SOLICITED GRANT FROM MAURITIUS RESEARCH COUNCIL

Project Title:

Experimental Enzymatic Degumming Of Raw Fibres From Banana Trunks & Pineaplle Leaves & Characterisation Of The Degummed Fibres

Final Report

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September 2013

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1.0 INTRODUCTION

Natural fibres of plant and animal origin are extensively used in a large variety of products such as ropes, mats, clothes, household furnishings and car interiors. Each year, around 35 million tonnes of natural fibres are harvested from a wide range of animals and plants – e.g. sheep, rabbits, goats, camels, alpacas, cotton bolls, abaca and sisal leaves, coconut husks, jute and hemp (FAO-2009). In the 19th century, there existed a fairly important natural fibre industry in Mauritius in which fibres were extracted from the giant Mauritian Hemp mainly for exportation. The industry slowly died out due to competition from petroleum based synthetic fibres which presented much more advantages such as strength, elasticity and versatility at that time (Meade, 1961; Royal Botanic Gardens, 1887). Nowadays, there are new trends to replace synthetic products with 'green' ones and this may provide a good incentive to revive the Natural Fibre industry in Mauritius.

Natural fibres have a net advantage compared to synthetic fibres since they are renewable, biodegradable and represent a sustainable source of raw materials. Plant fibres are schlerenchymatous cells with heavily lignified cell walls having a narrow lumen in cross section. Animal fibres consist mainly of particular structural proteins such as Keratin. This project focuses on plant fibres, which, from a biological viewpoint, can be divided into (i) structural fibres, (ii) gums and mucilages, and (iii) storage polysaccharides. Structural fibers make up the plant cell walls and include cellulose, lignin, many different hemicelluloses and pectins. The gums and mucilages have special functions such as the repair of injured areas. (Anderson & Wen-Ju, 1979). From an analytical standpoint plant fibres can be divided on the basis of water solubility into (a) insoluble fibres and (b) soluble fibres. The insoluble fibres include cellulose, lignin and many hemicelluloses. Whereas the soluble fibres include pectins, some hemicelluloses, gums, mucilages, algal polysaccharides, and the storage polysaccharides (Anderson & Wen-Ju, 1979).

Preliminary studies done by the Mauritius Research Council and collaborators have shown that raw fibres obtained from banana trunks and pineapple leaves can be blended with wool using the existing spinning machinery in Mauritius to produce yarns suitable for knitting. However the fabrics produced out of this blend are too coarse for garment production and are more

appropriate for uses such as upholstery and handicraft. In order to get finer quality yarns, the raw fibres must be degummed. As reported in the literature, very fine, silk like fibres can be obtained by digesting Banana and pineapple fibres with Pectinase enzyme (Shenboo, 2007; Jacob & Parukuttyamma, 2008). This practice is already being carried out in China and the Philippines to produce very fine and expensive garments from degummed pineapple or banana fibres. Furthermore, these fibres have also been reported to blend very well with other natural fibres such as cotton (Siripun & Palivanich, 2009). In this experiment, we propose to use the published experimental protocols (with minor modifications if need be) to degum banana and pineapple fibres using pectinase enzyme. The resulting softened fibres will be used to produce yarns which will be eventually knitted into high quality fabrics.

1.3 Aims

The aim of this study is to attempt to satisfactorily degum raw banana trunk fibres and raw pineapple leaf fibres by enzymatic digestion using pectinases for improved mechanical properties such as handle and stiffness for eventual yarn spinning.

1.4 Objectives

1. Define the optimal enzymatic reaction condition for degumming the raw fibres;
2. Carry out tests to degum the raw fibres to a satisfactory level as judged by the principal investigator;
3. Assess the properties of the degummed fibre and its suitability for yarn production either alone or in blends with cotton or wool,

2.0 LITERATURE REVIEW

2.1 Morphology of Pineapple and Banana Fibres

Plant fibres are thread like tissues obtained from different parts of the plant body. Banana fibre is a bast fibre extracted from the stem or bast of the banana tree and pineapple fibre is extracted from the leaves of the pineapple plant. Bast and leaf fibres are made mostly made up of sclerenchymatous cells. Sclerenchyama are dead cells with thick and heavily lignified cell walls which serve as support tissue to the plant. The cells walls are bonded together through the middle lamella, a thin extracellular adhesive layer between the walls of adjacent cells (Dutta, 1996).

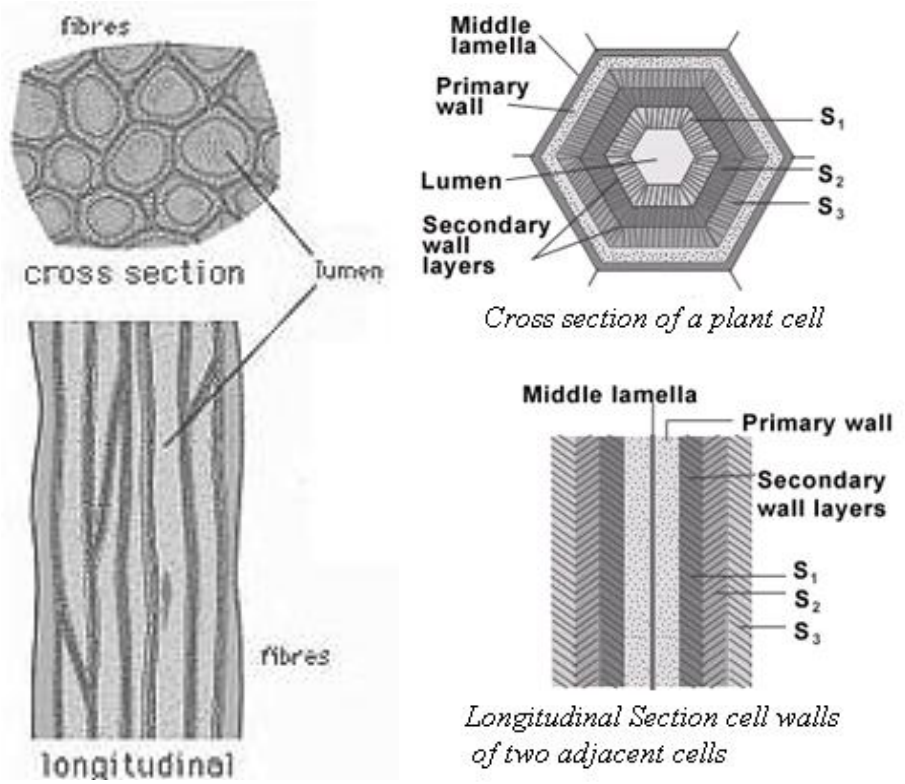


Figure 1 Cross Section and Longitudinal Section View of Scherenchyama Tissue [1]

2.2 Constituents of Banana Stem and Pineapple Leaf Fibres

Table 1 illustrates the varying proportions of cellulosic and non-cellulosic materials in the cell walls of pineapple and banana fibres.

Table 1 Constituents of Pineapple and Banana fibres (%) [Yu, C., 2001]

Fiber	cellulose	hemicellulose	pectin	lignin	water soluble materials	fat & wax	ash
Banana	50-60	25-30	3-5	12-18	2-3	3-5	1-1.5
Pineapple	56-62	16-19	2-2.5	9-13	1-1.5	4-7	2-3

Plant fibres like banana and pineapple are lignocellulosic that is, they are made predominantly of cellulose impregnated with lignin (Dutta, 1996). It can be seen from Table 1 that pineapple fibre has higher cellulose content and lower lignin content than banana fibre.

2.2.1 Cellulose

Cellulose is an insoluble carbohydrate and is the chief constituent of the cell-wall. It is a soft, elastic transparent substance and is readily permeable to water but insoluble in it. Cellulose is a polysaccharide and its molecules are mostly long, straight chains of D-glucose units (Dutta, 1996)

2.2.2 Hemicellulose

Hemicelluloses are similar to cellulose, but more soluble and less complex. (Little and Jones, 1980). Hemicelluloses have a heterogeneous composition of various sugar units. The main chain sugars of hemicelluloses are modified by various side groups such as 4-*O*-methylglucuronic acid, arabinose, galactose, and acetyl, making hemicelluloses branched and variable in structure (Timell, 1967; Wilkie, 1979 cited Aro *et al* 2005).

