International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Bio-Enzymatic Surface Modification of Banana Fibre and Improvement in Blending Potentiality with Other Natural Fibre

Indrani Sarma[1], A.C. Deka[2], N.K. Goswami[3]

[1,2,3]The Energy and Resources Institute, North Eastern Regional Centre, Guwahati-781036

Abstract:
Besides the nutritive value of banana fruits the biomass can also be used as natural resources for fibre extraction with indisputable advantages over synthetic reinforcement materials such as low cost, low density, non-toxicity, comparable strength, and minimum waste disposal problem. Microbial pectinolytic enzymes are known to play an important role in a number of industrial processes including degumming of natural fibers. In the present study the microbial and enzymatic retting process continued for optimization of quality banana fibre extraction. The isolated 17 fungal strains having different degree of Pectin degrading properties of which 6 strains also having laccase production properties. Among the strains studied *Phoma herbarum* and *Aspergillius niger* showed higher enzymatic production on pectin plate assay. In bio-enzymatic treatment enzyme produce from *Phoma herbarum* and *Aspergillus niger* showed higher degumming response at 45ºC with a treatment duration of 6h.The optimization of pectinase production by submerged fermentation by using Plackett-Burman Design. The highest enzyme activities, were recorded as 7.5 (U/ml) from *Phoma herbarum* (ENZ-5/2h-treatment) shows better results with degumming time six (6) and recorded higher Tenacity (225.155/den) and Toughness (2.206g/den) which was followed by *Aspergillus niger (*7.0 U/ml, ENZ-1/2h treatment) with Tenacity (147.851/den) and Toughness (2.669g/den).

Keywords: Banana Fibre, Bioenzymatic, Pectinolytic, Degumming, Blending

Introduction:

Banana belongs to the family Musaceae, is world second largest and widely cultivated fruit crop grown on about 8.8 million hectares in the tropical and subtropical countries (Mohapatra *et al*, 2010, Mohiuddin *et al*., 2014) with valuable application in food industry. It is native to the Malaysia-Indonesian region of South-East Asia. Cultivation of banana is one of the most popular agricultural practices in India and India is the leading producer of this perennial crop. About 27 % of world's banana production is from India. An area of around 830.5 thousand ha of land is used for banana cultivation with a production of around 29,779.91 thousand tons. Maharashtra, Tamil Nadu, Gujarat, Andhra Pradesh, Assam and Karnataka are the major banana producing states. Besides India other major banana cultivating countries viz., Japan, Brazil, Ecuador, Peru, Mexico, China, Indonesia, Philippines and West African countries. Banana grows all over the southern and eastern India and has a production of 30,808 thousand tonnes having a yield of 34851 (kg/hc) (Soraisham *et al*., 2021) of which only 10% or less of the banana waste (Pseudostems) are extracted for fiber (Elayaperumal, 2016). The banana trunks are

thrown as agricultural waste due to lack of awareness about the extraction of fibre and post harvesting technology. Natural fibres give a lot of benefit over synthetic fiber due to their biodegradability, recyclability, renewability, low cost, high specific mechanical properties and low density (Mukhopadhyay *et al*., 2008; Peças *et al*., 2018; Kulkarni *et al*., 1983;Gupta *et al*., 2021;Preethi *et al*., 2013). Natural fibres differ from one other in their inherent physical, mechanical, and surface properties. Fibres extracted at different countries from its pseudostem are equally good in textile properties *viz*., good absorbent, highly breathable, quickly drying; high tensile strength and biodegradable (Vigneswaran *et al*., 2015) to have no negative effect on the environment.

It is estimated that, after harvest of fruits, huge quantity of biomass residues (60t/ha – 80/t ha) is left over as waste. In comparison to synthetic fibres the natural fibres are with numerous advantages like it arise from their renewability, low cost, wide availability and stiffness etc (Khan and Alam, 2012). All banana varieties have fibers in abundance (Vigneswaran *et al*., 2015). Banana fiber is a biodegradable and recyclable natural fiber that provides certain advantages such as low cost, low density, non-toxicity, comparable strength and minimum waste disposal problems. Natural Fiber based products are now getting more preference across the globe over synthetic fiber products due to their comfort, bio-degradability and the manufacture of which add more to the carbon credit.

