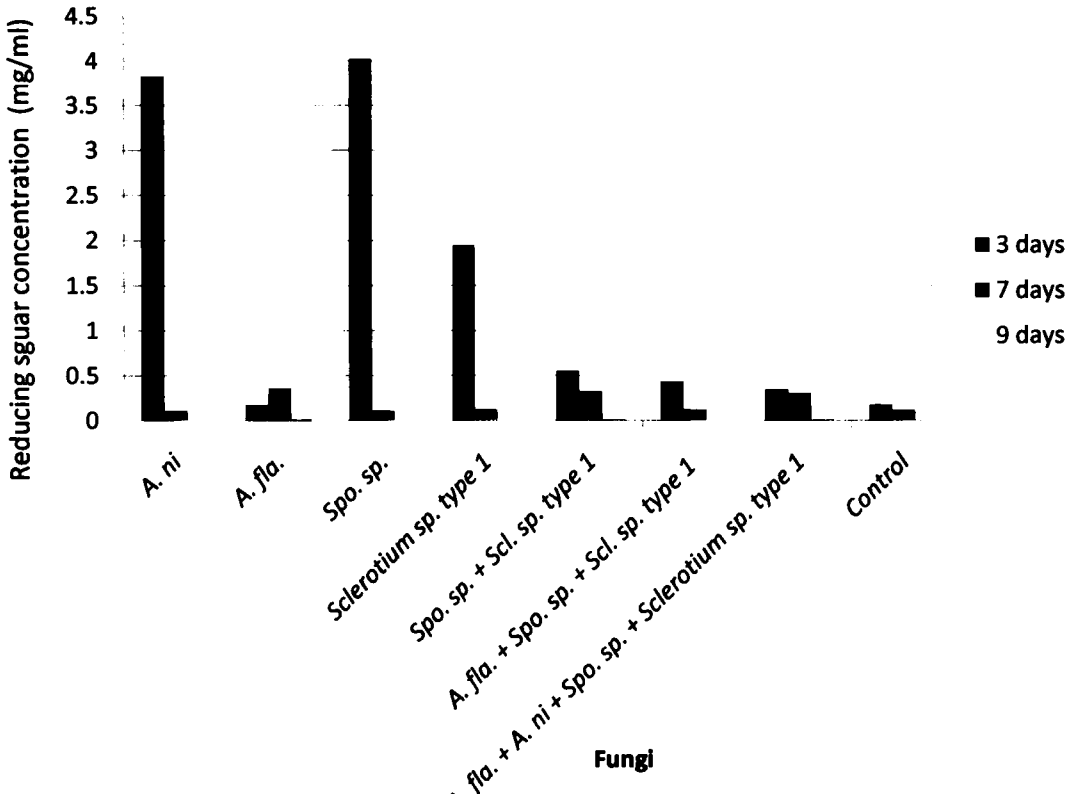


Fig. 22: Reducing sugar in submerged condition after 3, 7 and 9 days after retting

Fiber weight of jute varied from 1570 to 1656g in the fungal treated green ribbon (Table 34) However, the weight remains to 1800g where no fungal inoculums was added. The weight of fiber ranged between 1600 and 1656g where individual fungus was added. The order of weight of fiber due to fungal treatment was as follows: *A, flavus* (1656) > *Sporotrichum sp.* (1615) > *Sclerotium sp.* type 1> *A. niger* (1600).

However, the weight of fiber recorded was slightly lower where fungal mixture was added and the value varied between 1570 (*A. niger* + *A. flavus* +*Sporotrichum sp.* + *Sclerotium sp.* type 1) and 1585g (*Sporotrichum sp.* + *Sclerotium sp.* type 1). The results revealed that the overall variation in weight of fiber among the fungal treatments was not appreciable. The best weight may be considered to be 1800g in the control and 1570g recorded in treatment where all the four fungi were added.

The bundle strength of the jute fibber was found to be influenced by fungal treatments and varied between 9.00 to 10.49 lbs/mg (Table 34). The bundle strength of the fibber obtained from control treatment was 6.86 lbs/mg. Highest bundle strength (10.49 lbs/gm) was found in fibber collected from whole green ribbon treated with four fungi together and this was followed by a combination of three fungi (10.20 lbs/mg). Almost close bundle strength of fibber (10.00 lbs/mg) was recorded for treatment where only *A. flavus* was inoculated.

Fibber grade improved due to fungal treatment of green jute ribbons from C (control) to higher grades (Table 34). Species of *A. niger* and *A. flavus* alone and the combinations of three and four fungi produced A grade fibber while all other fungal treated green jute ribbons produced B grade fibber.

3.6.6 Soluble protein content

Soluble protein in the culture filtrate was analyzed by the method of Bradford (Analytical Biochemistry 72, S.248-2564, 1976) using bovine serum albumin (Sigma, USA) as standard.

Soluble protein released in culture filtrate measured after 3,7 and 9 days under solid state and submerged conditions also showed the highest amount after 3 days with a sharp decline after 6 days of inoculation in most of the fungal inoculated treatments (Table 17, Figs 23 and 24). However after 9 days, instead of decreasing an increase in the content of the same was found, where the jute ribbons were inoculated with *Sporotrichum sp.* and *Sclerotium sp.* type 1 separately and in combination in both solid state and submerged conditions of inoculation.

Tabic 17. Soluble protein content after 3, 7, and 9days in solid state and submerged condition of retting of jute.

Fungi		3days (mg/ml)	7days (mg/ml)	9days (mg/ml)
A. niger	Solid	3.610	0.030	0.004
	Submerged	5.700	0.080	0.030
A. flavus	Solid	3.610	0.320	0.085
	Submerged	3.210	0.720	0.610
Sporotrichum sp.	Solid	4.280	0.004	0.018
	Submerged	3.920	0.033	0.070
Sclerotium sp. type 1	Solid	0.910	0.032	0.072
	Submerged	0.790	0.090	0.110
Sporotrichum sp. + *Sclerotium sp.* type 1	Solid	4.950	0.072	0.110
	Submerged	4.510	0.070	0.110
A. flavus + *Sporotrichum sp.* + *Sclerotium sp.* type 1	Solid	0.230	0.270	0.045
	Submerged	0.280	0.320	0.041
A. flavus + *A. niger* + *Sporotrichum sp.* + *Sclerotium sp.* type 1	Solid	4.950	0.190	0.099
	Submerged	4.610	0.100	0.091
Control	Solid	4.280	0.190	0.180
	Submerged	3.91	0.16	0.190

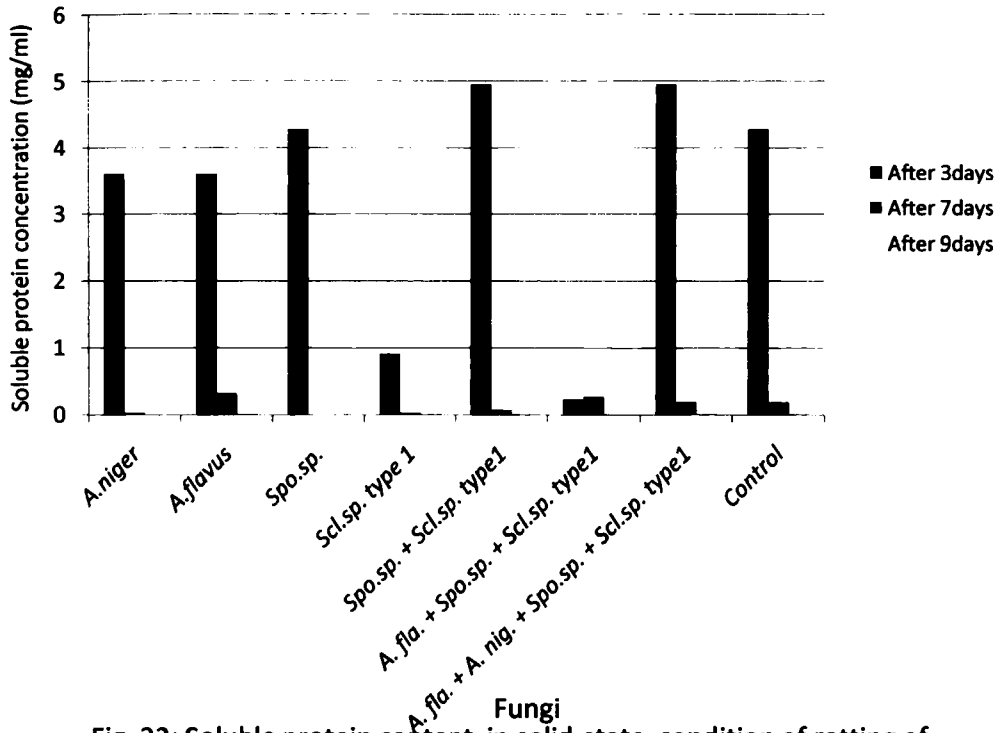


Fig. 23: Soluble protein content in solid-state condition of retting of jute after 3, 7 and 9 days.

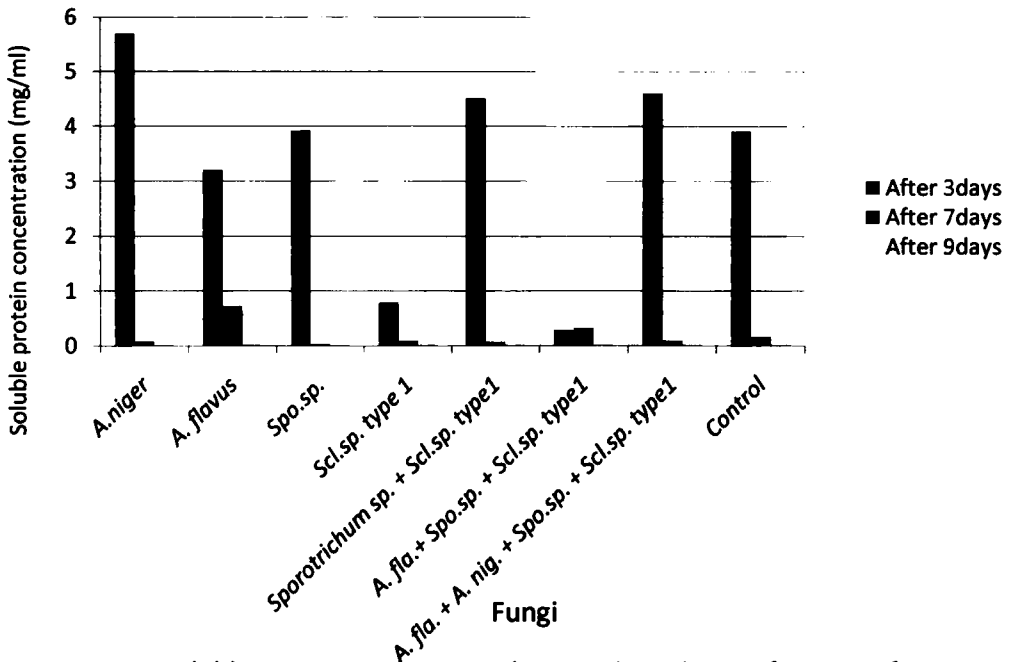


Fig. 24: Soluble protein content in submerged condition of retting of jute after 3, 7 and 9 days.

3.6.7 Solid and submerged retted fiber

The efficiency of 4 and 7 days fungal inoculum in various combinations on retting of green jute ribbon and quality of fiber under solid state fermentation have been studied (Tables 18,19 and 20, Figs 25, 26 and 27). Results showed that retting period of green jute ribbon varied from 9 to 12 and 11 to 14 days when treated with various combinations of 4 and 7 days potential fungal inoculum respectively. However, the time period required for retting of non-fungal treated green jute ribbon was almost close i.e. 23 and 22 days, and 22 and 23 days for solid state and submerged condition respectively. This indicates that fungal inoculum aged 4 days was comparatively more potent in retting of jute fiber than 7 days old ones.

Fiber weight measured also showed the variable weight due to variation in applied fungal inoculum and ranged from 8.85 to 10.55 and 18.20 to 23.70 g for 4 and 7 days potential inoculum respectively (Tables 18, 19 and 20, Figs 25, 26 and 27) indicating the fact again that 4 days inoculum were more effective to decompose the degradable components associated with jute fiber.

Similarly, bundle strength of fiber collected from of jute retting under solid state condition varied from 8.11 to 10.23 and 7.78 to 10.00 lbs/g for 4 and 7 days old inoculum respectively (Tables 18, 19 and 20, Figs 25, 26 and 27). Fiber color recorded were cream, off white and gray; and bright cream, cream and off white for various combinations of fungal inoculum aging 4 and 7 days respectively (Tables 18, 19, 20, Figs. 25, 26 and 27). Fiber grade also varied from A to C grade irrespective of the age of the fungal inoculum used (Tables 18, 19, 20, Figs 25, 26 and 27).

Table 18. Comparative stndy of solid (using 4 days inoculum) state and snbmerged state fermentation of CVL-1 green jute ribbon using 4 days inoculum.

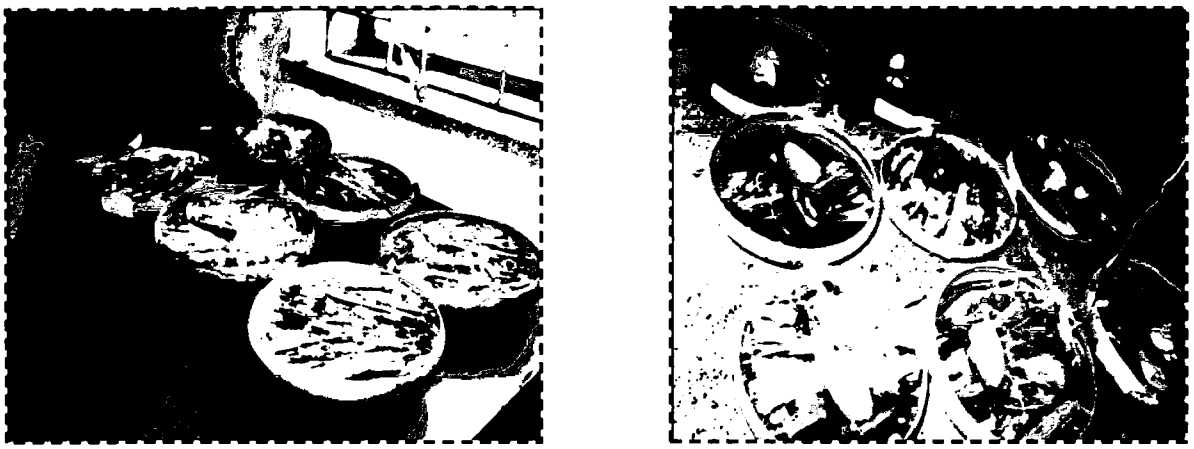
Fungi	Solid state					Submerged state				
	Retting time (days)	Fiber wt. (g)	Bundle strength (Ibs/mg)	Fiber quality	Fiber Color	Retting time (days)	Fiber wt. (g)	Bundle strength (Ibs/mg)	Fiber color	Fiber grade
A. niger	9	18.70	10.23	Cream	B	10	15.50	10.38	Bright cream	A
A. flavus	10	22.80	10.21	Cream	B	10	17.50	10.56	Bright cream	A
Sporotrichum sp.	12	20.30	9.17	Cream	B	12	17.50	9.47	Cream	B
Sclerotium sp. type 1	11	18.00	8.11	Off-white	C	11	17.60	9.67	Cream	B
Sclerotium sp. type 1 + *Sporotrichum sp.*	10	18.10	8.80	Off-white	C	12	17.30	8.71	Cream	B
A. flavus + *Sporotrichum sp.* + *Sclerotium sp.* type 1	11	29.10	8.67	Off- white	C	13	21.00	10.30	Cream	B
A. flavus + *A. niger* + *Sporotrichum sp.* + *Sclerotium sp.* type 1	9	17.10	9.85	Gray	D	12	17.00	9.82	Cream	B
Control	23	37.70	6.6	Gray	D-	22	26	6.00	Brown	D

Table 19. Comparative study of solid state and submerged state fermentation of CVL-1 green jute ribbon using 7 days inoculum.