Due to the complex chemical nature and heterogeneity of hemicellulose, its complete breakdown requires the action of a complex of several hydrolytic enzymes with diverse modes of action and specificity. (Beg *et al* 2001; Biely 1985; Belancic *et al* 1995; Dey D *et al* 1992 cited Muhammad 2010) However, owing to their branched structure they are easily hydrolyzed to their monomer components consisting of xylose, manose, glucose, galactose, arabinose and small amounts of glucoronic acid and methyl glucoronic acid (Taherzadeh and Karimi, 2007 cited Muhammad 2010).

2.2.2.1 Pretreatment of Hemicellulose

Prior to enzymatic hydrolysis, lignocellulosic plants can be pre-treated to disorganize the micro- and macro fibrils, in order to release the polymer chains of hemi-cellulose and modify the pores in them to allow the enzymes to penetrate into the fibres.

Steaming with or without explosion is a popular pre-treatment method. It removes the major part of hemicelluloses from the material and makes it more susceptible to chemical or enzymatic digestion. Hemicellulose sugars solubilise extensively during steam explosion The material can be treated with high-pressure steam of 160-260 °C, from several seconds to few minutes before the material is exposed to atmospheric pressure (Galbe and Zacchi 2002; Ruiz *et al* 2006 cited Muhammmad 2010).

In this work on the enzymatic hydrolysis of hemicelluloses in birch wood, Muhammad (2010) pretreated birch wood chips in rotating autoclave cylinders at a temperature of 160- 165 °C with a water to dry wood ratio of 2:1 for 90 minutes. Xylose concentration in the extracted liquors was measured and was found be around 18 %. Results also showed that the cellulose mostly remained intact.

2.2.3 Pectic Compounds

Pectic compounds are a group of complex carbohydrates, derivatives of polygalacturonic acid present in cell walls and an abundant constituent of the middle lamella. Pectic substances mainly consist of galacturonans and rhamnogalacturonans.

Pectic compounds occur in four forms: protopectin, pectinm pectic acid and pectinic acids.Protopectin present in the primary cell-walls acts as a binding material, holding together the cells. It is water insoluble and on restricted hydrolysis yields pectin or pectic acids. Pectic acid is the soluble polymer of galacturonans that contains negligible amount of methoxyl groups. Pectinic acids is the polygalacturonan chain that contains >0 and <75% methylated galacturonate units.

Pectin (Polymethyl galacturonate) is the polymeric material in which, at least, 75% of the carboxyl groups of the galacturonate units are esterified with methanol. It confers rigidity on cell wall when it is bound to cellulose in the cell wall. ((Little and Jones, 1980; Dutta, 1996; Pectinase database, 2007)

When the fibers are treated with pectinase, the interlamellar pectin which cements the fibers is destroyed. This results in the separation of fibers (Jacob *et al*, 2006; 2008).

2.2.4 Lignin

Lignin is mainly present in the secondary wall of plant cells. It is a hard and chemically complex compound and is permeable to water. It contributes to the rigidity of the plant body (Little and Jones 1980; Dutta 1996). Lignin is extremely resistant to enzymatic and chemical degradation. It is reported that chemical bonds exist between lignin and hemicelluloses and even cellulose (Taherzadeh and Karimi, 2007 cited Muhammad 2010).

2.2.5 Fats, Wax & Ash

Fats, wax and ash are wood compounds soluble in water and in neutral organic solvents. They represent a minor fraction of non-cellulosic component (Taherzadeh and Karimi, 2007 cited Muhammad 2010).

2.3 Enzymatic Degumming

Degumming is defined as the removal of heavily coated, non-cellulosic gummy material from the cellulosic part of plant fibers (Said *et al*, 1991 cited Jacob *et al*, 2006).

Pectinolytic enzymes have been successfully used in the degumming of plant fibers such as ramie, sunn hemp, jute, flax and coconut fibres (Bruhlmann *et al* 1994; Zhang *et al* 2000 cited Pedrolli *et al* 2009). Enzymatic degumming can be done by adding pectinolytic mixtures or by fermentation using pectinase producing microorganisms (Sharma 1981; Molina *et al*, 2001 cited Pedrolli *et al*, 2009). Since the content of cell wall polysaccharides varies in composition and quantity, efficient hydrolysis would require multi-enzymes complexes expressing different enzyme activities (Novozyme, 2008).

2.3.1 Enzymes

Enzymes are proteins with highly specialized catalytic functions, produced by all living organisms and are categorized according to the compounds they act upon. A catalyst is any substance which makes a chemical reaction goes faster, without itself being changed and can be used repeatedly in a chemical reaction as it does not get used up. Enzymes are very much the same except that they can be easily denatured by some means. An ordinary catalyst may be used

for several different chemical reactions, but an enzyme only works for one specific reaction (Tzanov 2003 cited Vigneswaran *et al*, 2011). Table 2 shows the major enzymes used in the textile wet processing industry.

Table 2 The Major Types of Enzymes Used in the Textile Wet Processing Industries [Vigneswaran *et al.*, 2011]

TYPE OF ENZYME	APPLICATION
Amylases	To decompose starches in sizing preparations
Catalases	Act on hydrogen peroxide to decompose it into water and oxygen
Protease, lipases and pectinase	When combined, act on proteins, pectins and natural waxes to effect scouring
Laccases	Decompose indigo molecules for wash-down effect on denim
Cellulases	Break down cellulosic chains to remove protruding fibers by degradation & create wash-down effect by surface etching on denims etc.

All enzymes are made of protein; that is why they are sensitive to heat, pH and heavy metal ions. Under unfavorable conditions of pH or temperatures, enzymes chemically remain in same form but their physical configuration may get altered i.e. they get "denatured" and lose their activity. For this reason live steam must never be injected in a bath containing enzymes and any addition of chemicals to the enzymes bath must be done in pre-diluted form (Vigneswaran *et al.*, 2011).

Substrate quality, substrate concentration, enzyme activity, applied pre-treatment method and hydrolysis conditions such as pH and temperature are the major factors in enzymatic hydrolysis. Use of surfactants can also affect the hydrolysis by modifying the cellulose surface properties such as adsorption of surfactants to lignin, which reduces unproductive binding of enzymes to lignin (Taherzadeh and Karimi, 2007 cited Muhammad 2010).

Commercial sources of enzymes are obtained from three primary sources, i.e., animal tissue, plants and microbes. These naturally occurring enzymes are quite often not readily available in sufficient quantities for industrial use. By isolating microbial strains that produce the desired enzyme and optimizing the conditions for growth, commercial quantities can be obtained. (Vigneswaran *et al*, 2011).

2.3.2 Cellulases

Cellulases are enzymes that degrade cellulose. The commercially available cellulases are a mixture of enzymes: endogluconases, exogluconases and cellobiases. Endogluconases randomly attack the cellulose and hydrolyse the 1-4 β glucosidic linkage of cellobiose chain. Exoglucanases hydrolyse the 1-4 β glucosidic linkage of cellulose to release cellotiose from the cellulose chain and cellobiases are enzymes which hydrolyse cellobiose into soluble glucose units. All these three enzymes act synergistically on cellulose to hydrolyse them (Vigneswaran *et al* 2011). Cellulases are used for tissue and fibre modification in the pulp and paper industry. They are also commonly used in fabric finishing, for example in biopolishing. Bio-polishing is a technique used in the finishing treatment of cellulosic fabrics with cellulase enzymes. It upgrades the quality of the fabric by removing the protruding fibers from the surface and modifying the surface structure thereby making it soft and smooth. The enzyme hydrolyses the microfibrils protruding from the surface of yarn because they are most susceptible to enzymatic attack. This weakens the microfibrls, which tend to break off from the main body of the fibre and leave a smoother yarn surface (Novozymes, 2008). Cellulases used for this process are too large to penetrate the interior of cellulosic fibres. Hence, only 1, 4 β-glucosidic bonds at the surface of cellulose fiber are affected (Vigneswaran *et al* 2011).