India has a big role to play in such a situation by utilizing its large reservoir of fibers in the most diverse terrine of our country. Among the available agro based, natural cellulosic fibres, potentiality of banana fibres as a natural fibre is high. Among the natural fibers the Cotton is widely utilized with 90% share in textile industries(Khan *et al*., 2020; Felgueiras *et al*., 2021), however, there is increased demand for exploration of fiber-yielding crops as alternative natural fibers for textile production. There are two alternatives for cotton like utilization of fiber-yielding crops such as jute, hemp, flax, ramie etc. or use of agro waste derived natural cellulosic fibers, which have increasingly been utilized as textile fibers over the last two decades. Banana fibers are agro waste derived natural fiber with 60-65% cellulose, 6-19% hemicellulose, 5-10% lignin, 3-5% pectin, and 3-6% extracts- bio substances(Bhatnagar *et al*., 2015, Gupta *et al*., 2021, Vajpayee *et al*, 2020). The cellulose microfibrils helical weave of banana fibers embedded within hemicellulose and lignin amorphous matrix(Priyadarshana *et al*., 2022). The presence of non-cellulosic components on the banana fiber's surface mainly alter the surface characteristics including wettability and other essential surface properties(Oliveira *et al*, 2012, Vajpayee *et al*, 2020). Alkali treatment is traditionally used to remove the non-cellulosic substances from raw cellulosic fibers or fabric surface(Twebaze *et al*, 2022) with imparting hydrophilicity and other surface properties required for further wet chemical processing like antimicrobial properties (Sheikh and Bramhecha, 2019). However, in traditional fibre processing system a numbers of extremely harsh chemicals are utilized with higher amount of water and energy consumption which may results in environmental pollution and health hazard(Gulzar *et al*., 2019). Moreover, along with generation of large amount of wastewater during traditional scouring, there may be oxidative damage to the cellulosic fabric if the process of traditional scouring is not appropriately controlled(Singh *et al*., 2020; Topalovic *et al*., 2007, Tzanov *et al*., 2002)..

In recent years as an alternative to harsh chemicals exploration of alternative biotechnological approaches have been expedite by various workers in various stages of textile wet processing with a view to obtain natural fabric with good physic-mechanical properties along with high wettability and dye absorbance(Aly *et al*., 2004, Shabbir *et al*., 2020). Enzymes obtained from numerous sources have been utilized for textile wet processing for scouring, degumming, washing, polishing, peroxide degradation

etc. and work in mild setting with aid in speeding up the substrate specified reaction(Banat *et al*., 1996, Bhat, 2000, Madhu and Chakraborty, 2017). The use of enzyme in textile wet processing are becoming more popular mainly due to their lower production costs, enhance fabric quality and functionality, environmental safeguard, reduce the issues with health and safety(Aly *et al*., 2004).

Enzymatic scouring, or bio-scouring, uses enzymes to target and release non-cellulosic impurities from cellulosic fabric in a relatively environmentally friendly manner (Madhu and Chakraborty, 2017, Rahman *et al*., 2020). The cellulase and pectinase enzyme has been reported as being ideally suited for treating natural fibers for their unique catalytic activities(Cao and Tan, 2002, Wu *et al*., 2023). The enzymatic extraction of banana fibers from pseudostem has been reported earlier by various workers (Balakrishnan *et al*., 2021, Paramasivam *et al*., 2022, Subash and Perumalsamy, 2022). However, limited work has been reported on the natural fibers surface modification(Gupta *et al*., 2021). It was reported that enzymatic treatment of banana fiber with pectinase (5%) able to improve the fineness of fibers for taxtile applications(Balakrishnan *et al*, 2019). The combination of enzymes like cellulose and pectinase reported to be suitable for removal of non-cellulosic impurities and enhance softness of banana fibers (Shroff *et al*., 2015) while laccase and pectinase was also reported as suitable degumming agents to obtain better quality banana fibers (Paramasivam *et al*., 2022). Similarly, treatment of banana fibers with other enzymes like laccase and xylanase was also reported to improve the banana fiber quality with a lignin removal efficiency of 29% for laccase and 27.7% for xylanase enzyme(Vishnu Vardhini and Murugan, 2017). It was also reported that laccase enzyme play an important role on improving the surface properties of banana fiber(Paramasivam *et al*., 2022). In another bioscouring study of banana fibric with xylano-pectinolytic enzymes a reduction (50%) in scouring chemical was reported to achieve the quality similar to 100% chemical treatment and bleaching (Kaur *et al*., 2020).

Surface properties of the enzyme-treated and untreated banana fabric were examined using Scanning Electron Microscopy (SEM) and other characterization techniques to understand the effect of the pectinase and leccase enzyme treatment on the surface and bulk properties of the banana fibers.