Serial no.	Fungi	Solid state					Submerged state				
		Retting time (days)	Fiber wt. (g)	Bundle strength (lbs/mg)	Fiber Color	Fiber grade	Retting time (days)	Fiber wt. (g)	Bundle strength (lbs/mg)	Fiber color	Fiber grade
1	*Aspergillus niger*	11	21.70	10.00	Bright cream	Fiber not yet produced	10	20.0	10	Bright cream	A
2	*Aspergillus flavus*	13	23.70	8.72	Off-white	C	11	21.70	10	Bright cream	A
3	*Sclerotium sp.* type 1	13	18.20	8.60	Off-white	C	13	17.10	8.72	Off-white	C
4	*Sporotrichum sp.*	12	18.40	9.12	Cream	B	14	15.70	8.66	Off-white	C
5	*Sclerotium sp.* type 1 + *Sporotrichum sp.*	11	20.40	7.78	Off-white	C	13	18.2	9.11	Cream	B
6	*A. flavus* + *Sclerotium sp.* type 1 + *Sporotrichum sp.*	14	23.40	8.85	Off-white	C	12	21.80	7.77	Off-white	C
7	*A. flavus* + *A. niger* + *Sclerotium sp.* type 1 + *Sporotrichum sp.*	14	23.40	9.28	Cream	B	13	11.80	8.85	Cream	B
8	Control	22	20.40	6.43	Brown	D	23	16.10	6.64	Brown	D

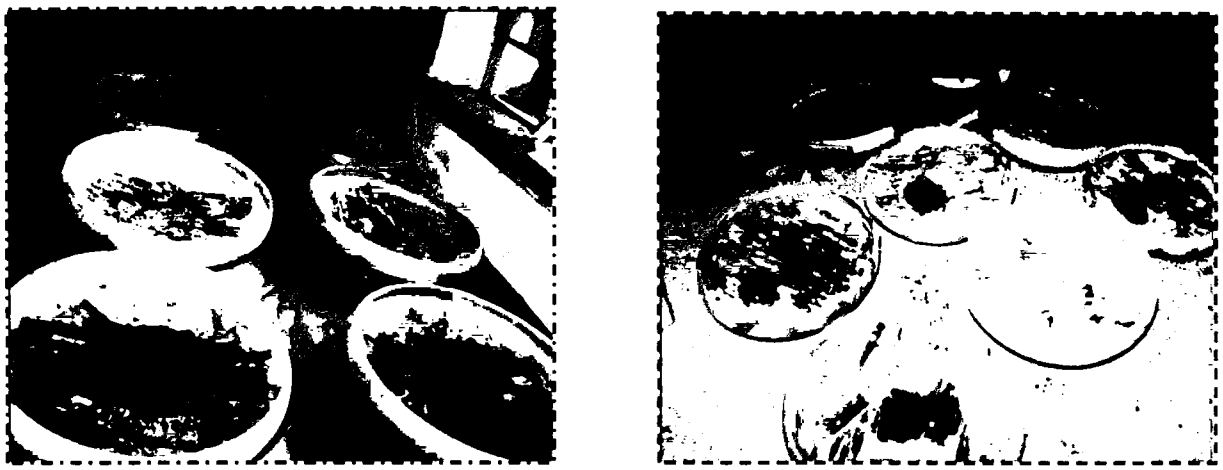
Table 20: Comparative study of 4 and 7 days inoculums during retting of CVL-1 jute under solid state and submerged conditions.

Retting of jute ribbon									
Condition of retting	**Fungi**	**4 days inoculums**				**7 days inoculums**			
		Retting period	**Bundle strength (lbs/mg).**	**Fiber color**	**Fiber grade**	**Retting period**	**Bundle strength**	**Fiber color**	**Fiber grade**
Solid	*Aspergillus niger*	9	10.23	Cream	B	11	10.37	Bright cream	A
Submerged		10	10.34	Bright eream	A	10	10.34	Bright cream	A
Solid	*Aspergillus flavus*	10	10.21	Cream	B	13	8.83	Off white	C
Submerged		10	10.66	Bright cream	A	11	10.43	Bright cream	A
Solid	*Sporotrichum sp.*	12	9.17	Cream	B	13	8.63	Off white	C
Submerged		12	9.69	Bright cream	B	13	9.75	Off white	C
Solid	*Sclerotium sp.* type 1	11	8.11	Off white	C	12	8.60	Cream	B
Submerged		11	9.84	Cream	B	13	9.94	Off white	B
Solid	*Sporotrichum sp.+ Sclerotium sp.* type 1	10	8.80	Off white	C	11	9.17	Off white	C
Submerged		12	8.67	Cream	B	13	9.94	Cream	C
Solid	*Aspergillus flavus +Sporotrichum sp.+ Sclerotium sp.* type 1	11	10.34	Off white	C	14	9.31	Off white	C
Submerged		13	9.85	Cream	B	12	8.89	Off white	C
Solid	*Aspergillus niger+ Aspergillus flavus +Sporotrichum sp.+ Sclerotium sp.* type 1	9		Gray	D	14	9.69	Cream	B
Submerged		12	9.75	Cream	A	13	10.29	Cream	B
Solid	Control (without inoculum)	23	6.6	Gray	D	22	6.43	Brown	D
Submerged		22	6.0	Brown	D	23	6.64	Brown	D



Solid state Submerged state

Fig 25: Early state of green ribbon retting (Using 4 days inoculum)



Solid state Submerged state

Fig 26: Final state of green ribbon retting (Using 4 days inoculum)

Solid state Submerged state

Fig 27: Fibers from green ribbon retting (Using 4 days inoculum)

However, under submerged condition, the age potentiality of the fungi was assessed on retting of green jute ribbon and quality of fiber (Tables 18, 19 and 20, Figs 25, 26 and 27). Results revealed that a variation was observed in retting period of jute fiber ranging from 10 to 12 and 10 to 14 when inoculated 4 and 7 days old fungal inoculum respectively. However, time period required for jute ribbons was found to be almost double i.e. 22 to 23 days where no fungal inoculum was added i.e. in control.

Fiber weight recorded under submerged condition of retting also varied appreciably due to variation in age of the fungal inoculum from 4 to 7 days ranging from 7.50 to 10.00 and 11.80 to 18.00 g respectively (Tables 18,19 and 20, Figs. 25,26 and 27). The weight recorded following inoculation of green jute ribbons with 4 days fungal inoculum was decidedly lower than that of 7 days old ones. However, weight of fiber was found to be recordedly lower in fungal treated jute ribbons in comparison to the control which yielded about 16.00 to 16.10 g under the respective submerged set of the treatment.

Bundle strength of fiber also showed a notable variation between the respective treatments due to variation in age of the added fungal inoculum under submerged condition (Tables 18, 19 and 20, Figs. 25, 26 and 27). The variation was found to be 7.50 to 10.30 and 7.77 to 10.00 lbs/g where the jute ribbons was treated with 4 and 7 days old inoculum respectively. Bundle strength was assessed to be generally higher in treatments receiving 4 days inoculum than that of 7 days inoculum.

Fiber color also remained between bright cream to cream color due to treatment of 4 days fungal inoculum (Tables 18,19 and 20, Figs. 25,26 and 27). However, the treatment of jute ribbons with 7 days old fungal inoculum produced fiber with bright cream, cream and off-white color against brown color in the control. Due to inoculation of jute ribbons with 4 days inoculum fiber grade was mostly A and B except the treatment inoculated with *(Sclerotium sp.* type 1 + *Sporotrichum sp.).* However, in 7 days old inoculum, the frequency of A grade remained same but that of B grade reduced with the increase in the frequency of C grade. Control treatment produced D grade fiber.

3.6.8 Major chemical constituents of solid and submerged retted fiber

Jute fiber collected following various combinations of fungal inoculum treated under solid state fermentation and submerged conditions of retting has been analyzed for cellulose, hemicellulose (xylan), lignin and pectin contents (Tables 21, Figs. 28 and 29).

3.6.8.1 Cellulose content of solid and submerged retted fiber

Cellulose content in jute fiber due to fungal treatments showed about 7.33 and 5.52% higher over the control in solid state fermentation (SSF) and submerged conditions respectively (Table 21, Figs 28 and 29) Cellulose content in jute fiber varied from 59.78 to 65.87 % due to treatment of *Sporotrichum sp.* and *A. niger* respectively in SSF condition (Table 21). Similarly, the range of cellulose content varied between 62.00 and 63.54% caused by *A. niger* + *A. flavus* + *Sclerotium sp.* type 1 and *Sporotrichum sp.* respectively in submerged condition. The results showed that content of cellulose are relatively higher in jute fiber while the retting was done in soil state fermentation irrespective of the fungal treatments used. However, the variation among the treatments is not very much appreciable so far cellulose content is concerned in both the conditions of retting.

3.6.8.2 Hemicellulose content of solid and submerged retted fiber

Hemicellulose content in jute fiber reveled about 2.79 and 4.80 % lower when compared with control in solid state fermentation and submerged conditions of retting respectively (Table 21, Figs 28 and 29).The hemicellulose content in jute fiber accounted about 21.07 and 23.63% when the jute ribbon treated with *A. niger* + *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 and *Sporotrichum sp.* + *Sclerotium sp.* type 1 respectively under solid state fermentation. Contrary to this, the range of hemicellulose varied between 21.15 and 22.50 % in submerged condition due to treatment of *Sporotrichum sp.*+ *Sclerotium sp.* type 1 and *A. niger* + *A. flavus* + *Sporotrichum sp.*+ *Sclerotium sp.* type 1 respectively. Like cellulose, hemicellulose content in jute fiber was found to be lower in general while retting was completed under submerged condition. The variation exerted by the fungus among the treatments was not very much countable.

3.6.8.3 Lignin content

Lignin content of jute fiber was found to be influenced by fungal treatments over the control while retting was accomplished under solid state fermentation and submerged conditions (Table 21, Figs. 28 and 29). The results showed that lignin content of fiber was 2.50 and 1.90% lower than the control due to variation in retting processes from solid state fermentation to submerged conditions. The highest and the lowest values of lignin was recorded to be 14.21 and 12.19 % in treatments receiving *Sporotrichum sp.* + *Sclerotium sp.* type 1 and *A. flavus* + *Sporotrichum sp. and Sclerotium sp.* type 1 respectively in solid state fermentation. However, the maximum and minimum values for lignin content were found to be 13.35 and 12.48 % in fiber collected from green jute ribbon treated with *A. niger* + *A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1 and *Sporotrichum sp.* respectively under submerged condition. It could be noted that the variation in lignin content of fiber among the fungal treatments and between conditions of retting was not very much appreciable.

Table 21: Major chemical constituents (%) during retting of jute fiber in solid state fermentation and submerged conditions.

Fungi	Solid state				Submerged state			
	Cellulose	Hemicellulose	Lignin	Pectin	Cellulose	Hemicellulose	Lignin	Pectin
Aspergillus niger	65.87	21.92	12.79	0.82	62.85	21.85	13.30	0.62
Aspergillus flavus	63.34	22.30	14.08	0.90	62.93	21.85	13.09	0.12
Sporotrichum sp.	59.78	23.54	12.90	0.84	63.54	21.38	12.48	0.92
Sclerotium sp. type 1	63.10	22.38	13.90	0.96	63.10	21.92	13.21	0.72
Sporotrichum sp. + *Sclerotium sp.* type 1	64.40	23.62	14.21	0.91	62.00	21.15	13.00	0.88
A. flavus + *Sporotrichum sp.* + *Sclerotium sp.* type 1	65.50	21.07	12.19	0.80	62.10	22.00	13.00	0.86
A. niger + *A. flavus* *Sporotrichum sp.* + *Sclerotium sp.* type 1	63.10	21.07	12.69	0.91	62.00	22.50	13.35	0.61
Control	58.54	23.86	14.69	1.80	58.02	25.95	14.38	1.26

3.6.8.4 Pectin content

Pectin content measured in jute fiber decreased by 1.00 and 1.14 % over the control due to fungal treatments in solid state fermentation and submerged conditions respectively (Table 21, Figs 28 and 29).Pectin content accounts 1.80 and 1.26 % in jute fiber not treated with fungus in solid state fermentation and submerged conditions respectively. However, the value of pectin decreased to less than 1.00 % in fiber ranging from 0.80 (*A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1) to 0.96 % (*Sclerotium sp.* type 1) and 0.12 (*A. flavus*) to 0.92% (*Sporotrichum sp.* accounted for solid state fermentation and submerged conditions of retting respectively. The results further showed that the variation in pectin content among the fungal treatments under solid state fermentation was not very much appreciable. However, an opposite trend was observed in pectin content in fiber of jute retted under submerged condition.

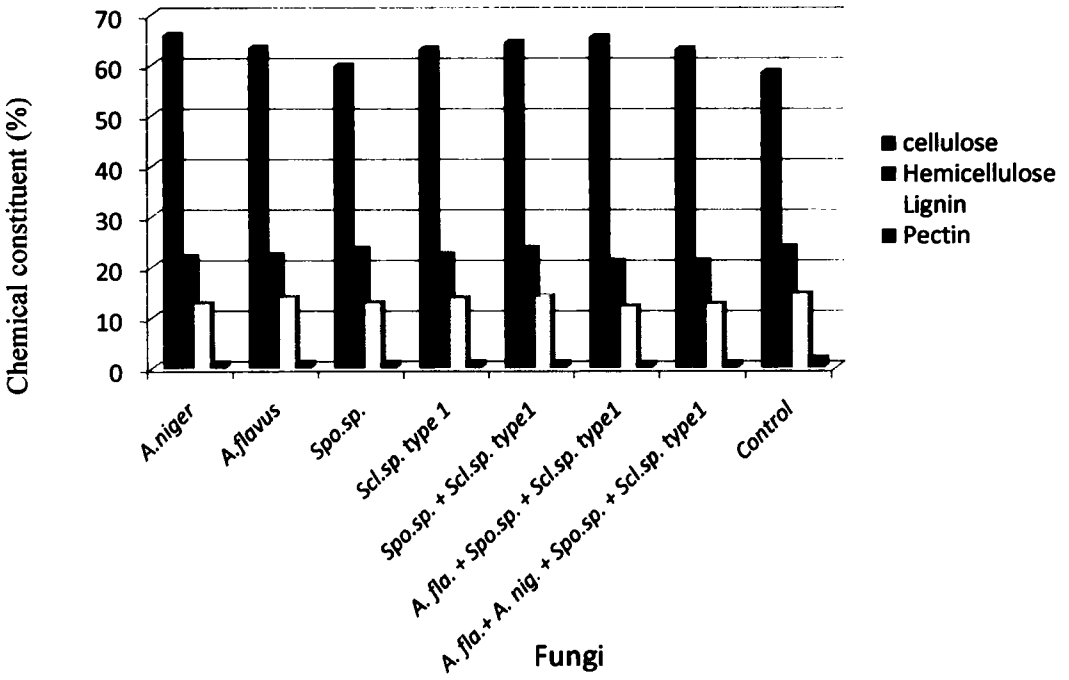


Fig. 28: Chemical constituents of jute fiber during retting under solid state condition.

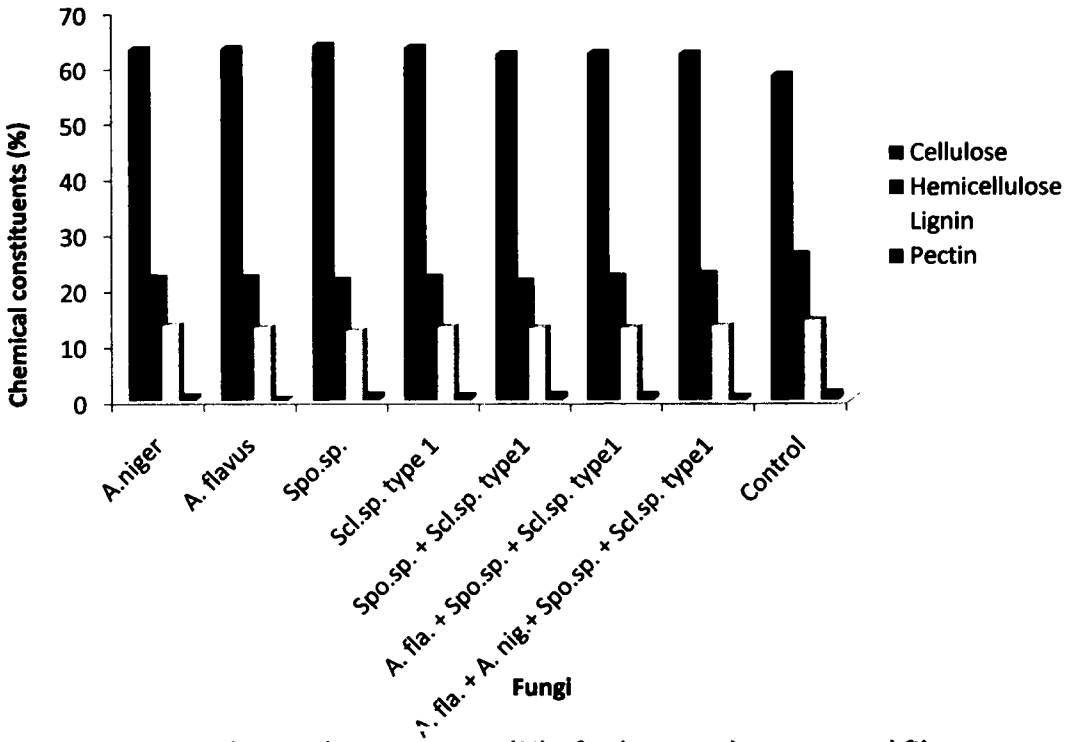


Fig. 29: Chemical constituents (%) of submerged state retted fiber.