2.3.3 Hemicellulases

Hemicellulases hydrolyse hemicelluloses to their monomer components by the concerted action of endo-enzymes cleaving internally the main chain, exo-enzymes liberating monomeric sugars and ancillary enzymes cleaving the side chains of the polymers or oligosaccharides leading to the release of various mono- and disaccharides depending on the hemicellulose type.

As an example, the breakdown of xylan (main hemicellulose in hardword) involves at least endo-1,4-β-D-xylanases and β-xylosidases acting on the main sugar chain and depending on the type of xylan, the side-chain cleaving enzymes such as α-glucuronidase and acetyl xylan esterase (De Vries and Visser 2001 cited Aro *et al* 2005).

Muhammad (2010) used two commercial enzyme mixtures, Celluclast 1.5 L (with β-xylosidase activity) and Pulpzyme HC (with β-1, 4 xylanase activity) for the enzymatic hydrolysis of hemicellulose in birch wood. Different ratios of the enzymes were applied at different temperatures (ranging from 40-60 °C) and pH range 5 to 7 in an autoclavable bioreactor system

for 48 hours. To stop further hydrolysis and deactivate the enzymes, the samples were incubated for 10-15 minutes at 100°C, and then freezed. Results showed that hemicelluloses was successfully hydrolysed with high yields of xylose.

2.3.4 Pectinases

Pectinases are produced by plants and microorganisms such as moulds, yeasts and bacteria. The common source of commercial pectinases is the filamentous fungi aspergillus sp (Torres *et al.*, 2005 cited in Jacob *et al.*, 2006; 2008). Pectinases can be produced either by submerged or solid state fermentation (Aquilar and Huitron 1990, Acuna-arguelles *et al.*, 1995 cited Arunachalam and Asha 2010).

Pedrolli *et al.* (2009) report that pectinases are a big group of enzymes that breaks down pectic polysaccharides of plant tissues into simpler molecules like galacturonic acids. Since pectic substances are a very complex macromolecule group, various pectinolytic enzymes are required to degrade it completely. These enzymes present differences in their cleavage mode and specificity. Pectinase enzymes can catalyze pectic substance degradation through depolymerization (hydrolases and lyases) and deesterification (Pectin esterases) reactions.

Pectin depolymerases are further classified as polygalacturonase (PG) and pectin lyases (PL). Pectin esterase has the ability to de-esterify pectin by the removal of methoxy residues. Thus on the whole, pectinases are hydrolytic enzymes, which hydrolyze the pectin molecules and are readily soluble in water (Ramanujam *et al.*, 2008 cited Arunachalam and Asha 2010).

Acid pectinases are widely used in extraction, clarification, and removal of pectin in fruit juices, in maceration of vegetables to produce pastes and purées, and in winemaking. In several processes, pectinolytic enzymes are applied associated with other cell wall degrading enzymes such as cellulases and hemicellulases. The mixture of pectinases and cellulases has been reported to improve more than 100 % juice extraction yields (Alkorta *et al.*, 1998; Baht 2000; Kashyap *et al., 2001* cited Pedrolli *et al.*, 2009).

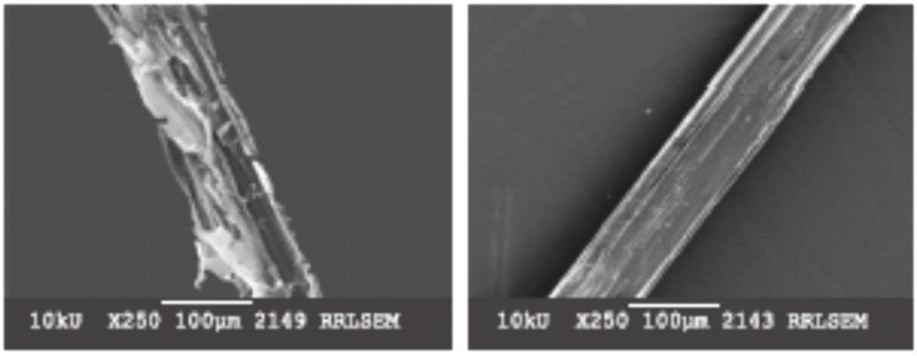
Alkaline pectinases have been used in the pretreatment of waste water from vegetable food processing that contains pectin residues; the processing of textile fibers such as flax, jute and hemp, coffee and tea fermentation, vegetable-oil extraction and the treatment of paper pulp **(**Zhang *et al* 2000; Kashyap *et al* 2001; Kapoor *et al* 2001; Hoondal *et al* 2002 cited Pedrolli *et al* 2009).

2.3.5 Pectinases in Banana Fibre Degumming

In one of their works on the degumming of banana fibres, Jacob et al (2006) produced the pectinase *polygalacturonase* through fermentation of cultures of the bacterial strain *Streptomyces lydicus*. Banana fibers were collected by hand stripping, dried and then treated with the crude pectinase (15 U enzyme/25 mg of fiber) for 90 min at 45 °C and 150 rpm in a rotary shaker at pH 7.5. The reducing sugar level was checked at regular intervals to study the effect of enzyme on break down of pectin present in the fibers. It was noted that reducing sugar level increased gradually indicating the effectiveness of the treatment. It was concluded from scanning electron micrographs of both treated and untreated fibers that the cells were separated after treatment as a result of pectin hydrolysis.

Jacob et al (2008) further experimented on a novel process of simultaneous extraction and degumming of fibre bundles from fresh pseudo stem of bananas. *Streptomyces lydicus* was again allowed to grow on wheat bran medium to produce *polygalacturonase* in which banana leaf sheath pieces were incorporated and the fibre bundles were separated after a two-step fermentative process.

The treatment medium contained 10 g of wheat bran (500- 1000 μm) and 0.1 g of pectin, moistened by a salt solution of (g/L) K2HPO4: 4 and KH2PO4: 4 (pH-6.0). The leaf sheath pieces (30 x 10 x 2 mm) were incorporated in the medium, autoclaved for 45 minutes and inoculated with 5 mL of broth culture. Flasks were incubated at 30ºC for three days and the wheat bran was washed off to take out the leaf sheath. The treated leaf sheath was re-fermented for another three days in fresh solid-state medium. Wheat bran was washed off and the fibre bundles were separated by hand stripping.Electron micrographs of the bundle taken after biological extraction showed removal of the non-cellulosic gummy wastes was extremely good as compared to the control (fiber bundle obtained from fresh leaf sheath by hand stripping was considered as the control)



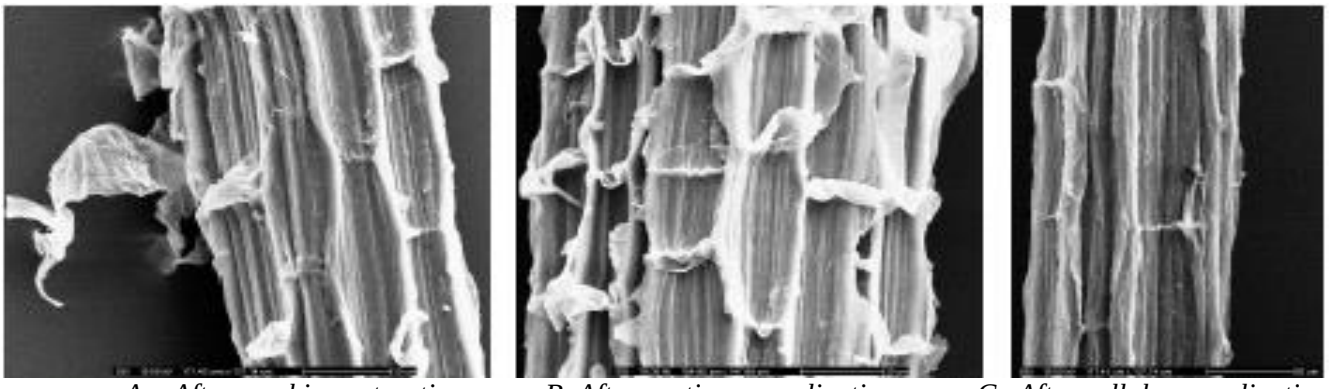
A. Control: fiber bundle obtained from fresh leaf sheath by hand stripping was considered as the control.