Proximate chemical analysis of banana pseudostem indicate the presence of- 59.18 % cellulose, 17.5 % hemicellulose, 54.6% Alpha-cellulose, 1.4% ash and 18.2 % lignin(Goswami *et al*., 2008). As the manufacturing of synthetic fibers from petroleum based products are found to be harmful, non-biodegradable and consume more energy. So there is scope for exploration of low cost natural fibres with good mechanical properties to increase its use in the composites field (Ortega *et al*., 2016). These leads for the development of agro based quality fibers for textile application. Among the available agro based, natural cellulosic fibres, potential of banana fibres as a natural fibre is high. Its sustainability is undeniable and has unexplored potentiality in textile field. In India there are vast resources to extract fibres from banana stem (Pappu *et al*., 2015). Many cottage industries in south India uses banana fibre for making handicraft products. In some parts of the world like Japan and Nepal this plant is used for making textile materials and accessories.

Relatively higher tensile strength and stiffness of banana fibre make it promising fibre material (Justiz Smith *et al*., 2008). The earlier studies reported that enzymatic extraction results in longer fibre extraction and results in more yarn production (Sarma and Deka, 2016). Moreover, the higher yarn strength of banana fiber will facilitate for blending with other natural or synthetic fibers for blended fabric and textile production. The jute-banana fibre blended yarn made with judicious blend ratio could successfully be used as hessian weft and sacking warp. Among the enzymes pectinases have great biotechnological potential with involvement in many industrial processes including processing of fibres.

Fungi are the maximum producer of secondary metabolites and extracellular enzymes including pectinases. After extracting the natural banana fibres, degumming is essential for removal of heavily coated, non-cellulosic gummy material from the cellulosic part of plant fibres. Pectinolytic enzymes have been applied to the degumming of jute, sunn hemp, flax, ramie and coconut fibres for textile application (Zhang *et al*., 2000). When the fibres are treated with pectinase, the middle lamella get destroyed facilitating separation of fibres.

Among the various degumming methods the biological enzymatic treatment methods are preferred over chemical methods due to its eco-friendly in nature. The chemical methods results in release of toxic treated effluents which causes environmental pollution. Moreover, mechanical methods fail to remove the sufficient gummy material from the fibre bundle surface besides the requirement of high energy inputs. In comparison to chemical and mechanical methods the bio-enzymatic degumming method serves as a good alternative to reduce the fiber damage and pollution. Thus, the aim of this work was to explore the potentiality of the mycogenic pectinase enzymes from different fungal isolates for extraction and degumming of banana fiber and followed by its surface modification by laccase enzyme to facilitate blending with other natural fibre like jute for its textile and other commercial uses.

MATERIALS AND METHODS

Mechanical, chemical and biological treatments are followed for extraction of banana fibres.

Mechanical extraction of banana fibre was carried out in banana fibre extraction machine. Among the chemical banana fiber extraction and degumming treatment was carried out with 10% w/v Commercial soda, ammonium oxalate and sodium sulphite. In biological treatment, mycogenic enzymatic retting is used for fibre extraction and degumming.

Isolation of fungal isolates

Soil samples and old banana pseudo stem scrapings, collecting from banana orchards was taken for isolation of microorganism by serial dilution and streaking method. Culture established on petriplates containing pre-sterilized potato dextrose agar (PDA) medium (with 0.1% ampiciline) and incubated at 28°C for 3 days. Pure culture of fungi were established with subsequent single spore inoculation technique. The established fungal strains were maintained at 4ºC in slants for further study. Identification of genus was based on morphological and biochemical characteristics and maintained on PDA slants as stock cultures.

Screening of potential fungal strain with pectinolytic activity

Preliminary screening of fungal isolates for pectinase production was carried out by disc plate method (Acuna-Argulles *et al*., 1994).

Pectinase Screening Agar Medium (PSAM) containing 3 g-l (NH4)2HPO4, 2 g-l KH2PO4, 3 g-l K2HPO4, 1 g-l MgSO4, 25 g-l Agar, 10 g-l Pectin with pH 4.5 was used for the selective growth of the fungal strains which release pectinase. After preparation and sterilization of PSAM medium a loopful of conidia of pre-identified pure fungal isolate was transferred aseptically to the petriplates with sterilized PSAM medium by streak plate method. The inoculated plates were incubated at 28±1ºC temperature in BOD incubator. After incubation for 48 hours at 30ºC the culture plates were screened for identification of Zones of hydrolysis. The utilization of Pectin by fungal isolate was detected by flooding the culture plates with freshly prepared Iodine - Potassium iodine solution (Hawksworth *et al*., 1983). After treating with Iodine - Potassium iodine solution results in development of colour to the medium containing pectin and demarcation of pectin degradation by forming a translucent halo in the pectin degraded

region. A set of culture plate having media without pectin or any carbon source was also maintained as control. The clear zone around the growth of fungi was indicated the activity of pectinase enzyme and the pectinase production efficiency of the fungal isolate(Beg *et al*., 2000). Clear zones of each fungal isolate were measured and recorded to identify the fungal isolate with higher enzyme production efficiency.