3.7 Characterization of selected 4 fungal enzymes

3.7.1 Time course study on enzyme production

Solid state fermentation for a period of 9 days with *A. niger*, *A. flavus, Sporotrichum sp. and Sclerotium sp.* type 1 using optimized medium was performed. The incubation temperature was 35°C. The results are depicted for xylanase (Table 22, Fig. 30), CMCase (Table 23, Fig. 31) and pectinase (Table 24, Fig 32). Synthesis of xylanase and CMCase by added fungi commenced within 3 days. Xylanase activity of *A. niger*, *A. flavus Sporotrichum sp.* and *Sclerotium sp.* type 1 fungi reached its maximum at day 6 (Fig. 22) while that of CMCase reached its highest level at 6 days of incubation (Fig. 31). After that, enzyme activity decreased at 9 days of incubation very sharply from 46 to 17.87,17.60 to 9.07,30.00 to 14.00 and 11.00 to 6.00 U/ml for xylanase activity of the fungi and followed the sequence *A. niger* > *Sporotrichum sp.*> *A. flavus*> *Sclerotium sp.* type 1.

Table 22. Xylanase activity of the fungi at different time course.

Time (days)	*A. niger* (U/ml)	*A. flavus* (U/ml)	*Sporotrichum sp.* (U/ml)	*Sclerotium sp.* type 1 (U/ml)
0	0	0	0	0
3	21.60	13.60	16.00	9.00
6	46.00	17.60	30.00	11.00
9	17.87	9.07	14.00	6.00

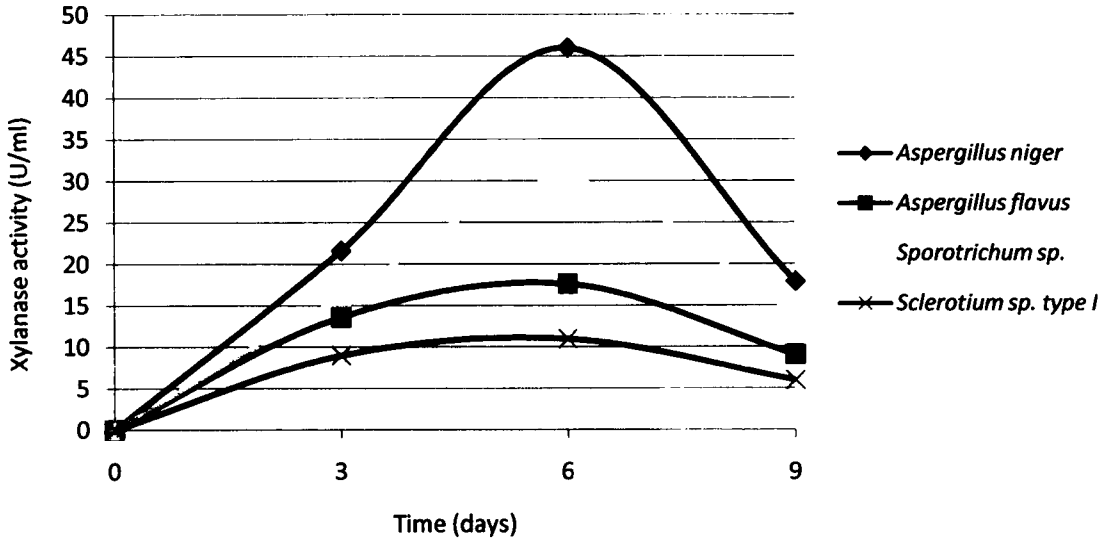


Fig. 30: Xylanase acivity of the fungi at different time course.

CMCase activity of the selected four fungi measured for 0, 3, 6 and 9 days showed a similar trend like xylanase activity as stated above (Table 23, Fig. 31). CMCase activity increased up to 6days of incubation in case of all the inoculated fungi and then fall at 9 days. The decrease in CMCase activity was found to be gradual for *A. niger* and *Sporotrichum sp.* i.e. the value dropped from 5.994 to 5.624 and 4.50 U/ml to 3.12 U/ml respectively. In contrast, the reduction in activity was recorded to be very drastic with a concomitant fall from 1.332 U/ml to 0.148 U/ml and 1.10 U/ml to 0.13 U/ml for *A. flavus* and *Sclerotium sp.* Type 1 respectively. CMCase activity of inoculated fungi can be ranked as: *A. niger* > *Sporotrichum sp.* > *A. flavus* > *Sclerotium sp.* type 1.

Table 23: CMCase activity of the fungi at different time course.

Time (days)	*A. niger* (U/ml)	*A. flavus* (U/ml)	*Sporotrichum sp.* (U/ml)	*Sclerotium sp.* type 1 (U/ml)
0	0	0	0	0
3	2.516	1.258	2.01	1.00
6	5.994	1.332	4.50	1.10
9	5.624	0.148	3.12	0.13

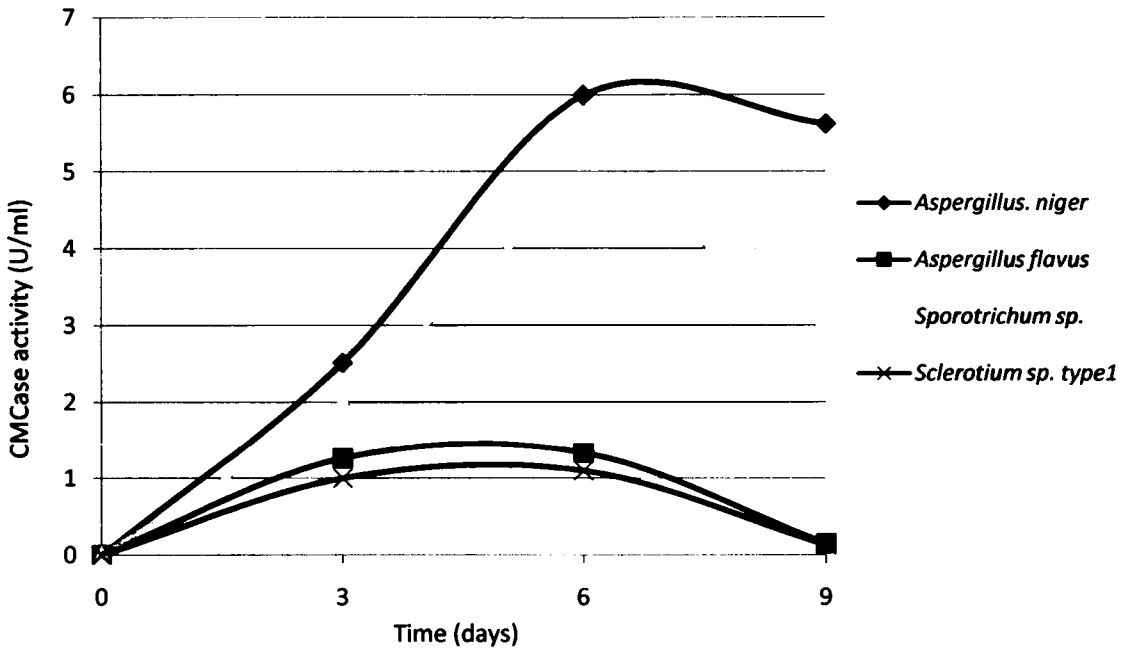


Fig. 31: CMCase activity of the fungi at different time course

Pectinase activity of the inoculated fungi followed at different time course also revealed a very similar trend like xylanase and CMCase activity of the fungi (Table 24; Fig. 32). Pectinase activity of fungi also increased rapidly reaching to maximum at 6 days and then followed a sharp fall at 9 days in case of all the fungi under study. Measurement of the activity showed around 50% fall in the value from 6 to 9 day in all the fungi. The order of efficiency of the fungi followed the sequence as: *A. flavus* > *Sporotrichum sp.* type 1 > *Sclerotium sp.* > *A. niger.*

Table 24. Pectinase activity at different time course.

Time (days)	*A. niger* (U/ml)	*A. flavus* (U/ml)	*Sporotrichum sp.* (U/ml)	*Sclerotium sp.* type 1 (U/ml)
0	0	0	0	0
3	1.11	1.60	1.12	1.02
6	2.18	2.70	2.80	2.90
9	1.20	1.70	1.60	1.80

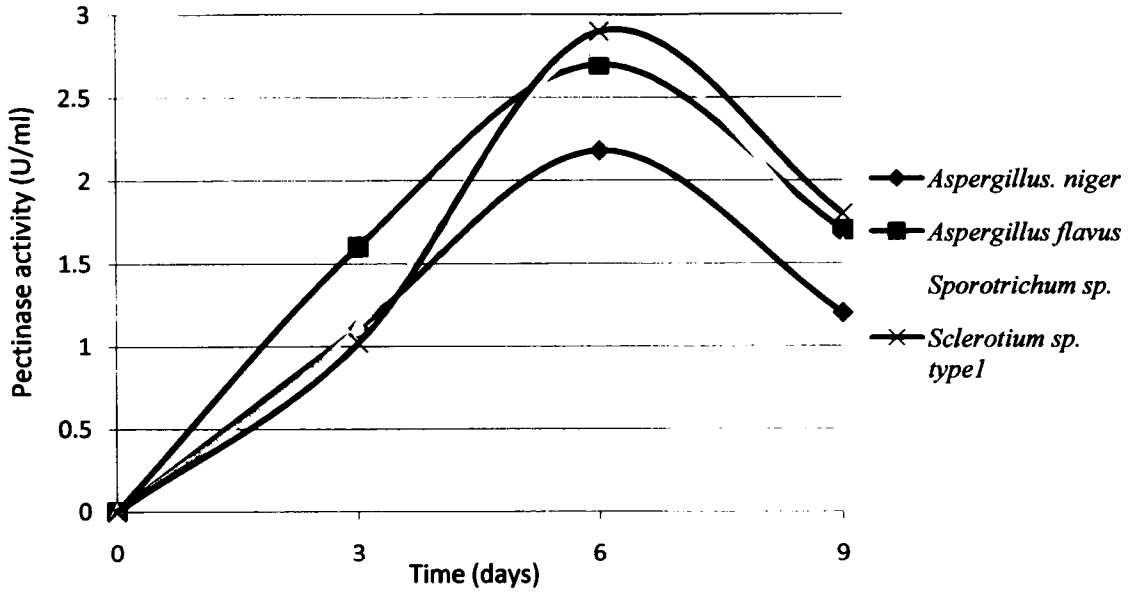


Fig. 32: Pectinase activity of the fungi at different time course.

3.7.2 Determination of optimum pH, optimum temperature and pH stability.

Solid state fermentation was carried out with *A. niger* and *A. flavus, Sporotrichum sp. and Sclerotium sp.* type 1 using wheat bran as a sole carbon and nitrogen source following the method described section 2. After 3, 6 and 9 days the extracts were collected for the enzyme assay and the enzymes namely xylanase CMCase and pectinase were characterized with respect to pH optima, temperature optima, and pH stability.

3.7.2.1 *pH optima*

The pH activity profile of xylanase and CMCase measured at pH range 2.5 to 7.5 are depicted in (Table 25, Figs. 33 and 34). The pH optimum for xylanase and CMCase of the fungi *A. niger, A. flavus, Sporotrichum sp., Sclerotium sp.* type I showed an in

general increase in their activity with the increase in pH from 2.5 of the extract and reached to a maximum at certain and pH (5.5) and then decline with further increase in pH to the highest level of 7.5. The pH optima for xylanase of *A. niger, A. flavus, Sporotrichum sp., Sclerotium sp.* type 1 was between pH 4.5 and pH 6.5. Xylanase of *A. niger* showed the highest activity at pH 5.5 (34.94 U/ml). Xylanase of *A flavus* showed the highest activity at pH 4.5 (13.068 U/ml). However, the maximum activity of xylanase for *Sporotrichum sp.* pH was at 5.5 (30.12 U/ml) and that for *Sclerotium sp.* type 1 was also at pH 5.5(29.20 U/ml).

CMCase of A. *niger, A. flavus and Sclerotium sp.* type 1 showed the highest activity at pH 4.5 amounting 7.99, 1.85 and 1.48 U/ml respectively. In contrast *Sporotrichum sp.* showed the highest activity at little bit higher of pH 5.5 accounting 3.01 (U/ml).

Table 25: pH optima of xylanase activity by different fungi after 3 days of growth.

pH	*A. niger* (U/ml)	*A. flavus* (U/ml)	*Sporotrichum sp.* (U/ml)	*Sclerotium sp.* type 1 (U/ml)
2.5	14.94	4.80	10.21	3.00
3.5	20.54	8.00	20.13	19.00
4.5	30.67	13.068	25.60	20.06
5.5	34.94	9.87	30.12	29.2
6.5	8.00	8.80	22.00	24.10
7.5	7.47	5.07	6.00	6.00

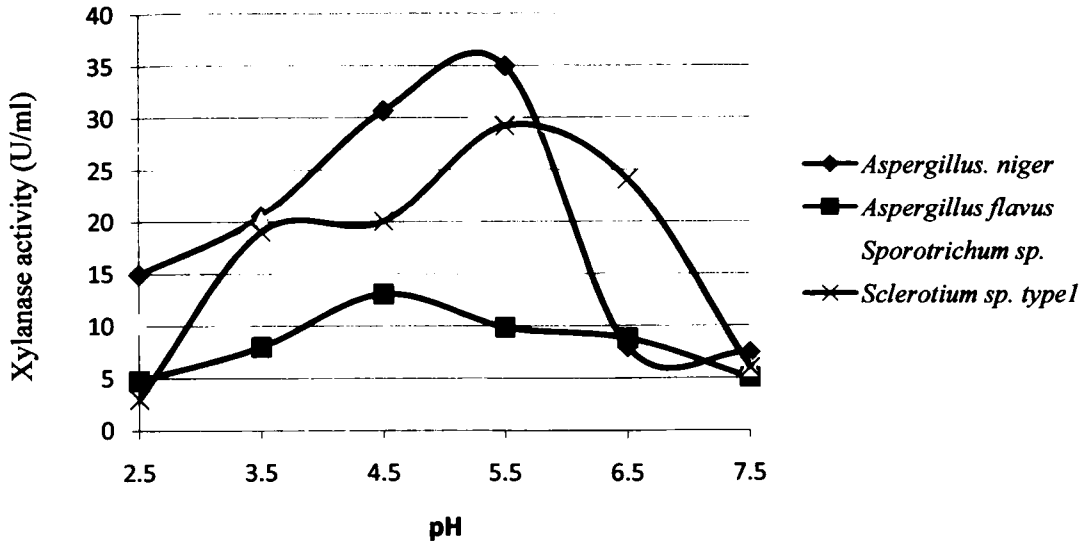


Fig. 33: pH optima of xylanase activity of different fungi after 3 days of growth.

Table 26: pH optima of CMCase activity by different fungus after 3 days of growth.

pH	*A. niger* (U/ml)	*A. flavus* (U/ml)	*Sporotrichum sp.* (U/ml)	*Sclerotium sp.* type 1 (U/ml)
2.5	0.00	0.00	0.00	0.00
3.5	0.851	0.74	0.82	0.62
4.5	7.99	1.85	0.69	1.48
5.5	6.216	1.11	3.01	1.10
6.5	1.11	1.11	1.00	1.10
7.5	0.814	0.74	0.41	0.32

Results presented in (Table 27 and figs. 34 and 35) revealed that the pH optima for the enzyme pectinase of the above mentioned fungi with respect to its activity represented a different trend i.e. the actively fall in initially with the increase in pH from 2.5 to 3.5 in the order of *Sporotrichum sp.* <*A. flavus* < *Sclerotium sp.* type 1 < *A. niger*. This was followed by a general increase in the same of the enzyme for all the fungi very rapidly reaching to the maximum at pH 5.5 for all the fungi except *A. niger* whose enzyme showed its activity at maximum at slightly higher pH of 6.5. The activity of the enzyme pectinase then falls sharply with the increase in pH of the medium to 7.5. However, the

pH optima range of the enzyme pectinase of the four fungi under study may be mentioned as 4.5 to 6.5.