B. Biologically extracted fiber bundle: fiber bundles were hand stripped after biological treatment.

Figure 2 Scanning Electron Micrographs of Banana Fibre Bundles [Jacob et al., 2008]

2.3.6 Scouring of Pineapple Fibres With Pectinase and Cellulase

Sricharussina *et al* (2009) studied the effect of enzyme scouring on the dyeing of pineapple leaf fibres with natural dyes. The fibres were extracted from the leaves by a decorticating machine. The machine broke the cell walls joining the sclerenchyma cells of the leaf fibres thereby releasing fibre bundles. Residual cell wall fragments remained on the surface of the fibre bundles (Figure 3A).



A. After machine extraction *B. After pectinase application* *C. After cellulase application*

Figure 3 Scanning Electron Micrographs of the Morphology Of Pineapple Leaf Fibres [Sricharussina *et al.*, 2009]

The fibres were scoured for 2 h in either pectinase (Pectinex Ultra SPL*) at 45 °C for 2 h at pH 4.5, or cellulase (Lava Cell NNM) at 55 °C at pH 7. A bath was prepared with the enzyme, 2% owf, and 1 ml/l wetting agent in a liquor ratio of 1:40. After the treatment, the fibres were boiled for 10 min to deactivate the enzyme, and then washed and dried.

Pectinex Ultra SPL is an active pectolytic enzyme preparation that contains mainly pectintranseliminase, polygalacturonase, and pectinesterase and small amounts of hemicellulases and cellulases.

The treated pineapple leaf fibres were spun into yarns with a semi-automatic spinning machine. Commercial natural dyes with appropriate mordanting processes were applied to the yarn samples to a shade of 2.5% owf with a liquor ratio of 1:40 at 60 °C for 30 min.

Sricharussina *et al* (2009) concluded that the enzyme treatments effectively removed non-cellulosic substances from the pineapple fibres resulting in a hydrophilic surface and improved wettability. The pectinase enzyme penetrated the cuticle of the leaf fibres through cracks and hydrolysed the pectic substances in the fibre, which resulted in the removal or partial removal of the cuticle (Figure 3b).The cellulase enzyme penetrated the fibre the same way as the pectinase did. The cellulase catalysed the hydrolysis of both the primary and secondary cell walls (Saleem et al, 2008 cited in Sricharussina et al, 2009). The outside layer of the fibre was loosened and fell away, Figure 3c.

Dye uptake results, colour measurements, and light fastness tests also showed that cellulase and pectinase-based scouring can effectively prepare pineapple leaf fibres for dyeing.

2.3.7 Effects of Degumming

Biofibres like pineapple leaf fibres and banana fibres do not show the general relationship between crystallinity and strength observed in pure cellulose fibres such as cotton and rayon. Their strength is rather determined by the presence of non-cellulosic materials, mainly lignin, and the dimensions of the cells in the tissue. The structural changes caused by the loosening of pectin lamellae can alter the mechanical properties of the fibres. Under stress, cracks propagate through the weakened bonding between cells, causing intercellular fracture (Reddy and Yang, 2005 cited Sricharussina *et al* 2009).

3.0 EXPERIMENTAL

3.1 Materials

Banana stem and pineapple leaf fibres were provided by the MRC.

3.1.1 Banana Fibres

The banana fibres, around 90 cm long were coarse, stiff and lustrous. They were irregular, varying in ‘thicknesses along the length. Some fibres were thick, coarse bundles glued together whereas others were very fine.

3.1.2 Pineapple Fibres

The pineapple fibres, around 1 m long, were also coarse but stiffer than the banana fibres. They were more filament-like, regular in thickness along their length and less tangled than the banana fibres. There are more visible impurities on the fibre surface.

3.1.3 Sansevaria Fibres

Sansevaria fibres, extracted from the leaves of the Sansevaria plant were provided at a later stage by the MRC. These were around 50 cm in length and more ‘silky’, soft and flexible than the banana and pineapple fibres. They were very regular along their length are almost completely free of bundles of fibres and were mostly single filament fibres.

3.2 Enzyme

The enzyme required was a commercial pectinase, the main function of which would be to degrade pectic materials. Based on the literature reviewed, it was decided to use a pectinase with polygalacturonase activity.

The enzyme selected was **Pectinase from *Aspergillus Niger*** sourced from SIGMA-ALDRICH by a local supplier: Supplies Solution, Curepipe. Technical information provided by the supplier indicates that the enzyme is polygalacturonase. It degrade pectic materials by the random hydrolysis of (1->4)-alpha-D-galactosiduronic linkages in pectate and other galacturonans (hydrolysis of O-glycosyl bond).

Enzymatic Activity ≥ 1.00 U/MG.

1 U corresponds to the amount of enzyme which liberates 1 UMOL galacturonic acid from polygalacturonic acid per minute at pH 4.0 and 50 °C.

3.3 Reagents

- ***Dynawet T*** from Dynachem, Mauritius is a universal wetting agent for all types of fibres.
- ***Acetic acid, 1 %*** was used to adjust pH of the degumming liquor.
- ***Levafix Blue*** is a commercial reactive dye used to dye cellulosics.
- The auxilliaries used during dyeing were ***Sodium Sulphate*** and ***Sodium Carbonate*** for optimum dye uptake.
- ***Lenetol HP-JET*** is a low foaming non-ionic detergent used for soaping off reactive-dyed cellulosics.

3.4 Equipment

Degumming and dyeing were carried out in glass beakers and in a pot dyeing machine. A bench pH meter was used to adjust pH of the degumming liquor.

Fibres were viewed in a laboratory microscope and photographed with a standard digital camera. Assessment was carried in a standard VeriVide light cabinet.

3.5 Part 1: Trial Enzyme Degumming of Banana Fibres

3.5.1 Materials

Bundles of raw banana fibres (~ 0.5 g) cut to around 8 cm long.

3.5.2 Degumming Recipe & Methodology

Two recipes with different enzyme concentration were used to degum the fibres. The experiments were carried out in exhaust method in glass beakers.

At the start of the experiment, the pH of the liquor was adjusted to around 4.5 - 4.7 using Acetic acid (concentration 1%). The temperature was kept at 50 °C for 2 hours with constant stirring for mechanical agitation.

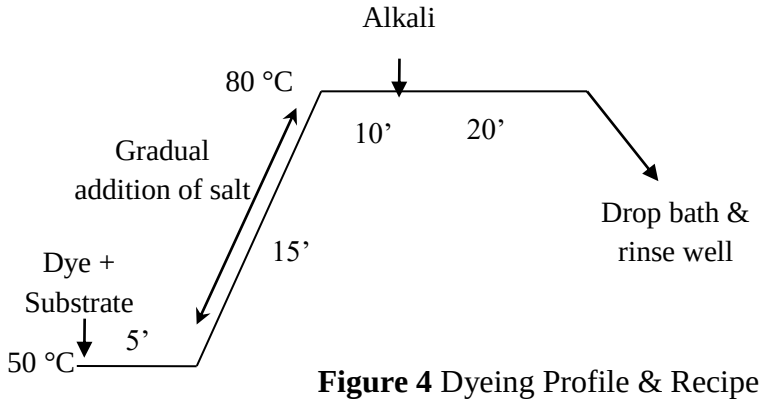
Table 3 Recipes 1 & 2

REAGENTS	% CONCENTRATION	
	RECIPE 1	**RECIPE 2**
Pectinase	2 % O.M.F	20 % O.M.F.
Dynawet T	2 % O.M.F.	2 % O.M.F.
MATERIAL TO LIQUOR RATIO	1: 40	1: 40

After the enzyme degumming process, the fibres were rinsed in cold tap water for a few minutes and allowed to air dry.