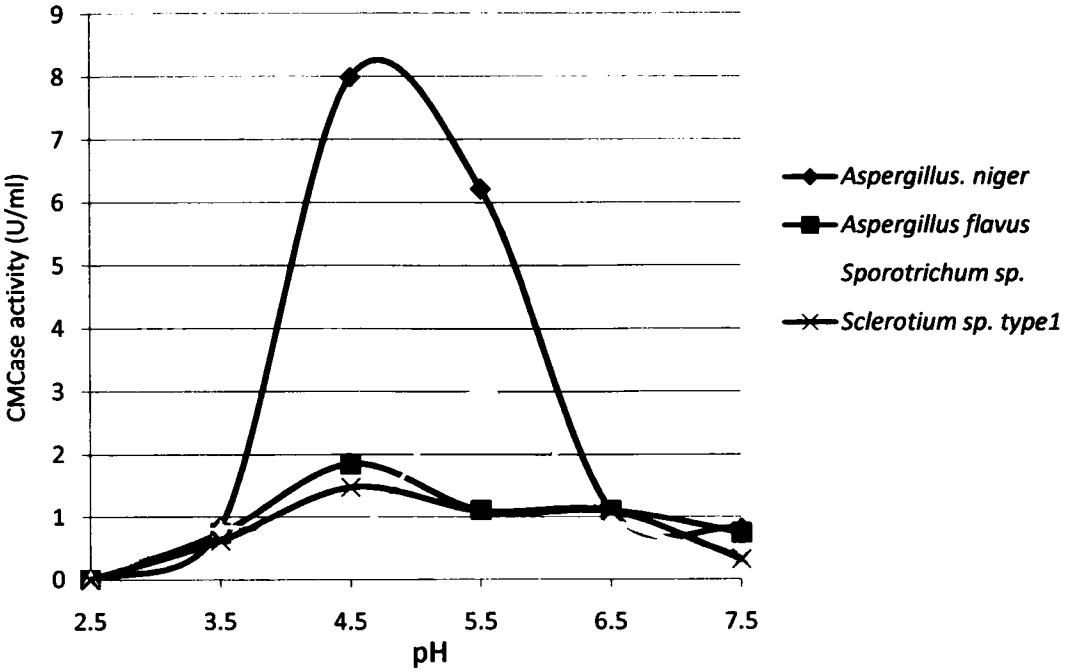


Fig. 34: pH optima of CMCase by different fungi at 3 days of growth.

Table 27. pH Optima of pectinase activity by different fungi after 3 days of growth.

pH	*A. niger* (U/ml)	*A. flavus* (U/ml)	*Sporotrichum sp.* (U/ml)	*Sclerotium sp.* type 1 *(U/ml)*
2.5	0.72	0.80	0.90	0.62
3.5	0.41	0.74	0.81	0.69
4.5	0.98	1.10	1.01	1.07
5.5	1.29	1.21	1.20	1.22
6.5	1.30	1.00	1.01	1.03
7.5	0.98	0.89	0.92	0.68

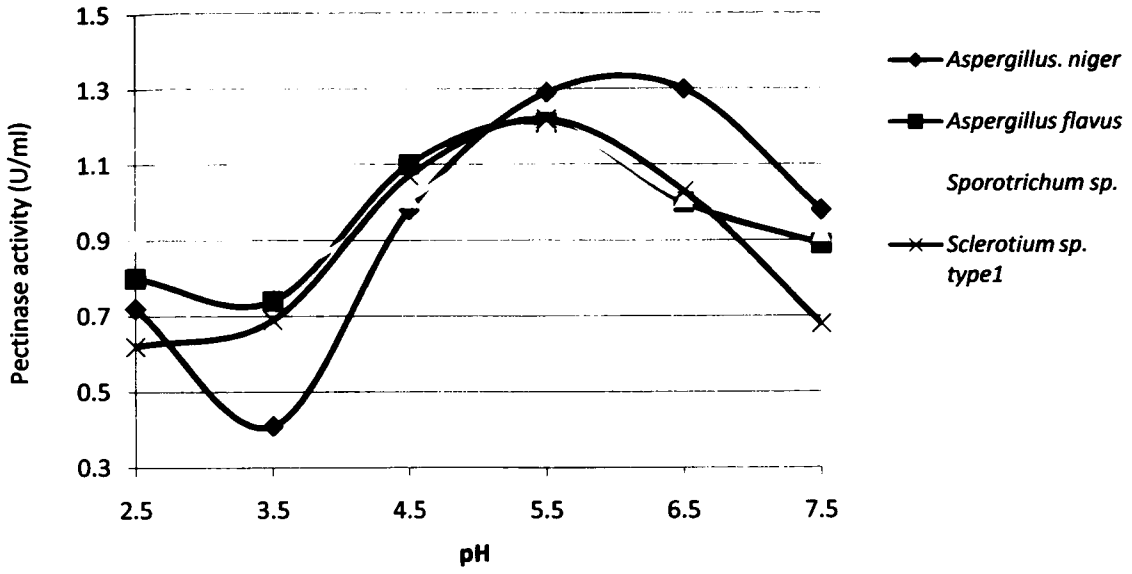


Fig. 35: pH optima of pectinase by different fungi at 3 days of growth.

3.7.2.2 pH stability by different fungi

The p^H stability of the enzymes-xylanase, CMCase and pectinase collected from four selected fungal isolates namely *A. niger*, *A. flavus*, *Sporotrichum sp.* and *Sclerotium sp.* type 1 were examined by pre-incubating them in appropriate buffer raging in pH from 3.0 to 10.0.

Activity of the xylanase, CMCase and pectinase measured under standard assay condition showed a wide variation with variation of the pH of the medium (Tables 28-30, Figs 36-38) the activity of the enzymes of all fungal isolates reveled a sharp increase with the increase in pH and become stable at pH 5.0 for xylanase and CMCase except the enzyme CMCase assayed from *A. flavus* which showed stability at pH 6.0.

However, the stability of the pectinase was found to be at 1 unit higher in comparison to the others i.e. at pH 6.0. This was followed by a sharp deactivation of the enzymes as was observed at pH 9.0 or above for xylanase and CMCase and at pH 10 for pectinase. The value of the enzymatic activity reached to almost zero at the highest pH of the medium irrespective of the enzymes and fungal strains used.

Results showed that the activity of xylanase, CMCase and pectinase of the strains were found to be almost stable at pH range between 3.0 to 8.0. A variation in activity of the enzymes towards stability was observed among the strains of four isolates.

Table 28. pH stability of xylanase by different fungi at 3 days of growth.

pH	*A. niger* (U/ml)	*A. flavus* (U/ml)	*Sporotrichum sp.* (U/ml)	*Sclerotium sp.* type 1 (U/ml)
3.0	6.40	4.00	6.20	3.00
4.0	9.33	8.35	9.31	7.31
5.0	12.93	10.13	10.92	8.12
6.0	9.33	8.00	6.32	7.00
7.0	6.67	7.07	6.60	7.01
8.0	6.67	2.40	6.39	2.20
9.0	2.93	2.13	2.11	2.11
10.0	1.60	0.53	1.00	0.50

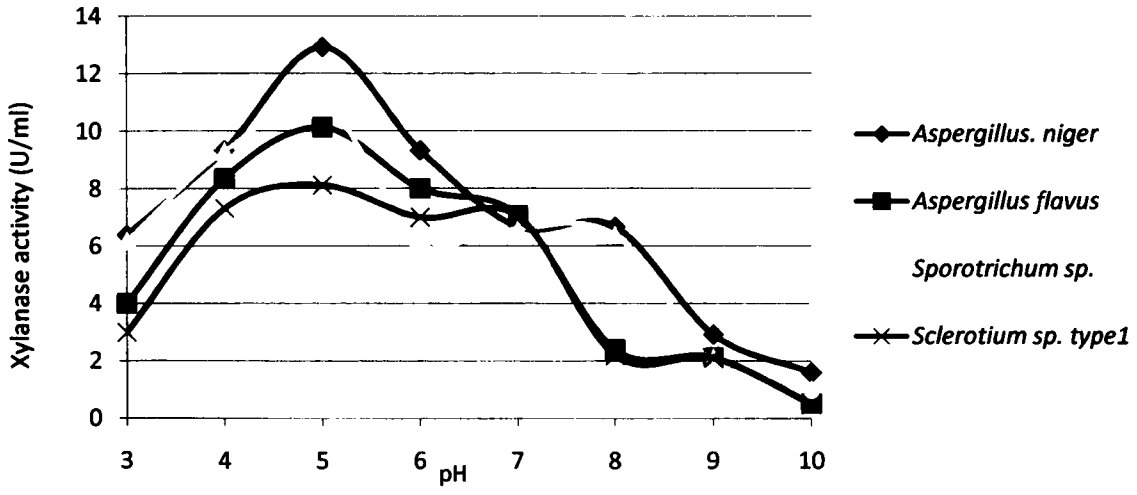


Fig. 36: pH stability of xylanase by different fungi at 3 days of growth.

Table 29. pH stability of CMCase by different fungi at 3 days of growth.

pH	*A. niger* U/ml	*A. flavus* U/ml	*Sporotrichum sp.* U/ml	*Sclerotium sp.* type 1 U/ml
3.0	2.516	0.148	2.410	0.140
4.0	3.145	0.259	3.120	0.210
5.0	4.292	0.333	4.112	4.022
6.0	3.626	3.019	3.326	3.011
7.0	2.997	2.183	2.991	2.100
8.0	2.183	1.080	2.182	1.010
9.0	0.430	0.638	0.420	0.328
10.0	0.000	0.000	0.000	0.000

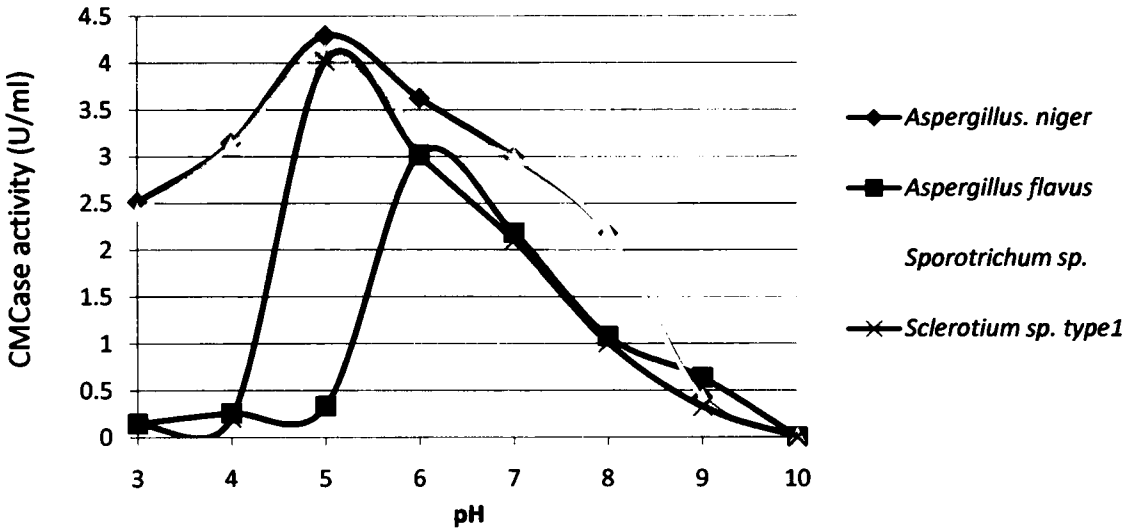


Fig. 37: pH stability of CMCase by different fungi after 3 days of growth.

Table 30. pH stability by of pectinase different fungi at 3 days of growth.

pH stability	Pectinase activity (U/ml)			
	Aspergillus niger	*Aspergillus flavus*	*Sporotrichum sp.*	*Sclerotium sp.* type 1
3.0	0.98	0.86	0.97	0.89
4.0	1.01	1.00	1.11	0.98
5.0	1.19	1.14	1.18	1.15
6.0	1.20	1.26	1.19	1.18
7.0	0.92	0.79	0.88	0.95
8.0	0.81	0.70	0.75	0.80
9.0	0.71	0.61	0.70	0.74
10.0	0.51	0.48	0.43	0.49

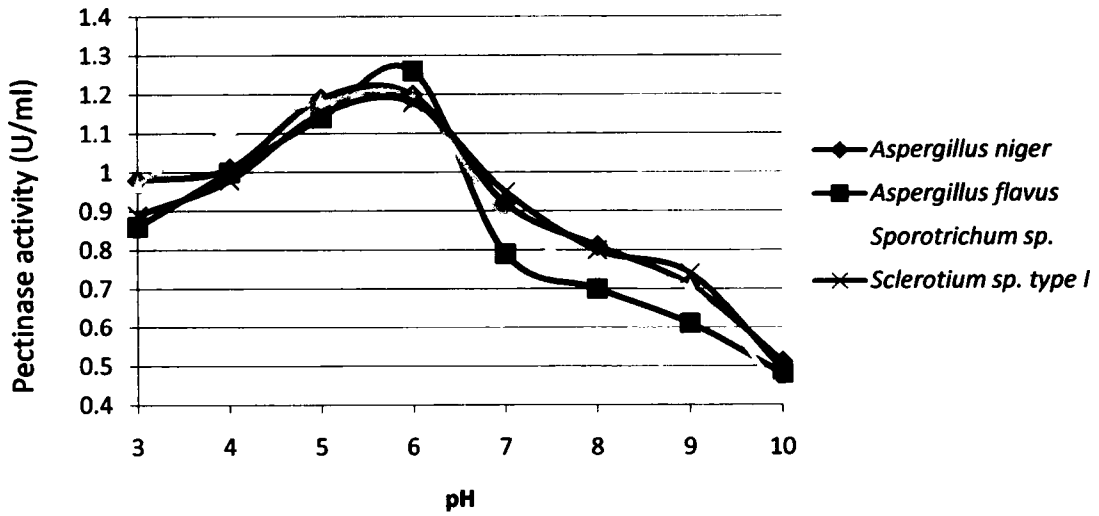


Fig. 38: pH stability of pectinase by different fungi at 3 days of growth.

3.7.2.3 Temperature optima by different fungus

The optimum temperature for xylanase activity was found to be at 50°C for the fungi *A. niger* and *A. flavus* (Table 31, Fig 39). However, the maximum activity of the enzyme for *Sporotrichum sp.* and *Sclerotium sp.* type 1 was recorded to be in the initial temperature i.e. at 40°C. The activity of the enzyme for the former two fungi showed an initial increase reaching to maximum at 50°C and then fall gradually. In contrast, the activity of the enzyme in case of later two fungi revealed a deactivation as the

temperature continued to rise to 80°C. A similar trend in activity of the enzyme pectinase was observed irrespective of the fungi used (Table 33, Fig. 41).

The optimum temperature for CMCase activity of the fungi was evidenced to be at 50°C. The nature and change of activity of the enzyme showed an initial increase leading to maximum at 50°C (Table 32, Fig. 40) and the fall sharply with increase in temperature irrespective of the fungi used in the experiment.

Table 31. Temperature optima of xylanase by different fungi at 3 days of growth

Temperature (°C)	*A. niger* (U/ml)	*A. flavus* (U/ml)	*Sporotrichum sp.* (U/ml)	*Sclerotium sp.* type 1 (U/ml)
40	22.14	1.6	21.10	11.0
50	40.8	2.667	20.10	10.98
60	22.14	1.07	11.12	5.02
70	11.47	0.53	9.50	0.50
80	6.40	0.27	4.20	0.12

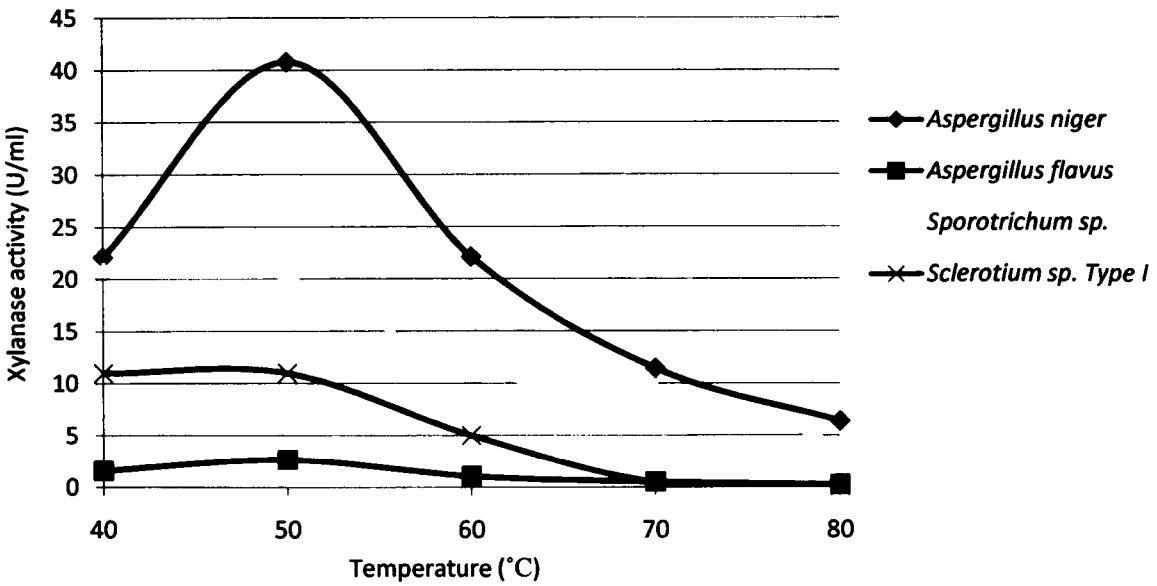


Fig. 39: Temperature optima of xylanase by different fungi at 3 days of growth.

Table 32: Temperature optima of CMCase by different fungi at 3 days of growth.

Temperature (°C)	*A. niger* U/ml	*A. flavus* U/ml	*Sporotrichum sp.* U/ml	*Sclerotium sp.* type 1 U/ml
40	2.997	2.849	2.12	2.42
50	3.848	3.515	3.81	3.41
60	1.998	1.70	1.91	1.60
70	1.258	1.147	1.25	1.12
80	1.184	0. 74	1.00	0.31

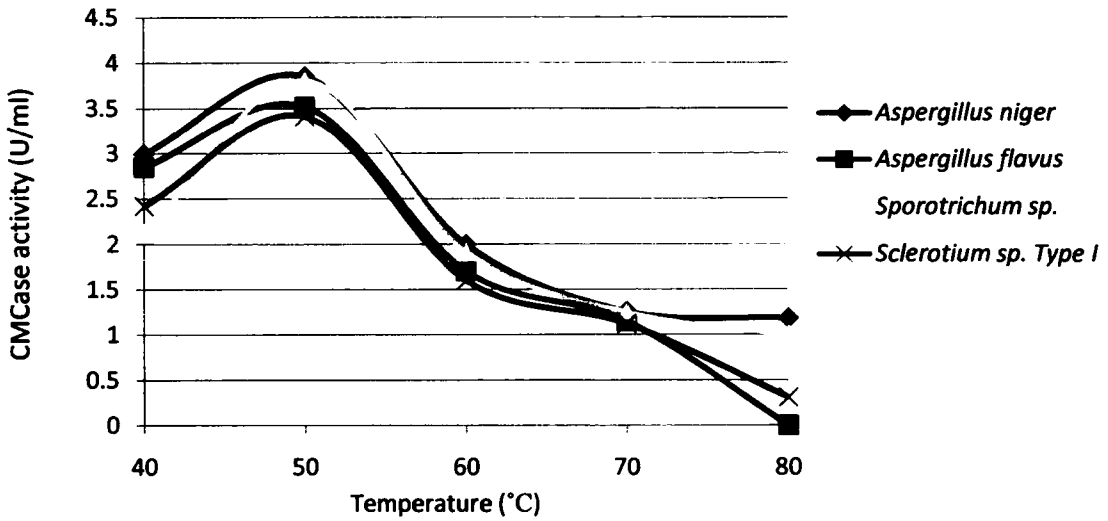


Fig. 40: Temperature optima of CMCase by different fungi at 3 days of growth.

Table 33. Temperature optima of pectinase by different fungi at 3 days of growth.

Temperature (°C)	*A. niger*	*A. flavus*	*Sporotrichum sp.*	*Sclerotium sp.*
40	1.06	1.12	1.20	1.29
50	0.99	1.00	1.00	1.09
60	0.81	0.96	0.94	0.91
70	0.71	0.61	0.48	0.82
80	0.60	0.51	0.30	0.40

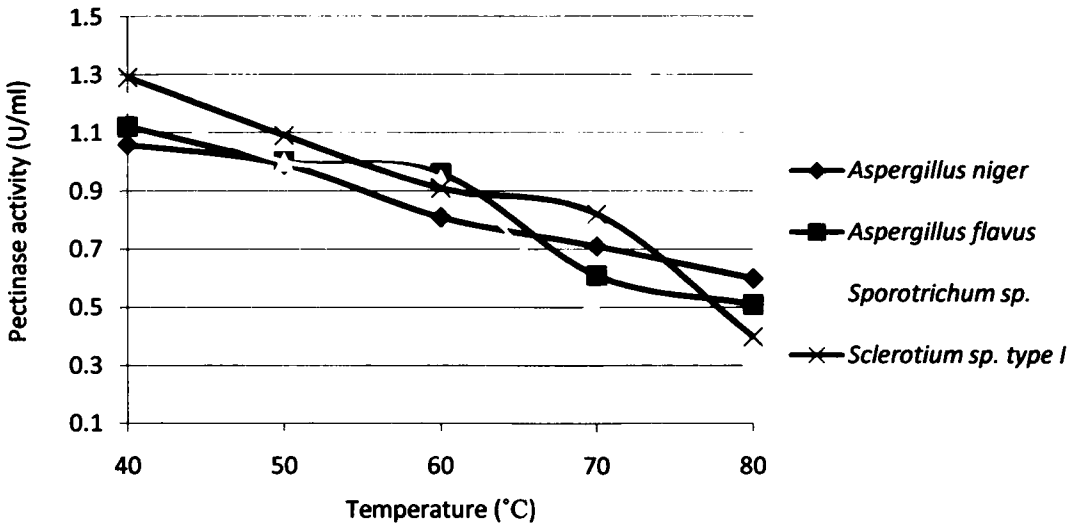


Fig. 41: Temperature optima of Pectinase by different fungi at 3 days of growth.

3.8 Application of inoculum on large scale under submerged condition:

3.8.1 Application of fungal inoculum in earthen vat.

Fungal crude enzyme extract from *A. niger* and *A. flavus* separately retted whole green jute ribbon at 11 days with A grade fiber of bright cream color, (Table 34) Fig. 42. Similarly both *Sporotrichum sp.* and *Sclerotium sp.* type 1 completed retting at 12 days and produced B grade fiber with cream color in appearance. Four fungal combination treated green jute ribbon retted at 9 days and was earlier than all other fungal treatments with production of A grade fiber with bright cream color. Moreover, this fungal combination completed retting within half of the time than that of the control. Three fungal (*A. flavus.* + *Sporotrichum sp.*+ *Sclerotium sp.* type 1) and two fungal (*Sporotrichum sp.* + *Sclerotium sp.* type 1) combinations required almost same time i.e. 10 and 11 days producing A and B grade fiber with bright cream and cream color respectively. It is evident from the data that green jute ribbons treated with various combinations of fungi completed retting within 9-12 days while in control it took 18 days.

Table 34. Application of fungal inoculum in whole green ribbon retting in earthen vat (chari) at normal temperature.

SL	Treatment	Green ribbon (g)	Inoculum (g)	Enzyme extract (ml)	Retting time (days)	Fiber (g)	Bundle strength (Ibs/mg)	Fiber color	Fiber grade
1	*A. niger*	3000	300	1500	11	1600	9.98	Bright cream	A
2	*A. flavus*	3000	300	1500	11	1656	10.00	"	A
3	*Sporotrichum sp.*	3000	300	1500	12	1615	9.60	Cream	B
4	*Sclerotium sp.* type 1	3000	300	1500	12	1609	9.00	"	B
5	*Sporotrichum sp.* +*Sclerotium sp.* type 1	3000	300	1500	11	1585	9.00	Cream	B
6	*A. flavus*+ *Sporotrichum sp.* +*Sclerotium sp.* type 1	3000	300	1500	10	1580	10.20	"	A
7	*A. niger* + *A. flavus*+ *Sporotrichum sp.*+*Sclerotium sp.* type 1	3000	300	1500	9	1570	10.49	Bright cream	A
8	*Control*	3000	300	1500	18	1800	6.86	Cream off white	C

Fiber weight of jute varied from 1570 to 1656g in the fungal treated green ribbon (Table 34) However, the weight remains to 1800g where no fungal inoculums was added. The weight of fiber ranged between 1600 and 1656g where individual fungus was added. The order of weight of fiber due to fungal treatment was as follows: *A. flavus* (1656) > *Sporotrichum sp.* (1615) > *Sclerotium sp.* type 1> *A. niger* (1600).

However, the weight of fiber recorded was slightly lower where fungal mixture was added and the value varied between 1570 (*A. niger* + *A. flavus* +*Sporotrichum sp.* + *Sclerotium sp.* type 1) and 1585g (*Sporotrichum sp.* + *Sclerotium sp.* type 1). The results revealed that the overall variation in weight of fiber among the fungal treatments was not appreciable. The best weight may be considered to be 1800g in the control and 1570g recorded in treatment where all the four fungi were added.

The bundle strength of the jute fibber was found to be influenced by fungal treatments and varied between 9.00 to 10.49 Ibs/mg (Table 34 Figs.42 and 43). The bundle strength of the fibber obtained from control treatment was 6.86 Ibs/mg. Highest bundle strength (10.49 Ibs/mg) was found in fibber collected from whole green ribbon treated with four fungi together and this was followed by a combination of three fungi (10.20 Ibs/mg). Almost close bundle strength of fibber (10.00 Ibs/mg) was recorded for treatment where only *A. flavus* was inoculated.

Fibber grade improved due to fungal treatment of green jute ribbons from C(control) to higher grades (Table 34). Species of *A. niger* and *A. flavus* alone and the combinations of three and four fungi produced A grade fibber while all other fungal treated green jute ribbons produced B grade fibber.

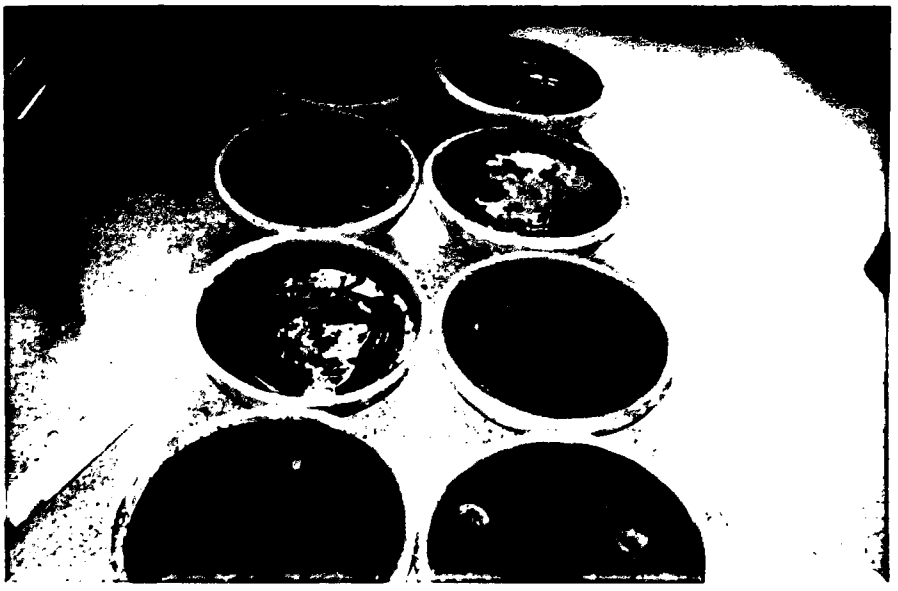


Fig. 42: Large scale retting of whole green ribbon with fungal inoculum in earthen vat

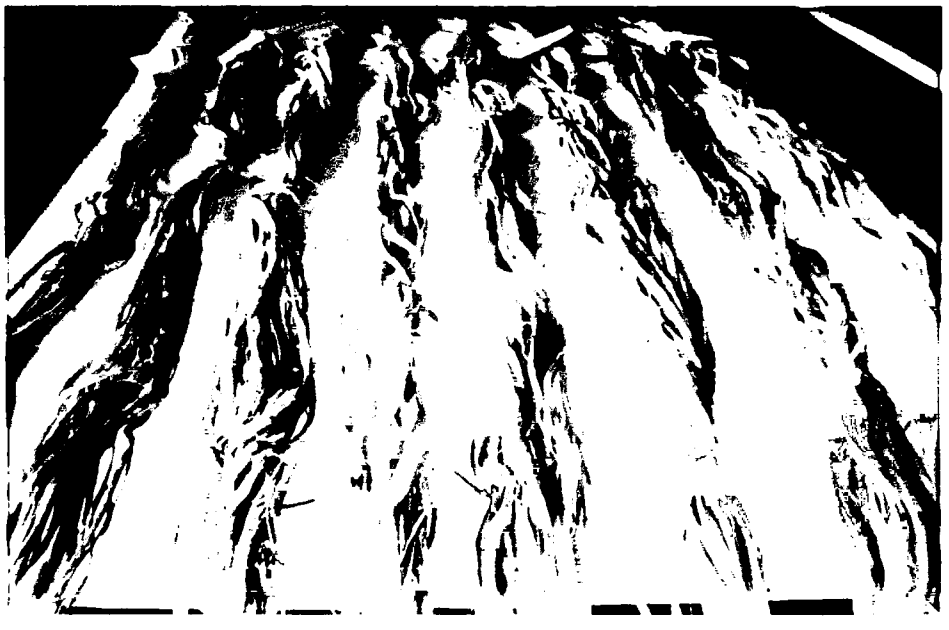


Fig. 43: Whole green ribbon retted fiber

Chapter 4
DISCUSSION

DISCUSSION

Jute is the major cash crop of Bangladesh but due to the paucity of retting water, cultivation of jute become troublesome for the farmers particularly in northern part of the country such as Rangpur, Kushtia, Dinajpur, Rajshahi. As an attractive alternative option is the retting of green jute ribbons (instead of jute plants) under moist conditions.

Jute is lignocellulosic material. The main constituents are hemicellulose (xylan), cellulose and pectin. Retting involves the separation of fibers from the pectic materials by the enzymatic action of microbes. Mesophillic fungi are potent lignocellulose degraders and secrete complex mixture of cellulose and xylanase concurrently (Sandana *et al.*, 1980; Dersochers *et al.*, 1981, Stewart and Hepstinall, 1988). For the improvement of fiber quality higher xylanolytic and lower cellulolytic fungi are needed under both moist condition and submerged conditions. It is to be mentioned that jute ribbons unlike jute plants will need less space as well as water even for submerged conditions.

In the present study, 21 fungi were collected from different sources of jute environments searching for as a part of potential jute retter. Of 21 fungi 10 fungi namely *Aspergillus niger*, *Aspergillus flavus*, *Macrophomina sp.*, *Botryodiplodia sp.*, *Sclerotium sp.* type I, *Sclerotium sp.* type 2, *Trichoderma sp.*, *Mucor sp.*, *Fuserium sp.* and *Sporotrichum sp.* were tested for retting enzymes under moist conditions. Wheat bran is indigenous and cheap and an ideal source of carbon and nitrogen. It provided the organisms a suitable medium to produce hydrolytic enzymes in large amounts using negligible amount of water 50% for their growth at 30°C. After 3 and 6 days of incubation, fermented slurry was collected to assess their retting ability. All the 10 isolates produced xylanase, CMCase and pectinase enzymes. Concentration of soluble protein and reducing sugar were also determined which showed good correlation for growth and enzymes production respectively.

In our study, out of the 10 screened fungi, only four fungal strains exhibited very good xylanase, pectinase and lees amount of CMCase activity through formation of clear zones around their colonies. They also exhibited a little CMCase activity which is suitable for not affecting or degrading the cellulose fiber of jute itself.

In order to study, the morphological feature and growth patterns, the fungi were grown on PDA. The fungus started with whitish mycelial growth at first and gradually turned into dark black (*A. niger)*, light green (*A. flavus*), white mat (*Sporotrichum sp.*) and white cottony (*Sclerotium sp.* type 1). In this study, it was found that the selected fungi confirmed its reported characteristics through formation of clear zones during screening test (Fig 4a). A sharp clear zone around the colony of *A. niger*, *A flavus*, *Sporotrichum sp.* and *Sclerotium sp.* type I on PDA plates having xylan as carbon source indicated its ability as producer of xylanase activity. *Trichoderma viride* has been found to produce xylanase in wheat bran medium at sixth day of incubation (Gomes *et al.*, 1993), Purkarthofer *et al.*, (1993), obtained highest xylanase activities at 7^{th} days of inoculation an wheat bran medium also. Appreciable amount of xylanase activity was demonstrated by the fungi on retting which promoted liberation of hemicelluloses from this jute ribbon. Xylanase activity was observed to increase after 6 days compared to 3 days by *A. flavus, Macrophomina sp.*, *Sclerotium sp.* type 1 and *Sporotrichum sp.*. They showed higher activity than the others. *A. flavus* showed the highest xylanase activity (396.81 U/g at 3 days and at 6 days 1382 U/g) as compared to the others. The second highest was produced by *Macrophomina sp.* which was 1164.81 U/g at 6 days of incubation.