3.5.3 Colouration of the Untreated and Treated Samples

Samples of raw and degummed banana fibres from Recipes 1 and 2 were dyed with the reactive dye Levafix Blue according to below recipe.



REAGENT	% CONCENTRATION
Levafix Blue Dye.	4 % O.M.F.
Sodium Sulphate	20 g/L
Sodium Carbonate	30 g/L
MATERIAL TO LIQUOR RATIO 1:200	

Figure 4 Dyeing Profile & Recipe for Reactive Dye

After dyeing, the fibres were rinsed in tap cold water and soaped in a 1 g/L Lenetol HP-JET non-ionic detergent solution at near boil for 10 minutes. The fibres were given a final rinse with cold tap water and dried.

3.5.4 Assessment of Degummed and Dyed Samples

The untreated and enzyme-treated samples were viewed in a light cabinet under proper lighting conditions (D65) and subjective assessment of the appearance and hand feel was done by

experienced assessors. Dry mounts of the samples were viewed and assessed under microscope (magnification x 100).

3.6 Part 2: Degumming of Banana Fibres, Pineapple & Sansevaria Fibres

3.6.1 Materials

Samples (~ 0.5 g) of banana and pineapple cut to around 8 cm long. Care was taken to select the most 'gummed' bundles for the banana and pineapple fibres. Being very flexible, the samples of sansevaria fibres (~ 0.5 g) were not cut and were treated in their whole length.

3.6.2 Degumming Recipe & Methodology

In this experiment, banana, pineapple and sansevaria fibres were degummed for prolonged period using 20% O.M.F enzyme. The enzymatic degumming process was carried out at 50^{o}C for 17 hours. The objective of prolonged enzyme treatment was to obtain an improved degumming effect on the fibres.

All experiments were carried out in a pot dyeing machine with constant agitation.

Table 4 Recipe 3

REAGENT	**% CONCENTRATION**
Pectinase	20 % O.M.F.
Dynawet T	2 % O.M.F.
MATERIAL TO LIQUOR RATIO	1:40

The fibres were degummed for 17 hours in 3 stages.

Stage 1: 6 hours

Stage 2: 6 hours

Stage 3: 5 hours

The pH of the bath was adjusted to around 4.2-4.8 using Acetic acid (1%) at the start of each stage. The pH at the end of every stage was monitored and recorded.

The temperature was kept constant at 50 °C.

After enzyme degumming process, the fibres were rinsed in cold tap water for a few minutes and allowed to air dry.

3.6.3 Assessment of Degummed Samples

Assessment of the degummed samples was carried out as detailed in section 3.5.4.

3.7 Part 3: Steam Pre-Treatment Followed by Prolonged Enzymatic Treatment

In this experiment, the fibres were exposed to steam for some time followed by enzymatic degumming for prolonged period using pectinase enzyme, at same temperature as described in Part I and II. The enzymatic degumming process was carried out at 50^{o}C for 6 hours. The objective of steam pre-treatment followed by prolonged enzyme treatment was to obtain an improved degumming effect on the fibres.

3.7.1 Materials

Samples banana, pineapple and sansevaria fibres selected as detailed in section 3.6.1

3.7.2 Steaming Pretreatment

About 100 ml of tap water was poured into a small pressure cooker and 1 g of fibres was placed above the water on a raised perforated steel plate. The water was brought to the boil and the fibres were exposed to the steaming environment for 30 minutes.

3.7.3 Degumming Recipe & Methodology

The fibres were degummed as per the Recipe 3 (refer to Table 4, section 3.6.2) but for only 6 hours. After enzyme degumming process, the fibres were rinsed in cold tap water for a few minutes and allowed to air dry.

3.7.4 Assessment of Degummed Samples

Assessment of the degummed samples was carried out as detailed in 3.5.4.

4.0 RESULTS & DISCUSSIONS

4.1 Part 1: Trial Enzyme Degumming Of Banana Fibres

4.1.1 Assessment of Raw and Degummed Fibres

Raw banana fibre samples enzyme-treated with 2% and 20% by mass of the fibres were for visual differences. It was found that there was no noticeable difference in visual appearance (lustre and colour) between the untreated and treated samples. The hand feel was also not noticeably different.

The fibres were viewed and photographed under microscope under magnification x100 (Figure 5).

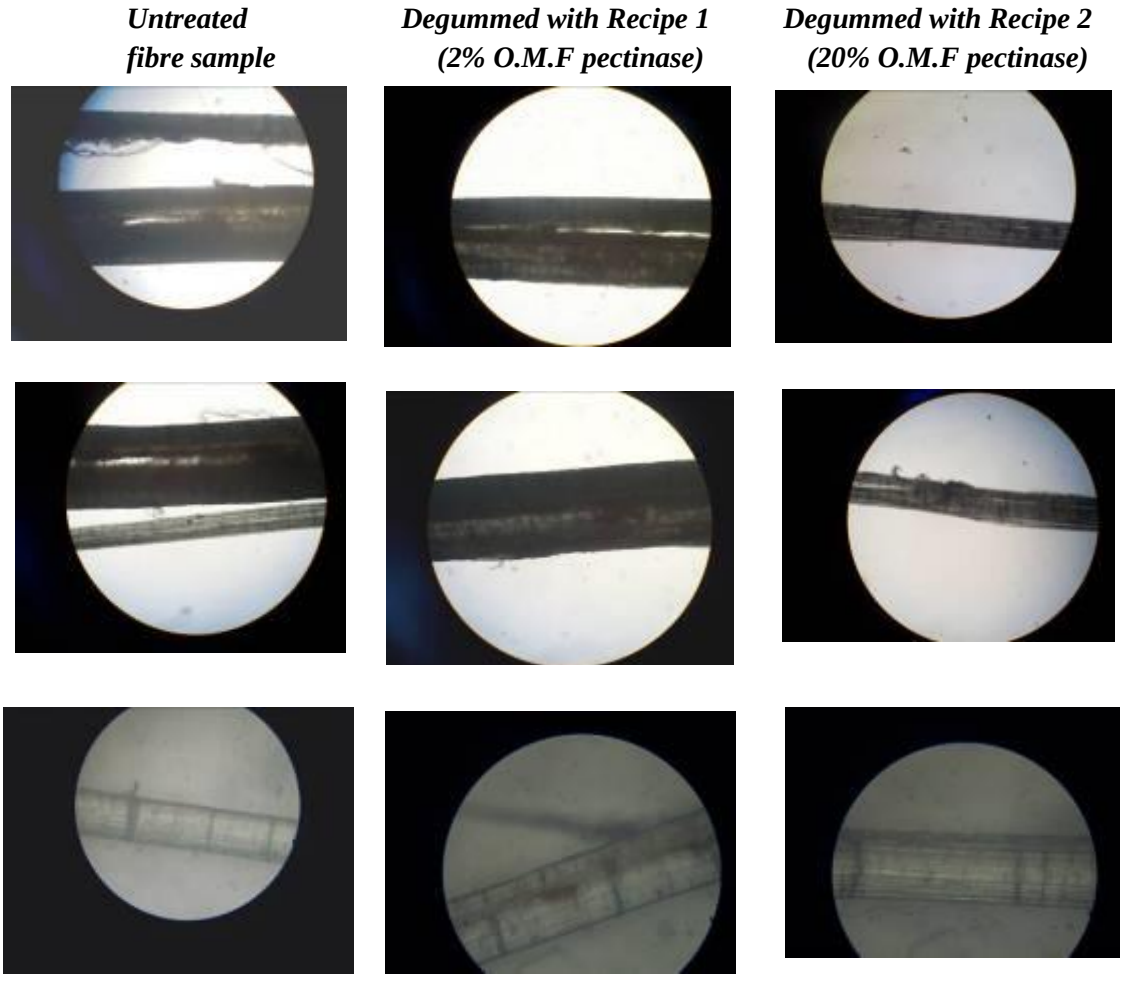


Figure 5 Photographs of Untreated and Treated Banana Fibre Samples as Taken Under Lab Microscope (x100)

The following observations were made under the microscope:

Untreated banana fibres: There are noticeable and varying traces of impurities on the surface of the fibres.

Banana fibres degummed with Recipe 1: Clearer than the untreated fibres but impurities were still present in some places along the length of the fibres. It is possible that these are associated with the surface pectin which has not been completely hydrolysed.

Banana fibres degummed with Recipe 2: More clear and translucent fibre surface than the [2%] degummed fibre. Great improvement in the surface appearance under microscope and this suggest that much of the surface impurities may have been removed.

4.1.2 Assessment of Dyed Raw and Degummed Fibres

Varying level of dye uptake may indicate the extent of degumming of the fibres. Therefore, the untreated samples and samples obtained from Recipe 1 & 2 were dyed using Levafix Blue reactive dye. The untreated and treated samples were visibly different after dyeing; the depth of shade was not significantly different. However, the untreated sample was yellower than the treated sample (Figure 6)

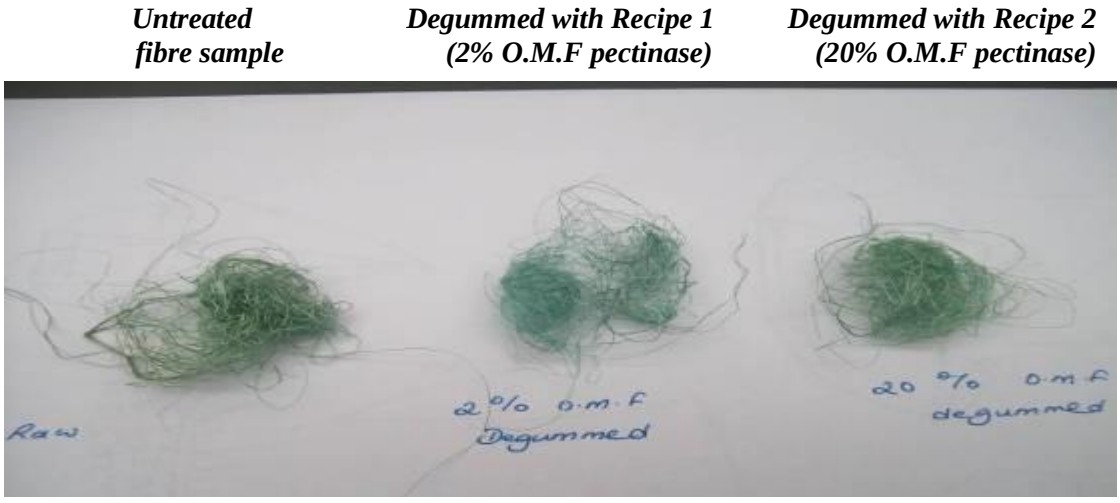


Figure 6 Photographs of Dyed Untreated and Treated Banana Fibre Samples

The fibres were then mounted and photographed under microscope with magnification, (x100). The results are shown in Figure 7. From the analysis of the photographs, the surface of untreated fibres appeared not to be very clean and it suggested the presence of loose impurities which may be fibrous or pectin-based materials. The treated samples were much clearer and this may indicate the significant removal of surface impurities, most of which are pectin-based.

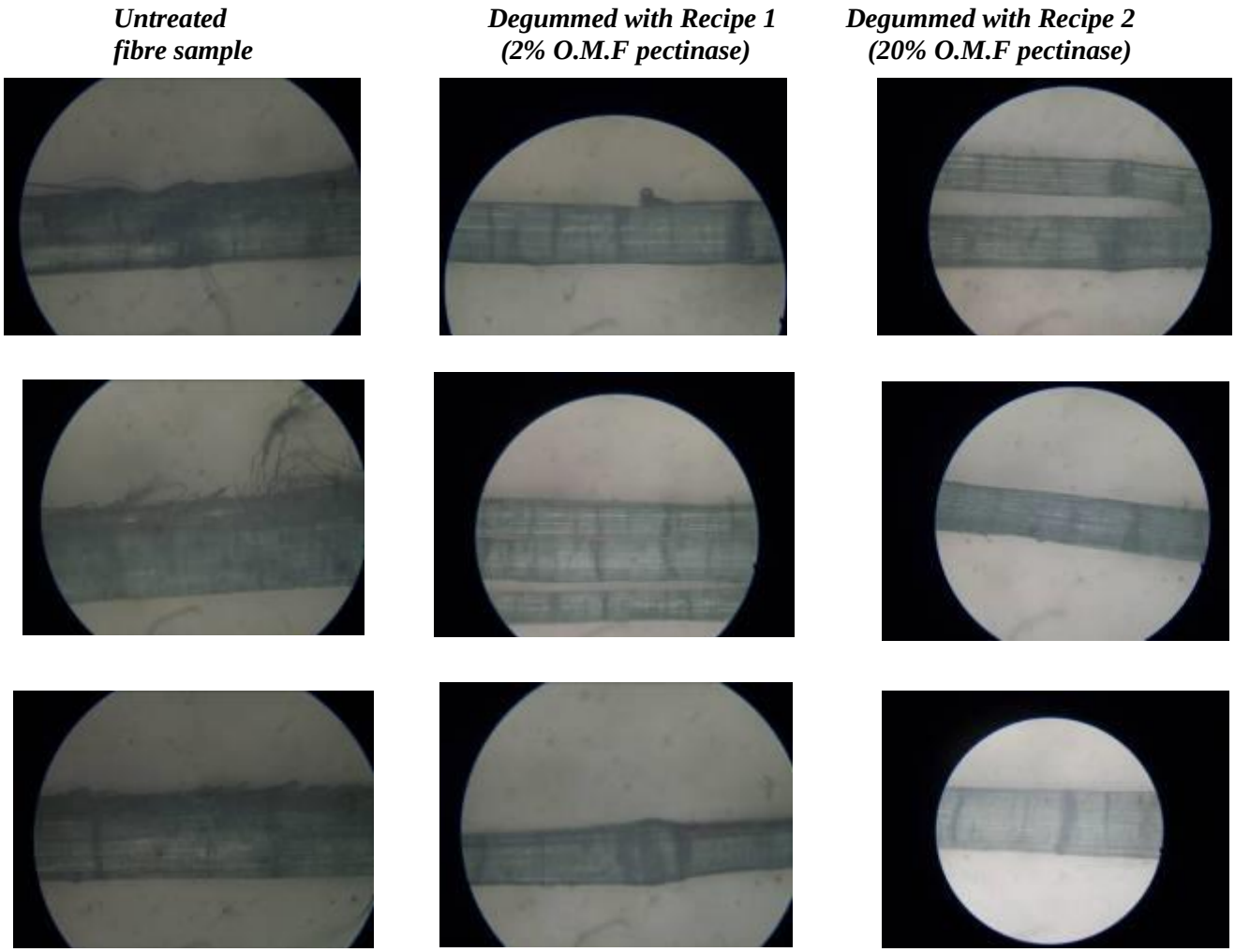


Figure 7 Photographs of Untreated and Enzyme Treated Fibres After Dyeing With Reactive Dye Under Lab Microscope (x100)

It may be concluded that the enzyme treatment, under the recommended conditions applied, has been quite effective in removing surface impurities from the fibres. Under industrial conditions, the results could be more satisfactory, with greater mechanical agitation and a greater mass of fibres may provide better surface frictional effect for removal of surface impurities during

enzyme treatment. The concentration of enzyme needed to be optimised for satisfactory degumming of banana fibres under laboratory conditions.

4.2 Part 2: Degumming of Banana Fibres, Pineapple & Sansevaria Fibres

Fibres were degummed for prolonged period of 17 hours in three stages. The objective of prolonged enzyme treatment was to obtain an improved degumming effect on the fibres. The pH of the baths were adjusted at the start of every stage, monitored and recorded throughout the experiment (Table 5).