CMCase activity of the ten isolates reduced after 6 days of incubation. This is better not to hydrolyze the jute fiber itself. Incubation time for maximum enzyme production varied with the type of organism. Maximum xylanase and cellulase production by *A. niger* has been reported at fifth and sixth days of cultivation, respectively (Wong *et al.*, 1994). While Anand *et al.*, 1990, reported a sharp increase in xylanase activity after 12-14 days of growth with their strain of *T. lanuginosus.*

The pectinase activity of the ten isolates of fungi at 3 days showed a variation among them 0.72 U/g (*Botryodiplodia sp.*) to 1.90 U/g (*Mucor sp.*). The order of efficiency in pectinase activity was found to be changed at 6 days. The activity ranges between 1.00 U/g (*Botryodiplodia sp.*) and 2.90 U/gm (*Sclerotium sp.* type 1). This increase in pectinase activity helped in the liberation of pectin from the ribbons during the progress of retting.

Ali and Islam (1965) found that enzymes polyglacturonic acid and pectin methyl esterase of the *Penicillum frequentants* macerated potato discs; jute bark and jute stem and also found that during retting the pectic cementing material was removed by pectinase enzyme. The non-fibrous part of the bark was found dissolved by pectic hydrolytic enzyme.

Jute ribbon consists of both fibrous and non-fibrous matter. During retting by hydrolytic enzymes the non-fibrous matter went under decomposition, the fibrous matter from the fiber got released from the non-fibrous matter. Basak in 1987 reported a microscopic examination of barky jute and enzyme treated jute and found huge aggregation of non fibrous debris mainly pectin on the epidermis which masked the fiber strands. They further reported that enzyme treated fibers were clearly visible with small amount of non fibrous aggregation and their findings correlate with the decrease percentage of non cellulosic materials of enzyme treated fibers. In this study, it was found that fungal single, di of tri combination retted green ribbon and removed non-fibrous material due there pectolytic and xylanolytic activities.

Moisture is one of the important factors for the growth of microorganisms and production of hydrolytic enzymes required for jute retting. The success of retting under SSF is, in particular, highly dependent on the optimum level of moisture. In this study inoculum was produced on wheat bran medium at different moisture levels such as 30%, 40%, 50%, 60% and 70% using four fungal isolates in SSF for green jute ribbon retting .The 4 isolates (*A. flavus, A. niger, Sporotrichum sp.* and, *Sclerotium sp.* type 1) showed their highest xylanse activity at 50% moisture levels (199.57, 198.57, 193.10 and 304.65 U/ml respectively (Table 6 and Fig 10). Shakila (1995) and Talukder

(2007) also found that the activity of xylanase by fungi *Thermomyces lanuginosus* and *Aspergillus sauzae* was maximum at 50% moisture level in wheat bran medium at SSF condition.

In all these isolates, pectinase activity increased with the increase of moisture level up to 50% but a lower result was obtained when moisture level was increased 70% (Table 9, Fig 12). Among 4 fungi, the maximum was of *A. niger* (1.29 U/ml) and then *Sclerotium sp.* type 1(1.23 U/ml). *A. flavus* (1.19 U/ml) and *Sporotrichum sp.* (1.10 U/ml) were found respectively.

The moisture in SSF is a critical factor that determines the success of a process observed by (Fenik sova, 1960). The importance of moisture level in SSF media and its influence on microbial growth, enzyme production and product biosynthesis may be attributed to the impact of moisture on the physical properties of the solid substrate (Nirahara, 1982). But excess moisture causes decreased porosity, alterations in the substrate particle structure, lower oxygen transfer and enhancement of the formation of aerial mycelia (Raimbault and Alazard, 1980). Feniksova, 1960 reported that lower moisture level leads to reduced solubility of the nutrient of the solid substrate, lower degrees of swelling and a high water tension.

Influence of natural flora on retting in presence of inoculum showed that non sterilized ribbon retted earlier (8 days) than sterilized ribbon (retted in 10 days) where the fungi was used singly or mixture of two fungi (*Sporotrichum sp.* + *Sclerotium sp.* type 1) and three fungi (*A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type 1) that retted ribbons within 8 days and *Sclerotium sp.* type 1 alone retted at 9 days respectively.

In the sterilized condition or retting speed was not much accelerated when inoculums of *Sporotrichum sp.*, alone and four fungal mixtures, retting took place at 10 days in both cases of non sterilized and sterilized condition, whereas sterilized ribbons showed delayed retting of 10-12 days than fungal treated ribbon in both the cases of non sterilized and sterilized condition of retting. It may be the result of influence of local

fungi which retted the non sterilized ribbon earlier than sterilized one. Fiber strength (bundle strength lbs/mg) was also found higher in non sterilized condition.

The mechanism of enzyme action during fermentation, xylanase activity in solid state fermentation after 3, 7 and 9 days of inoculation generally decreased very sharply with time in all combination of the fungi used (Table 12). Xylanase activity was comparatively better where fungi were applied singly in comparison to their multiple cultures when 4 days inoculums were used. Xylanase activity was relatively lower in case of multiple fungi was applied. Among the mixture, the combination of *Sporotrichum sp.* and *Sclerotium sp.* Type 1 was the best for xylanase activity

Xylanase activity under submerged condition inoculated for 3, 7 and 9 days also followed the same trend as in case of solid state condition. Here also the activity of xylanase enzyme decreased 52 U/ml at 3 days 4.60 at 9 days with time in case of *Sporotrichum sp.* and in the case of single fungal inoculums showed the same trend of result. Activity of CMCase was maximum at 3 days and decreased very sharply irrespective of conditions of inoculations that is green ribbon at submerged conditions. Under submerged condition of inoculation, the maximum activity was recorded for *Sclerotium sp.* type I (0.57 U/ml) but combination of four fungi resulted maximum activity (3.85 U/ml) under submerged condition. CMCase activity in both the conditions of single fungus or combinations at 3, 7 and 9 days of inoculation decreased with time. Activity of CMCase was maximum at 3 days and then decreased very sharply irrespective of conditions of inoculations.

The experiment of green ribbon retting in SSF condition we worked first in such a detailed way. It was evident from the experiments that retting was being accomplished by the enzyme produced by fungi and fungal retting was found quicker than normal bacterial (submerged) retting. Like our experiment, Echndi and Walker (1957) observed the same phenomenon in the case of retting with *Sclerotium rolfsii*. From the observation of our study of both solid and submerged retting it was evident that the retting action could be accomplished by the extra cellular enzymes like xylanase, CMCase and pectinase of the fungi. So, for the retting of green jute ribbons, all these

enzymes primarily acted together were responsible. Green jute plants contained cellulose, xylanase, hemicellulose, xylose, galactose, pectin, fat, sugars, lignin etc. were decomposed and removed. For the decomposition of the above constituents, enzymes such as xylanase, CMCase and pectinase worked well in the process of retting.

Both pectinase and pectinlyase combindly retted the green jute ribbon within the above mentioned time in solid and submerged conditions. In solid state, using 4 days inoculum retted earlier than submerged condition of retting. Fungal retting showed 11 days earlier than the control. In SSF condition better production of retting enzyme was observed probably because in some areas of ribbon, enzyme produced more where fungal growth was maximum as a result unequal distribution of retting enzyme was produced and fiber become some sort of deteriorated in quality than submerged retted fiber and other areas of fiber also.

Pectin lyase activity at pH 8.5 was increased after 6 days of inoculation and thereafter a decrease was observed in case of all fungi. Pectin lyase activity measured under submerged condition at pH 5.5 showed similar trends as was observed in green ribbons retting at solid state condition. Pectin lyase activity generally decreased with time in most of the cases except *Sclerotium sp.* type 1 where the activity increased up to 6 days of inoculation and then a fall was recorded. Single inoculation of the fungus achieved better Pectin lyase activity then their combined inoculation except *Sclerotium sp.* type 1.

Pectin lyase activity of the fungus at pH 8.5 under submerged condition also revealed a decrease in activity after at three days of inoculation. Except *A. niger* an increase in Pectin lyase activity at 9 days of inoculation was recorded where *A. flavus, Sporotrichum sp.* or a combination of 4 fungi. Pectinase activity of the fungi at 3,7 and 9 days during solid state retting of green jute ribbon showed a gradual decrease with time. Highest activity (2.0 U/g) was measured where all the 4 fungi were inoculated together. The lowest activity was encountered to the combination of two fungi (1.8 U/g). Under submerged retting fiber produced better than solid state fermentation

because, here both enzyme and inoculum was used besides here the entire enzyme particle are able to ret the solid non fibrous matter through diffusion process. In SSF process an insoluble substrate is fermented with sufficient moisture, but without free water. Fungi have been used for large-scale production of hydrolases by SSF, since they grow on relatively dry substrates on which bacteria cannot easily grow (Hesseltine, 1972). This process is slower because of diffusional barriers imposed by the solid nature of the fermented mass (Charles & Gavin, 1977).

In this experiment, under submerged condition, it found that pectinase production was almost same to 1.7 to 1.8 U/g at 7 days of inoculation. Enzymatic activity increased markedly while fungi were inoculated in combination from 2 species to 3 species together accounting 2.10 U/g to 2.13 U/g of pectinase at 7 days of inoculation.

The age of inoculum had a moderate effect during SSF retting. Three days inoculum was found to accelerate the production of exo-xylanases and endo-mannanase while xylanase and CMCase were best produced with 4 days old culture. (Mahmud, 2001). The highest amount of reducing sugar and soluble protein was found with 5 and 3 days old culture also reported by Mahmud,(2002).

Combination of all the fungi together showed the best performance of CMCase (3.859U/ml) under submerged condition and a combination of 3 fungi ranked to the second position (2.6263 U/ml). Pectinase activity in green ribbon in solid state condition at pH 5.5 showed a general decrease with time attaining the maximum at 3 days of inoculation. This was followed by a gradual decreased up to the end of the inoculation. Pectin lyase activity measured at pH 8.5 was increased up to 6 days at inoculation and there after a decrease trend was observed in case of all fungi.

For the improvement of fiber quality higher xylanolytic and lower cellulolytic fungi are needed. Hoq *et al.*, 1992, reported that the production of thermostable cellulose-free xylanse form *Thermomysis lanuginosus* has been investigated to optimize the production process in both solid state and submerged cultures. Choudhury (1951) observed that Pectinase and protopectinase in retting jute took much shorter time

without any injury to the fiber and the pectins' were divided in to three groups; a) Proto-pectin b) Pectins' and c) Pectic acids. Proto-pectinase was hydrolysed to pectinase and then pectinase was hydrolysed to galacturonic acid residues, were formed.

It is evident that fungal inoculation increased cellulose level in the jute fiber. Cellulose content in jute fiber varied from 59.78 to 65.87% due to treatment of *Sporotrichum sp.* and *A. niger* respectively in SSF condition. The range of cellulose content varied between 62.00% and 63.64% caused by *A. niger* + *A. flavus* + *Sclerotium sp.* type 1 and *Sporotrichum sp.* respectively. Cellulose content observed higher in SSF condition. Hemicellulose content in jute fiber revealed to be lower in general while retting was completed under submerged condition in comparison with SSF condition. Lignin and pectin content measured in jute fiber both in fungal treated solid and submerged fiber and control and found much reduction than control. So from the study of fungal and control treatment of both solid and submerged condition cellulose was found much more than control where no fungi were used and lignin and pectin content was found reduced in the case of treated fiber. The chemical constituent of jute fibre normally was cellulose 60.0%, lignin 13.5%, protein 1.0%, Hemicellulose 24.0%, Fat and Wax 0.5% and pectin 0.2% and minerals 0.8% was observed by Sengupta, (1947) in jute fiber.

Synthesis of xylanase and CMCase commenced within 72 hours and were reached within 6 days by all four fungi. Xylanases and cellualse are inextricably linked. Both cleave β-1, 4-glycosidic bonds by similar catalytic mechanism and are, therefore, closely related at the functional level. Furthermore, with few exceptions xylan degrading organisms are invariably cellualolytic and often secret complex mixture of cellulase and xylanase concurrently (Sandana *et al.,* 1980; Dersochers *et al.,* 1981; Stewart & Hepstinall, 1988). Cellulase and xylanase of microorganism reported so far are mainly from mesophilic fungi. For improvement of fiber quality higher xylanolytic and lower cellulolytic fungi are needed.

Fungal CMCase (Coughlan, (1985) and xylanase (Dekker and Richards, 1976; Vikari *et al.*, 1993) generally have lower pH optima for activity than those from bacteria.
In our study the optimum pH the enzyme of *A. niger, A. flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1 seems consistent with these. The pH optimum of xylanase and CMCase for *A. niger* optimum pH for the component of enzyme extract of *A. niger* were 4.5. Talukdar, 1997 reported that activities were measured at 40°c for 20 minutes incubation at pH 3-9 but found at pH 6-7 and temperature. Eshaque ,1991 also observed that fungus *Corticium rolfssii* grow on potato dextrose both at pH 4.5 (at 35°c) were found best for enzyme activates of retting green jute plants, green and dry ribbons and jute cuttings. Shakila 1995, found in wheat bran inoculum that the enzymes from both strains of *Thermomyces lanuginosus* were found highly stable at 50°C. Xylanase and CMCase retained more than 95% activities after 6 days exposure at 50°C. The enzymes retained more 80% of their original activities after heating at 60°C for 72 hours. Xylanase and CMCase were also sufficiently stable at 70°C for several hours.

In the study, the pH optima for xylanase of *A. niger* were between pH 4.5 and 5.5. The pH optimum for xylanase of *A. falvus*, *Sporotrichum sp.* and *Sclerotium sp.* type I were between pH 4.5 & 6.5. The pH optima for CMCase of *A. niger, A. flavus*, *Sporotrichum* sp. and *Sclerotium sp.* type I was between pH 3.5 and pH 5.5. Pectinase activity was found maximum at pH 4.0-5.5 by all four fungi. The activity was drastically inhibited at pH 9.0 and above.

pH of the growth medium has a significant effect on the synthesis and activity of different enzymes and may also be connected with growth and cell lysis, accumulation of reducing sugars, secretion of extracellular proteins etc. The culture pH 7-9 at 30^0C and pH 6-9 at 50^0C were found suitable for production of extracellular enzymes.

Xylanase of *A. niger, A. flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1were highly stable at pH range between 3.0 and 8.0 and rapid deactivation of xylanase was observed after pH 9.0. CMCase of *A. niger* was stable in the pH range between 3.0 and 8.0. The optimum temperature for enzyme activities of *A. niger, A. flavus,*

Sporotrichum sp. Sclerotium sp. type 1 were assayed over the temperature range of 40-80°C. Maximum activity of xylanase of both *A. niger* and *A. flavus, Sporotrichum sp., Sclerotium sp.* type 1 were found at 50°C. At 50°C, the activity of xylanase was found maximum. CMCase also showed highest activity at 50°C. But pectinase activity observed maximum at 50°C.

During the study, the optimum temperature for enzyme activities of *A. niger, A. flavus, Sporotrichum sp., Sclerotium sp.* type 1 was assayed over the temperature range of 40°-80°C. CMCase of *A. niger* and *A. flavus*, showed maximum activity at 50°C. Pectinase activity was observed maximum at 50°C of *A. niger, A. flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1.

Highest xylanase titers in fungal systems were reported to occur generally at temperatures that are optimum for cultures in SSF (Sudgen and Bhat, 1994 and Biswas *et al.*, 1990).

Several workers (Kluepfel *et al.*, 1986; Wang *et al.*, 1992; Okeke *et al.*, 1992) reported the optimum temperature for xylanase and cellulose activities in the range of 55°C - 60°C. Xylanases and CMCase of fungi *A. niger* and *A. flavus*, *Sporotrichum sp.* + *Sclerotium sp.* type 1 were showed maximum catalytic activities at 50°C. The enzyme activities were drastically decreased with the increase of temperature which indicates rapid inactivation of the enzymes. The optimum temperature of xylanase production by *Thermomyces lanuginosus* MH4 resembles to that the *Thermomyces lanuginosus* RT7 as reported by increased the release of extracellular protein, but it had no significant effect on the enzyme activity. Similar result was obtained by Alam *et al.*, (1994) with their strain of *T.langinosus* RT7 on wheat bran under solid state fermentation. Ilias, 1997, obtained cellulose-free thermo stable xylanase from thermophilic fungi *Themomyces lanuginosus* MH4 isolated from jute compost. *Thermomyces lanuginousus* Tsiklinskaya found capable of producing cellulose free thermostable xylanase in shake culture was observed by Gomes *et al.*, (1992), Talukdar (1997) found optimum temperature for the maximum activities of CMCase, pectinase and xylanase were 50°C, 40°C and 48°C respectively.

It has been found that temperature increase in composting systems; the number of viable fungi rapidly declines and disappears completely at 65°C (Finstein and Morris, 1975). Both mesophilic and moderately thermophilic fungus have been widely reported in self heating materials (de Bertoldi *et al.*, 1983, Kane and Mullins, 1973, Tansey, 1971, Stutgenberger *et al.*, 1970 and Chang 1967). In the preset study, all four fungi (*A. niger*, *A. flavus*, *Sporotrichum sp.* and *Sclerotium sp.* type 1 generated heat during incubation time particularly at 3rd and 4th days of incubation.

Xylanase of *A. niger, A. flavus, Sporotrichum sp.* and *Sclerotium sp.* type 1 were highly stable at pH range between 3.0 and 8.0. A rapid deactivation of xylanase was observed at pH 9.0 to above. *A. flavus* was highly stable at pH range between 4.0 and 9.0 but rapid deactivation of xylanase was observed at pH 3.0 to 8.0 was drastically inhibited at pH 9.0 and inhibited at pH 3 and pH > 8.0. Like xylanase pectinase activity was also observed stable in the pH range 3.0 and P^H 10. Deactivation of pectinase was found at pH 9.0 and above.

The ultimate goal of the study was production of quality fiber through fungal application. Whole green ribbon in submergence condition with mixed inoculum of four fungi showed a great promise of whole green ribbon retting at 9 days with 'A' grade fiber. *A. niger or A. flavus* separately retted green jute ribbon at 11 days producing "A" grade fiber. Similarly, *Sporotrichum sp.* and *Sclerotium sp.* type I completed retting alone at 12 days but fiber grade was "B". Three fungal (*A. flavus* + *Sporotrichum sp.* + *Sclerotium sp.* type I) and two fungal treatment (*Sporotrichum sp.* + *Sclerotium sp.* type I) retting took nearly same period of 10 and 11 days with "A" and "B" grade fibers. Bundle strength was highest (10.49 Ibs/mg) with 4 fungal treated samples of bright cream color fiber. Fungal treated fiber obtained superior and retted 2 days earlier than control.

CONCLUSION

CONCLUSION

Jute ribbon retting is a practical alternative to whole jute plants retting due to requirement of less space and volume.

Fifty percent (50%) moisture only required for ribbons retting under moistened (SSF) conditions or 1:3 (v/v) or three fold water required under submerged condition where as 1:20 volume of water requires for conventional retting.

It is revealed that the four fungal strains are found to be suitable for retting of green jute ribbons.

Wheat bran, as very cheap medium, was used successfully for producing retting inoculum. Retting enzymes such as xylanase, pectinase and cellulase were produced satisfactorily in the medium by the fungi. The retting enzymes were found stable under wide temperatures and pH ranges which are suitable for retting under varied environmental conditions. The retting system may be monitored by addition or reduction of moisture and temperature levels.

Hence, the retting process of moistened green jute ribbons has profound importance in respect of the scarcity of water to jute growers of Bangladesh.

Chapter 5
BIBLIOGRAPHY

BIBLIOGRAPHY

Ahmed, Z., Akhter, F., & Alamgir, M.1999 (June). Isolation of sound jute retting fungi from natural source and their effect on dry ribbon retting of CVL-I jute (*Corchorus capsularis*). Bang. J. Sci. Res. **17**(1): 107-109.

Ahmed, M. 1963. Studies on jute retting bacteria. J.Appl. Bacteriol., **26**:117-126.

Akhter, F., Mondal, R., & Asaduzzaman, M. 1999. Influence of various moisture levels and bacterial inoculum on retting of jute. J. Phytol. Res., India .**12**(1-2); 59-63,

Akther, F., Asaduazzaman, M. & Mondal, R. 2001. ``Use of indigenous material for the removal of shaymla colour of jute fiber (*C. capsularis* var. CVL-1) Journal of Phytological Research, India. Volume **14 (1- 2):** 62-66

Alam, S.M. 1970. Jute retting bacteria from certain ditches of East Pakistan. Pak. J. Sci. Indus. Res., **12**: 229-231.

Ali, M. Alam, M. S., Eshaque, A.K.M. & Khandakar, A.L. 1976. Studies on the Mechanization of Jute retting, Ban.J.Jute Fib. Res., **1**(2): 61-70.

Ali, M.M., Sayem, A.Z.M. & Eshaque. M. 1972. Effect of neutralization of retting liquor on the progress of retting and quality of fiber. Sci. Ind., **7**: 134-136.

Ali, M.M., 1958. Aerobic bacteria involved in the retting of jute. Appl. Microbiol., **6**: 87- 89.

Ali, M.M., & Islam, A. 1963. Pectic enzymes of *Penicillium frequentans* involved in the retting of jute. Pak. J. Sci. Indus. Res., **8**: 47-51.

Ali, M. M., & Alam, S. 1973. Effect of different pH values on jute retting. Ban. J. Bot. **2**: 19-23.

Ali; M. M., & Islam, M. A. 1965. Pectic enzymes of *Penicillium frequentance* involved in the retting of jute. Pak. J. Sci. & indus, Res. **8**: 47-51.

Ali, M.M., Sayem, A.Z.M. & Eshaque, M. 1970. Effect of neutralization of retting liquor on the progress of retting. Science and Industry, **7**: 134-136.

Alam, M., Gomes, I., Mohiuddin, G., & Hoq, M. M. 1994. Production and characterization of thermostable xylanases by *Thermomyces lanuginosus* and *Thermoascus aurantiacus* grown on lignocellulose. Enzyme Microbioal Technol. **16**: 298-302.

Alexopoulos, C.T. & Nims, C.W. 1979. Introductory mycology, 3rd ed., John Wiley and Sons, New York, 2-17.

Anonymous, 1968. Bangladesh Jute Research Institute (Agriculture), Dhaka-1207, Bangladesh.

Anonymous, 1969. Bangladesh Jute Research Institute (Agriculture), Dhaka-1207, Bangladesh.

Anonymous, 1970. Bangladesh Jute Research Institute (Agriculture), Dhaka-1207, Bangladesh.

Anonymous, 1971. Bangladesh Jute Research Institute (Agriculture), Dhaka-1207, Bangladesh.

Anand, J. & Vithyathi, P.J. 1990. Production of extracellular xylanase by thermophilic fungus *Humicola lanuginosus*. Experi. Biol. **28**: 434-437.

Asaduzzaman, M. S. Alam, Eshaque, A. K. M. & Ali, M.M. 1985. Relationship between plant maturity, lignin-pectin content and quality of jute fiber. Bangladesh J. Jute Fib. Res. **10**: 49-53.

Barnett, H.I., & Hunter, B.B. 1972. Illustarted genera of imperfect fungi, 3rd ed., Burgess Publishing Co., Minneapolis, 244.

Barenger, J. E., Frixon, C., Bigliardi, J. & Creuzet, N. 1985. Production, purification and properties of thermostable xylanase from *Closridium thermocellum*. Can. J. Microbiol. **31**: 635-643.

Bailey M.J., Bailey, P. & Poutanen, K. 1992. Introductory testing methods for assay of xylanase activity. J. Biotechnol.23: 257-270. Cannel, E. and Moo. Yong. M.1980. Process Biochem. **15**: 2-28

Bastawde, K. B. 1987. studies on xylanase by *Chinia sp.* Ph. D. thesis, Pune University, Puna, India.

Basak, M. K., Ghosh, S. K,. Jain A. K &. Ghosh A. 1993. Efficiency of microbes in softening of jute root cuttings. The Indian Textile J. **43**: 74-79.

Bateman, D. F. 1963. The macerating enzymes of *Rhizoctonia solania.* Phytopathology, **53** : 1178-1186.

Bateman, D. F. 1964. The cellulase and *Rhizoctonia sp.* infected disease of bean. Phytopathology, **54**: 1372-1377.

Bateman, D. F. 1968. The enzymatic maceration of plant tissue. Netherlands J. plant pathology, 74 (suppl).

Bashiruzzaman, M., Mian, A.J. & Haque, M.M. 1964. The chemical constituents and molecular weights of cellulose in different parts of Jute fiber. U. Textile Res. **34**: 910-911.

Baruah, P. & Baruah, H. K. 1944. Retting by Hipparol and its commercial applications. Science and culture, 10, P01-204.

Basak, M.K., Sao, K. P. & Bhaduri, S.K. 1987 Scanning electron microscopic studies on microbial softening of barky jute. Ind. J. Textile Res. **12**: 154-157.

Belily, P., Mislovicova, D. & Toman, R. 1985. Soluble chromogenic substrates for the assay of endo-1, 4- β-xylosidase and endo-1, 4, β-glucosidase. And Biochem. **144**: 142-146.

Bhattacharyya, S. K. 1973-1974. Retting of jute- A key process that needs more attention. Jute Bulletin. 194-198. Jute Technological Research Laboratories, I.C.A.R. Calcutta-700-040

Bhattacharrya, S. K. & Basu, M. K. 1981. Quality improvement of retted jute by Fungal Culture. Jute Technological Research Laboratories, I.C.A.R., Calcutta-700-040. Jute Development J., **17** :22-24.

Bhattacharja, H. P. 1961. The role of lignin in jute fiber. Jute and Gunny Review. Vol-13, No-7: 254-259.

Biswas, A.C. 1968. Study of an active aerobic jute retting bacterium. Pak. J. Sci. Ind. Res. **11**: 370-372.

Biswas, S.R., Jana, S.C., Mishra, A.K., & Nanda, G. 1990. Production, purification and characterization of xylanase from a hyperxylanolytic mutant of *Aspergillus ochraceus*. Biotechnol. Bioeng. **35**: 244-251.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. (Analytical Biochmistry **72**: 248- 254)

Callow, H.J. Determination of lignin content of jute. Texi, J. Inst. **41**, 255-266 **(1952).**

Chang, Y. 1967. The fungi of wheat straw compost II. Biochemical and physiological studies. Trans. Br. Mycol. Soc. **50**: 667-677.

Charles, M.& Gavin, J. R. 1977. Symposium on Indigenous Fermented Foods (GIAM 5) .Bangkok, Thailand. 21-26.

Chi, C. Y., Sun, I. M. & Wang, T. F. 1966. Jute in Taiwan. Rep. Taiwan Fiber Crop. Exp. Station Bul. No. **27**: 1-11.

Chaudhuri, S.D. & Ahmed, Q.A. 1968. Softening of jute cuttings by bacterial and fungal organisms and its commercial feasibility. Jute and Jute Fabrics Pakistan, **VII**: 230-233.

Chaudhury, S. D. 1951. A short review of biochemical study on jute retting Pakistan J. Sci. **5**: 11-17.

Chaudhuri, S.D. & Ali, M.A. 1962. Report on survey of cost of production of Jute in East Pakistan (1958 and 1959). Pakistan central jute Committee, Tejgaon, Dacca-5, Govt. of Pakistan 228 .

Coughlan, M.P.Biotechnol. Genetlc Engng Rev. 1985. 3: 39-109.

Debsharma, G. D. 1976. Biochemical investigations on jute retting. Indian J. Agr. Sci., **16**: 453-458.

De Bary, A. 1984. comparative anatomy of the vegetative organs of the phanerogams and Ferns. Eng. Trans.Oxford.

Dekker, R.F.H. 1985. Biodegradation of the hemicellulose. In biosysthesis and Biodegradation of wood components, ed Higuchi, T., 505-533.

Dekker, R.F.H. & Richanrds, G.N. 1976. Hemicellulases, their occurrence, purification, properties and mode of action. Advances in Carbohydrate chemistry and biochemistry. **32**: 277-352.

De Bertildi, M., Vllini, G., & Pera, A. 1983. The biology of composting: A review. Waste Manage. Res. **1**: 157-176.

Desrochers, M., Jurasek, L., & Paice, M. G. 1981. High production of β-glycosides in *Schizophyllum commune*: isolation of the enzyme and the effect of culture filtrate on cellulose hydrolyses. Appl. Environ. Microbiol. **111**: 222-228.

Dorgan, K.S. 1971. Use of fertilizers in Jute production. I.C.A.R. Technical Bulletin (Agric.) No. 30. Indian Council of Agricultural Research, New Delhi, 98.

Eames, A. J. & Mac Davils, L. H. 1951. An introduction to plant anatomy. Mac Graw Hill Book Company, inc. New York, 427.

Echandi, E. & Walker, J. 1957. Pectolytic enzymes produced by *Sclerotium sp.* Phytopathology **47**: 303-307.

Edgington, L. , Lorden ,V.& Dimond , A. 1. 1961.Resistance to fuserium will of tomato, phytopathology, **51** : 179-182.

Eriksson, K.E. & Jojnsrud, S .C. 1983. Mutants of the white rot *Funus sporotrichum pulvruluntum* with increased cellulose and b-glycosidase production Enzyme Microbial Technol. **5**: 425-429.

Eshaque, A.K.M Ph.D thesis 1982.Studies on the Jute retting enzymes from *Sclerotium Sp.* 126.

Esau, K. 1953. Plant Anatomy. John wily sons, Inc, New York, 753.

FAO, 1994: Jutc production (Agriculture section). Bangladesh Bureau of statistics. Website: http://www.cpd.org.bd/pub_attach/op78.pdf

Feniksova, R.V., Tikhorova, A.S., & Rakhleeva, B.E. 1960. Conditions for forming amylase and proteinase in surfacc culture of Bacillus subtilis. Mikrobiologica. **29**: 745-748.

Finstein, M.S., & Morris, M.1. 1975. Microbiology of municipal solid-waste composting. Adv. Appl. Microbiol. **19**: 113-151.

Fischer, K. D. 1965. Hydrolytic enzyme and toxin production by sweet potato *Fusaria.* Phytopathology, **55** : 396-398.

Flannigan, B & Sellers, P.N. 1977. Amylase, β-glucosidase and β-xylosidase activity of thermotolerent and thermophloic fungi isolated from barley. Transaction of the British Mycological Society. **69**: 316-317.

French, M. M. D. 1959. Experiments in the cultivation, harvesting and retting of jute in British Guiana. Trop. Sci., **1**: 231-285.

Frederick, M.M., Chin-hen, K., Frederick, J.R. & Reilly, P. J. 1985. Purification and characterization of endoxylanases from *Aspergillus niger* I two is enzymes active on xylan backbone near branch points. Biotechnol. Bioeng. **27**: 527-532.

Frendenberg, (1960) The chemistry of lignin, Accademy Press Inc. New York, pp. 532-533.

Ghose, T.K. & Bisaria, V.S. 1987. Measurement of hemicelluloses activities part 1: Xylanases. IUPAC. **59**: 1739-1752.

Ghose, S. N. & Bose, R. G.1973. Biodeterioration of jute, part-I, Nature of microbial growth. J. Text. Assoc., **34**: 11-16.

Ghosh, B.L. & Dutta, A. R. 1980. The enzymatic softening and upgrading lignocellulosic fibers. J. Tex. Inst., **71**: 108-116.

Gomes, I., Gomes, J. Steiner, W. & Esterbauer, H. 1992. Production of cellulose and xylanase by a wild strain of *Trichoderma viride*. Appl. Microbiol. Biotechnol. **36**: 701-707.

Gomes, J., Gomes, I., Esterabuer, H., Sinner, M. & Steiner, W. 1993. Production of high level of cellulose-free and thermostable xylanase by a wild strain of *Thermomyces lanuginosus* in laboratory and pilot scales using lignocellulosic materials. Appl. Microbiol. Biotechnol. **39**: 700-709

Gupta, P.C.D., Sardar, D. & Majumdar, A.K.1976. Chemical retting of jute. Food farming and Agriculture, **VIII**: 7-9.

Haarer, A. E. 1958. Decoration of stem fibers. Jute and Gunny Rev., **10**: 101-104.

Haque, M. S., Asaduzzaman, M., Alam, M. S. & Eshaque, A.K.M. 1989. Upgrading of SMR and seed-cut low-grade barky jute fiber through the application fo fungi. **14**: 53-58.

Haque M.S., Asaduzzaman M.., Akhter F., Hossain M.M &. Ahmed Z., 2001; Impact of Stem water ratio and separately retting the topand basal parts of jute on the quality of fiber. Pak J. boil. Sci. 4(10): 1191- 1193, 2001.

Haque M.S., Alam. S., Akhter F., Asaduzzaman, M. & Eshque A.K.M. 1992. Effect of different fungi on the retting of dry jute ribbons (*Corchorus capsularis* var. CVL-1) and fiber quality. Ban. J. Jute Fib. Res. **17** (1&2): 79.