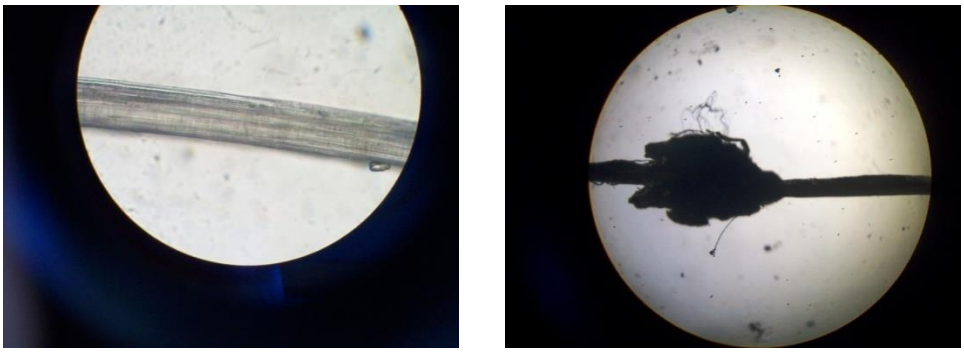
Table 5 pH Record of Degumming Bath at Start & End of Each Stage of Degumming Process

	STAGE 1: 6 HOURS		**STAGE 2: 6 HOURS**		**STAGE 3: 5 HOURS**	
FIBRES	**pH at start**	**pH at end**	**pH at start**	**pH at end**	**pH at start**	**pH at end**
Banana	4.21	3.97	4.77	3.67	4.66	3.61
Pineapple	4.19	3.98	4.69	3.67	4.45	3.56
Sansevaria	4.36	4.01	4.77	3.37	4.63	3.39

4.2.1 Assessment of Banana Fibres

A stiff and coarse bundle of fibres were specifically chosen for degummning. After the enzymatic treatment for 17 hours, the bundle of fibres had a much softer hand feel and the fibres may be more easily separated by hand.

This suggests that the pectinase enzymatic degumming process, under prolonged conditions, was quite effective in removing substantial among of gum impurities from the fibre bundle (Figure 8A). It was, however, observed under optical microscope that some of the fibres still carried impurities (Figure 8B).

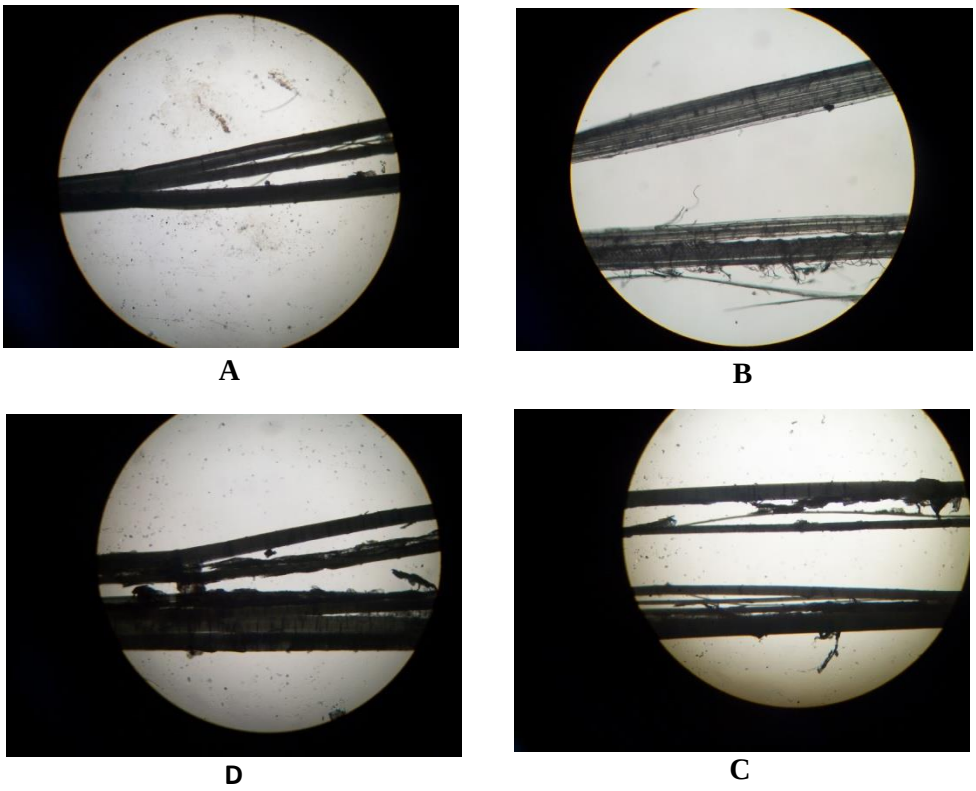


A.Clean section of banana fibre (Magnification x400)

B. Presence of impurities at one end fibre (Magnification x100)

Figure 8 Photographs of banana fibres: untreated and degummed for 17 hours under Lab microscope (x100) & (x400)

This may explain why the fibres were not completely separated from the bundle, the impurities were acting as 'glueing' agent between the fibres in the bundle. Figures 9 A-D show the magnified view of banana fibre bundle after pectinase enzymatic process; partial 'debundling' of the fibres.



A

B

D

C

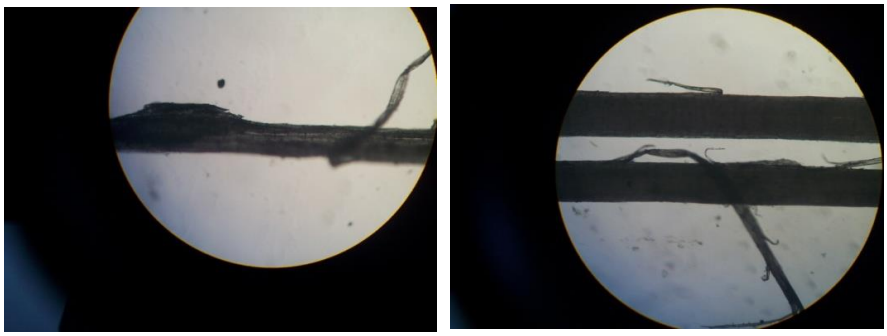
Figure 9 Photographs Showing Separation of Banana Fibres in Bundles Under Lab Microscope (x40) & (x100)

Literature has shown that banana fibres may contain as much as 25-30% of hemi-cellulose, 12-18% of lignin and 3-5% of pectin. In this experiment, the pectinase enzymatic treatment alone appears to be insufficient for the *complete degumming* of banana fibres.

It was, however, observed that the hand feel of the treated fibre bundle improved significantly after pectinase enzymatic degumming process. The fibres could also be more easily separated manually after degumming. Similar observations could be made for pineapple fibres, Figure 10.