Hancock 1964, 65. Enzymological aspects of some pathological processes associated with southern anthracnose, Spring black stem Stamphylium leaf spot alfalfa. Ph. D. Thesis, cornel University, 123.

Hancock, Joseph G & Miller R. G. 1965. Relative importance of polygalacturonate trans elimenase and other pectolytic enzyme in southern anthracnose, spring black stem, stamphylium leof spot of alfalfa. Phytopathology, **55** : 345-355.

Hagerdal, B.G.R, Ferchak, J.D., & Pye, E.K. 1978. Cellulolytic enzyme system of *Thermoactinomyces* sp. grow on microcrystalline cellulosic. Appl Environ Microbiol. **36**: 606-612.

Hesseltine, C.W. 1972. Biotechnol. Bioeng. **14** : 517-532.

Henderson, M.E.K. & Farmer, V.C. 1952. J.Gen. Microbiol, **12**: 37-46.

Hossain, Altaf, A..K.M. 1963. Studies of the population of certain microbes of Jute retting water. M.Ag. Thesis, Dacca University, Bangladesh, 1960.

Hoq, M.M., Alam, M.M., Mohiuddin, G. & Gomes, I. 1992. Enzyme biotechnology in developing countries: production an application of thermostable xylanase for jute fiber upgrading. In Harnessing biotechnology for the 21st century. Proceedings of the ninth International Biotechnology Symposium and Exposition. 568-573.

Ilias, M, 1997. Bioprocess for cellulase free xylanase production by *Thermomyces lanuginosus* MH4 (PhD. Thesis)/

Ishii, S. & Kiho, K. 1976. Evidence for a factor stimulates tissue maceration by pectolytic enzymes. Phytopathology, **66** : 1077-1081.

IUPAC Measurement of cellulose activities. Pure Appl. Chem. 1987.59:257-286.

Jalaluddin, M. 1965. Studies in jute retting aerobic bacteria. Econ. Bot. **19**: 184-393.

Jansen, E. F. Jang. R & Bonner, J. 1960. Bindings of enzymes to avena caleoptile cell-walls. plant physiol, **35** : 567-574.

Johansson, M. H. & Samuelson, O. 1977. Reducing end groups in birch xylan and their alkaline degradation. Wood Sci. Technol. **11**: 251-263.

Josyln, M. A. 1962. & Wood, 1960. The chemistry of protopectin : a developments. Advance Food Res. **11**, 107.

Kane, B.E., & Mullins, J.T. 1973. Thermophilic fungi in a municipal waste cmpost system. Mycologia. **65**: 1087-1100.

Kesker, S.S. Srinivasan, M.C. & Desphande, V.V. 1989. Chemical modification of a xylanase from a thermotolerent Streptomyces T7. J. Biochem. **261**: 49-55.

Kertesz, Z.I. 1957. The pectic substances. Inter Science publishers Inc. New York, 225 and 333-380.

Kerfesz, Z. E. & Mecolloch, 1950. Enzymes acting on pectic substances. Advances in carbohydrate chemistry, **5** : 79-103.

Kluefel, D., Shareck, F. & Morosoil, R. 1986. Characterization of cellulose and xylanase activities of *Streptomyces lividans*. Appl. Microbiol Biotechnol. **24**: 230-234.

Koneman, E.W., Roberts, G.D. & Wright, S.F. 1978, Practical laboratory of mycology, 2nd ed., Williums and Wilkins co., Baltimore, Maryland, USA.

Kundu, A. K. 1964. Factors influencing retting of jute. Jute Bull. December, 1964, **27** (9): 225-230.

Kundu, A. K. 1960. Studies on microbiological retting of Jute. D. phil. thesis, Cal. Univ. Rahman, A. 1963. A study of the flax retting members of the genus clostridium sp. Ph.D. University of leeds, 141.

Kundu, B. C. 1943. Anatomy of Jute Stem Forma Aion of net work of fibers. Bulletin issued by I. C. I. C. April, 1943 vol, **VIP**. 157-161.

Kunda, B.C., Basak, K. C. & Sarkar,P. B. 1952, Jute in India: a monograph. Indian Central Jute Committee, Calcutta, 112.

Linderberg, B., Rosell, K-G. & Svensson, S. 1973. Position of O-acetyl groups in birchwood xylan. Svensk. Pappersitidn. **76**: 30-32.

Lutzen, N.W., Nielson, K.M., Oxeuboellus, S. & Sternberg-Olsen, B. 1983. Cellulases: their application in the conversion of lignocellulose to fermentable sugars. Phil. Trans. Royal Soc. London. **300**: 283-291.

Majumder, A.K & Day, A. 1977. Chemical constitute of jute ribbon and the materials

removed by retting. Food farming and Agriculture.

Mandles, M. & Rees, E.T. 1960. Introduction of cellulose in fungi by cellobiose. J. Bacteriology. **79**: 816-826.

Mandels M. & D Stermberg. 1976. Recent advances in cellulose technology. J. Ferment. Technonl. **54**: 267-285.

Mahmud, S.A. 2002. Isolation and Screening of Microorgainsms capable of Degrading city organic wastes and the preparation of ''Mixed culture Inocula'' for the production of Bioorganic fertilizer.

McClendon, J.H., Somers, G.F. & Huberger, J.W. 1960. The occurrence of a variety of Enzymes hydrolyzing cell-wall poly-saccharides in applies rotted by *Botrysophaeria ribis*. Phytopathology, **50**: 258-261.

Miller, G.L. 1959. Use of DNS reagent for the determination of reducing sugar. Anal Chem. **31**: 426-428.

Mitscheriich, H. 1850. Ann. Chem. Pharm. **75**: 3056.

Mohiuddin, G.1992. A manual improved processing technique for low grade jute and cuttings. 6-19.

Mohiuddin, G: Talukder, S.H. & Hasib, A. S. 1981. Chemical constituents of Jute cuttings, Part I. Determination of pectin and wax. B.J. Jute & Fiber, Res. **6**(1 & 2): 77-80.

Mohiuddin, G., Chowdhury, I.M; Ruhuai Kabir, A.K.M, & Hasib, A.S. 1978. Lignin content of jute cuttings in the white and tossa varieties of Bangladeshi jute. B.J.Jute Fib. Res. **3**(1&2): 27-32. 1978.

Nirahara, H., Kayama, Y., Yoshida, T., Pichangkura, S., Ueda, R., & Taguchi, H. 1982. Growth and enzyme production in a solid-state culture of *Aspergillus oryzae*. J. Ferment. Technol. **60**: 311-319.

Okeke, B. C. & Paterson, A. 1992. Simultaneous production and induction of cellulolytic andxylanolytic enzymes in a *Streptomyces sp.* World Journal of Microbiology. **8**: 483-487.

Paice, M.G., Jurasek, L., Carpenter, M.R. & Smillie, L.B. 1978. Production characterization and partial amino acid sequence of xylanase A from *Schizophyllum commune*. Appl. Environ Microbiol. **36**: 802-808.

Paul, N. B. & Bhattacharyya, S. K. 1974. Upgrading of hard barky root cuttings of jute and mesta by a fungal culture. Indian J. Microbiol. **14**: 61-66.

Poutanen, K. 1988. Characterization of xylanolytic enzymes for potential applications (Ph.D. thesis) Technical Research Centre of Finland.

Puls, J. & Poutanen, K. 1989. Mechanism of enzymatic hydrolysis of hemicelluloses (xylan) and procedures for determination of the enzyme activities involved. In: Enzyme systems for lignocellulose degradation, edited by M.P. Coughtan Elsevier applied Science, London. 151-165.

Puls, J. & Schuseil, J. 1993. Chemistry of hemicellulose structure and enzymes required for hydrolysis, in hemicellulose and hemicellulases. (Coughlan, M. P. and Hazlewood, G.P. eds) 1-28.

Purkarthofer, H., Sinner, M. & Steiner, W. 1993. Cellulase free xylanase from *Thermomyces lanuginosus*: optimization of production in submerged and solid state culture. Enzyme, Microb. Technol. **15**: 405-410.

Rahman, A. 1963. A study of the flax retting members of the genus *Clostridium.* Ph.D.Thesis. University of Leeds. 141

Raimbault, M., & Alzard, D. 1980. Culture method to study fungal growth in solid fermentation. Eur. J. Appl. Biotechnol. **9**: 199-209.

Ryu, D.D.Y. & Mandles, M. 1980. Enzyme Microb Technol. **2**: 91-102.

Sarkar, P. B. 1963. The chemistry of jute lignin, part-II; Potash fusion of lignin, J. IndianChemical Soc., **10**: 263.

Sarkar, P. B. 1964. The chemistry of jute lignin, part III; Action of Nitric acid on lignin. J. Indian Chemical Soc., **11**: 407.

Sarkar, PB. 1931. J. Indian Chem. Soc. **8**: 397.

Saha, P.K., Paul, S.S. & Sarkar, P.B. 1952 Taxtile 79: 96.

Sandana, J. C. Shewale, J. G. & Despande, M. V. 1980. High cellobiase and xylanase production by *Sclerotium rolfsii* UV-8 mutant in submerged culture. Appl. Environ. Microbiol. **39**: 935-938.

Schuraz J., Billiani A., Honel A., Engner W.D, Janosi A; Hayn M. & Exterbauer H.

1985. Reactions mechanisms und strkturan derungen bein enzymatischem Abbau von cellulose, Acta polymeric **36**(2): 76-80.

Sen Gupta, J.C. 1947. Variation in osmotic values in jute plants. J. Indian Bot.Soc. 26: 157-175. Shei, J. C., Fratzke, A.R., Frederick, M.M., Frederick, J. R. & Reilly, P. J. 1985. Purification and characterization of endoxylanase from *Aspergillus niger* II. An enxyme of pl 4.5. Biotechnol Bioeng. **27**: 533-538.

Shakila N.K, 1995. Production and partial characterization of endoxylanase and endogluacanase by thermophilic fungi. (M.Sc. Thesis).

Siddiquellah, M. & Sen Gupta, A.B. 1958. Non-cellulose constituents and chemical modification of Jute. Jute and Gunny Rev. **10**: 1973-1981.

Stutzenberger, F.J., Kaufman, A.J., & Lossin, R.D. 1970. Cellu7lolytic activity inmunicipal solid-waste composting. Can. J. Microbiol. **16**: 553-560.

Stewart, J. C. & Heptinstall, J. 1988. Cellulase and hemicellulase from *Aspergillus fumigatus* Fresenuis, Methods in Enzymology. **160**: 264-274.

Sudgen, C., & Bhat, M.K, 1994. Cereal straw and pure cellulose as carbon source for growth and production of plant cell wall degrading enzymes by *Sporotrichum thermophile*. World J. Microbiol. Biotec6hnol. **10**: 444-451.

Talukdar S.H., Ph. D. thesis 1997. The enzymatic up gradation of jute cuttings.

Takanashi, S. & Tusjisaka, Y. 1973. Structure of oligosaccharides for the enzymatic hydrolysate of rice straw arabinoxylan by a xylanase of *Aspergillus niger*. Agriculture and Biological Chemistry. **37**: 1385-1391.

Tansey, M.R. 1971. Agar-diffusion assay of cellulytic ability of thermophilic fungi. Achieves of Microbiology. **77**: 1-11.

Tiaz, L. & Honigman, W.A. 1976. Production of cellwall hydrolyzing enzymes by barley aleurone layer in response to gibberelic acid. Plant physiology. **58**: 380-386.

Urabanek H., Zalewska-sobzak J., & Boriwinska, A. 1978. Isolation and properties of Extracellular Cellulase Hemicellulose complex of phoma herbernica. Arch. Microbial **118**: 265-269.

Viikari,L., Tenkanen, M., Buchert, J., Ratto,M., Bailey, M., Siikaho,M., Linko,M.1993. Hemicellulases for industrial applications. In: Saddler JN (ed), Bioconversion of forest and

agricultural plant residues, C.A.B. International, Wallingford, England,131± 182.

Wang, P. & Broda, P. 1992. Stable defined substrate for turbidimetric assay of endoxylanases. Apple Environ Microbiol. **58**: 3433-3436.

Wilkie, C.R., Mairella, B., Sciamanna, A., Tanquu, K., Whey, D. & Wong, H. 1983. Enzymatic hydrolysis of cellulose: Theory and application. New Jescy USA.

Woodward, J. 1984. Xylanases: functions, properties and applications. Topics in enzyme and fermentation biotechnology. **8**: v9-30.

Wong, K.K.Y., Tan, L.U.L. & Saddler, J.N. 1988. Multiplicity of β-1, 4-xylanase in microorganisms: functions and applications. Microbiol. Rev. **52**: 305-317.

Wong, K.K.Y., Tan, L.U.L., & Saddler, J.N. 1994. Application of hemicellulose in food, feed, pulp and paper industries. In: Hemicellulose and Hemicellulase. M. P. Coughlan, and G.P. Hazlewood, (eds.) Portland Press, London. 127-143.

Website: (http://en.wikipedia.org/wiki/jute)

Chapter 6
APPENDIX

APPENDIX

1. Potato Dextrose Agar (PDA) medium

Potato	*: 200g*
D-glucose	*: 20g*
Agar	*: 20g*
Dist. Water to make	*: 1000ml*
pH adjusted to	*: 5.4*

Sterilized at 121°C under 15 psi for 30 min

2. Wheat bran medium

Wheat bran	*: 12.50g*
Dist. Water	*: 6.25ml (50%),*

Sterilized at 121°C under 15 psi for 30 min

3. Wheat bran medium

Wheat bran	*: 50 g*
Dist. Water	*: 25ml (50%)*

Sterilized at 121°C under 15 psi for 30 min

4. CMCase agar medium

CMCase	*: 10.0g*
Yeast-N-base	*: 6.7g*
Oxgall	*: 10.0g*
Yeast extract	*: 1.0g*
Agar	*: 20.0g*
Dist. Water to make	*: 1000.0 ml*

Sterilized at 121°C under 15 psi for 20 min.

5. Xylan agar medium

Xylan	*: 10.0g*
Yeast-N-base	*: 6.7g*
Oxgall	*: 10.0g*

Yeast extract : *1.0g*

Agar : *20.0g*

Dist. Water to make : *1000.0 ml*

Sterilized at 121°C under 15 psi for 20 min.

6. *Pectin agar medium.*

Polygalacto uronic acid : *10g*

Na No_3 : *10g*

KH_2PO4 : *2.0g*

$MgSo4, 7H_20$: *0.5g*

Yeast extract : *0.2g*

Agar : *20g*

Sterilized at 121°C under 15 psi for 20 min

7. *Dinitrosalicylic acid*

DNS : *7.48g*

NaOH (Merck, Germany) : *13.98g*

Potassium sodium tratarate tetra hydrate : *216.10g*

Phenol : *5.15g*

Sodium meta bi-sulfite : *5.86g*

Dist. Water to make : *1000ml*

8. *Citrate buffer (0.05 m, pH 6.5)*

Sodium citrate (Merck, Germany) was dissolved in dist, water to make 0.05 M solution and the pH was adjusted to 6.5 with 0.05 M citric acid.

9. *Bradford reagent:*

Stock solution: 300g Serva blue G (Coomassie Brilliant G-250) was dissolved in 300g methanol (BDH, England). 600ml 85% phosphoric acid (BDH, England) was then added and stirred well.

Test solution: 150mg stock solution was diluted with 850ml dist, water and stirred well. The solution was then filtered with Whatman filter paper.

10. Tris-Hcl buffer (0.2M, pH 7.0-9.0)

Thris (hydroxymethyl-aminomethane) was dissolved in dist, water to make an 0.2 M solution and the pH was adjusted to the appropriate value with 0.2 M hydrochloric acid.

11. Glyeine-NaOH buffer (0.05 M, pH 10.0-11.0)

Glycine was dissolved in dist, water to make an 0.05 M solution and the pH was adjusted to the appropriate value with 0.05 M NaOH.

12. HEPES Buffer: 23.83g of HEPES is to be dissolved in I L distilled water. Concentrated NaOH (saturated solution) is to be added to increase pH to 8.0.

13. Cetylrimethyl ammonium bromide: 1% (W/V) solution is to be prepared dissolving I g cetyltrimethyl ammonium bromide in 100ml distilled water.

14. Clear zone test.

a) For CMCase activity: PDA with 1% CMCase the plates are flooded with 10ml 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 PDA with 1% xylan plates are to be flooded with 96% ethanol. Although clear zones are visible within 3-4 hours of incubation, prolonged incubation enhances the contrast.

c) *For pectinase activity: PDA plates with 1% pectin the plates should be flooded with 1% (W/V) cetyltrimethyl ammonium bromide solution. Zones of hydrolysis are apparent after 5-10 minutes of incubation.*