4.2.2 Assessment of Pineapple Fibres

A. UNTREATED FIBRES



B. TREATED FIBRES

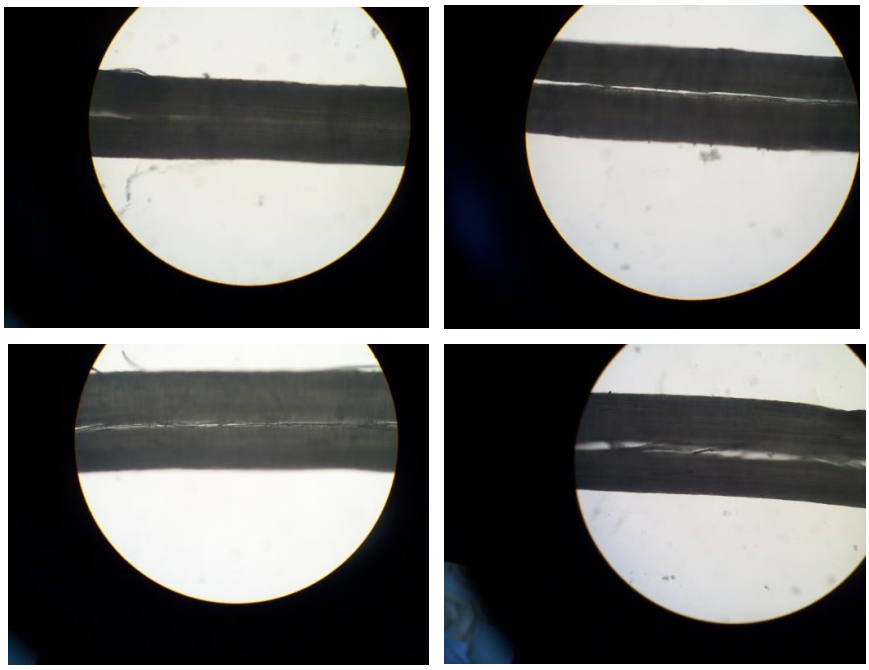


Figure 10.Photographs of A (Untreated) & B (Treated) Pineapple Fibres Under Lab Microscope (x100)

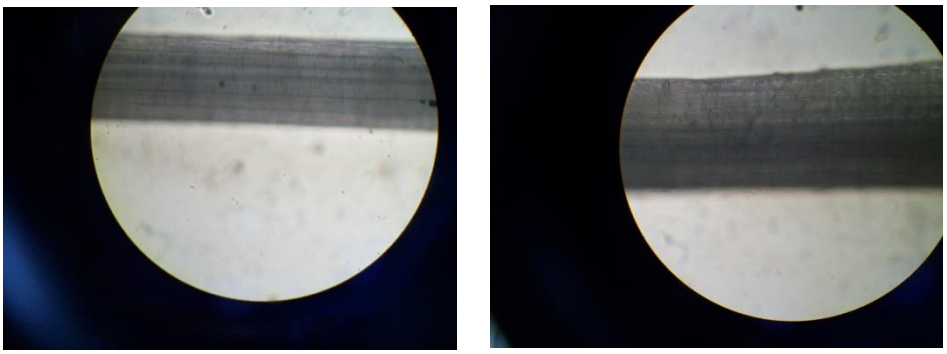
Under microscopic observation, treated fibres are clearer and cleaner than the untreated fibres but the fibres are partially held up in bundles

4.2.3 Assessment of Sansevaria Fibres

The sansevaria fibres are, by appearance, cleaner fibres than banana or pineapple fibres and as such there are no significant changes in the visual appearance of the fibres after enzymatic degumming. The handle of the untreated fibres is also good and that of the enzymatically-treated fibres are improved slightly. Besides, the untreated sansevaria fibres are almost completely free of bundles of fibres and exist, predominantly, as single filament fibres.

In the bath, at the end of the degumming process, there were a number of broken down Sansevaria fibres in the solution. This may suggest that the sansevaria fibres are more 'brittle' than the other two fibres (banana and pineapple fibres) and more susceptible to breakage under hot wet treatment with agitation.

A. UNTREATED FIBRES



B. TREATED FIBRES

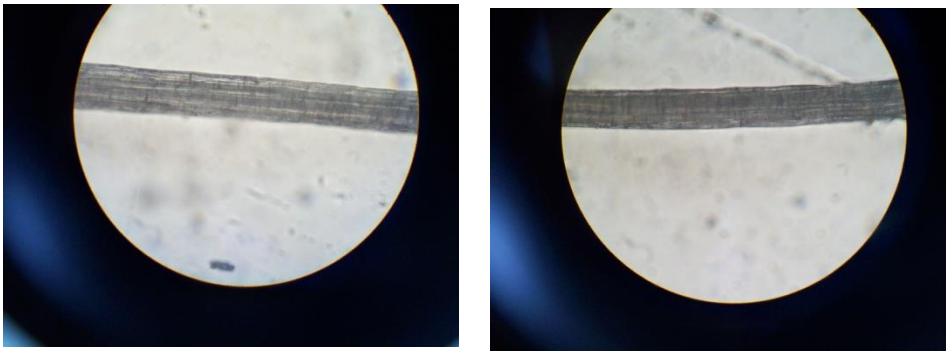


Figure 11 Photographs of A(Untreated) & B (Treated) Sansevaria Fibres Under Lab Microscope (X400)

4.3 Part 3: Steam Pre-Treatment Followed By Prolonged Enzymatic Treatment

Fibres were pre-treatment with steam prior to enzymatic treatment.

4.3.1 Assessment of Banana and Pineapple Fibres

Stiff and coarse bundles of raw fibres were specifically chosen for the degumming process. After steaming, the fibrous material had a softer hand feel and the bundle of fibres could bend more easily under flexing action. The change in softness was more pronounced for the banana than for the pineapple fibres. This suggested that the steaming process was quite effective in softening the bundle of fibres.

It was also observed that the hand feel and bending stiffness of the treated fibre bundles further improved after prolonged (6 hours) pectinase enzymatic degumming process which was carried out after steaming. The result of the combined steaming and (6 hours) enzymatic degumming process is comparable to that obtained using prolonged (17 hours) enzymatic degumming of the fibres.

4.3.2 Assessment of Sansevaria Fibres

The sansevaria fibres are, by appearance, cleaner fibres than banana or pineapple fibres and as such there were less significant changes in the visual appearance and hand feel of the fibres after steaming and enzymatic degumming. The handle of the untreated fibres is quite good and that of the steam treated followed by enzymatically-treated fibres improved slightly. Besides, the untreated sansevaria fibres are almost completely free of bundles of fibres and exist, predominantly, as single fibre.

The main challenge in this project was to free banana trunk and pineapple fibres completely from the bundles by removing all traces of ‘gum’ holding the fibres together. This would, as a consequence, improve the handle of the fibres and produce a more refined and flexible fibres for spinning. It has been shown that the three natural fibres Banana, Pineapple and Sansevaria fibres have been satisfactorily degummed using Pectinase (from *Aspergillus Niger)* enzymayic process. The fibre degumming improved with process time. It has also been demonstrated that when the raw fibres were exposed to steam followed by enzymatic treatment, the degumming effect was quite good. One of the

advantages of steaming the fibres prior to enzymatic degumming is that it reduced the time of enzymatic process from 17 hours to 5-6 hours.

5.0 CONCLUSIONS

It has been shown that the three natural fibres such as Banana, Pineapple and Sansevaria fibres may be satisfactorily degummed using Pectinase (from *Aspergillus Niger)* enzymayic process. The fibre degumming improves with process time. It has also been demonstrated that when the raw fibres were exposed to steam followed by enzymatic treatment, the degumming effect was quite good. One of the advantages of steaming the fibres prior to enzymatic degumming is that it reduces the time of enzymatic process from 17 hours to 5-6 hours. In commercial application, this may be an important aspect as it may affect the cost and viability of the process.

Of the three fibres, Sansevaria seems to be the most promising one. The degummed fibre is relatively soft and long to be spun with other fibres. It may find commercial application in blends with other natural fibres such as wool or cotton.

6.0 FURTHER WORKS

From the results of the set of experiments carried out on the banana, pineapple and Sansevaria fibres, it is recommended that the process be pilot tested in an industrial environment on a larger (mass) sample of fibres. The extent of degumming of the fibres may then be assessed and compared to laboratory trials. Experienced spinners may also be called for to give their views on the suitability of the degummed fibres for spinning with other fibres such as wool and cotton.

One of the immediate uses of the degummed or raw fibres may be in the development of biodegradable nonwovens for mulch production for plant and/or soil protection in agriculture.

7.0 ACKNOWLEDGMENTS

The Principal Investigator would like to thank the Mauritius Research Council for the research grant for this project, Dr V. Bissonauth for the administrative and technical support and for providing the samples of fibres. The researcher is also very grateful to the Faculty of Engineering, University of Mauritius for providing the necessary technical assistance for the project, and to Mrs A. Vencatasamy, Technician at DASED, for her work as Research Assistant for the project.

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