Primary screening of efficient Pectinase producing fungal isolate

Isolates that showed the positive result for production of pectinase enzyme were inoculated on the same (PSAM) media using a sterilized cork borer. A disc of fungal isolate was transferred to the media at the centre of the petridish and incubated for 5-6 days at 30°C temperature. The diameter of the fungal colony and the total zone of enzyme activity including the growth diameter were measured in each case. The 'Relative Enzyme Activity' (REA) was calculated on the basis of enzyme activity zone. The fungal isolates with biggest zone of inhibition were selected for further identification of the best pectinase enzyme producer.

Production of pectic enzymes by submerged fermentation

The submerged fermentation test was performed for the primarily screened fungal pectinase positive isolates. The Pectinase production efficiency of fungal isolates was studied by maintaining the culture in broth media contained (g/L) (NH4)2SO4 2; K2HPO4 2; KH2PO4 2; yeast extract: 3 and pectin: 5 with pH maintained at 7.0 for submerged fermentation. The screened potent strains with pectinase activity were inoculated into 100 mL of the broth and incubated at 30°C and 175 rpm for 24 h in a rotary shaker.

Pectin Enzyme Assay

The liquid enzyme mixture and the mycelia mat was separated from fermented broth by filtering through Whatman No1 filter paper. The extract was further centrifuged at 8,000 rpm for 20 minutes to separate cell debris and other contaminants. The crude enzyme extract was then used for measuring pectinase activity. The pectinase actiovity in the fermented broth was monitored by using the dinitrosalicyclic acid(DNS) assay methods. Two millilitres of fermented broth was taken out into a sterile tube and centrifuged at 8000rpm, for 20 min. The supernatant was used as the crude enzyme for analysis. 1ml of crude enzyme and 1ml of 3% pectin were mixed in a sterile tube and incubated at 50 ºC for 15 min and after incubation, 1 ml of DNS reagent was added to the mixture to stop the hydrolysis reaction. The well shaken tube was then placed in a boiling water bath for 30 min for colour development. The absorbance was then spectrophotometrically read at 540nm by running the enzyme and substrate blanks in parallel. The blank containing 1ml of 0.5% pectin, 1ml of sodium acetate buffer (0.1M, pH 4.2) and 2ml of DNS reagent was used as a control.

Plackett-Burman Design were followed for optimization of pectinase production by submerged fermentation by using 6 variables such as Glucose, Peptone, K2HPO4, MgSO4, pH and CuSO4 at 2 levels and 8 runs.

Partial purification for enzyme characterization by Ammonium sulphate fractionation:

Fungal supernatant was partially purified by ammonium sulphate precipitation and activity was again checked. Solid ammonium sulphate was added to the crude extract to 40-80% saturation. The precipitate was collected by centrifugation, dissolved in minimal volume of 0.1% Tris-Hcl buffer (pH 9) and dialyzed against same buffer.

Degumming of banana fibres by mycogenic Enzyme treatment:

Banana pseudo stem treatment were performed in 5L Beaker (Borosil) containing the crude extract of fungal isolates. The pieces of banana pseudo stem (30 x 10 x 2 mm) were incorporated in the crude

enzyme and were incubated at 30ºC for three days. After 3 days, the pieces of pseudo stem were washed thoroughly with clean water and the fibre bundles were separated by hand stripping. The resulted hand stripped banana fibres again washed and air dried at room temperature.

Evaluation of physical strength:

The extracted Banana fibres through different treatments (enzymatic, chemical and control) was evaluated for the physical strength properties using standard Universal Testing Machine (UTM) methods, to ascertain the effect of enzyme and chemical treatment on the quality of the extracted banana fibre. Three sets of fibres were evaluated for each of the test parameters. Other fibre properties were also recorded such as tensile strength, fineness, diameter, fibre pre-treatment and residual gum content. The tensile properties of the fibres bundles were also carried out on Universal Testing Machine (UTM) machine. The higher breaking strength was observed in the fibers with diameter ranged between 200-250(µm). From the findings it was observed that Young's modulus and tensile strength decreased with increasing fibre diameter.

Blending of Banana fibre is done with jute at different blending ratio like Banana : Jute(25:75), (50:50) and (75:25).

Result and Discussion:

A total of 17 fungal strains showed different degrees of enzyme producing properties. Out of 17 strains 9 numbers of strains showed Pectin degrading properties. The optimization of pectinase production by submerged fermentation was carried out using Plackett-Burman Design. Plackett-Burman Design were followed for optimization of pectinase production by submerged fermentation using 6 variables such as Glucose, Peptone, K2HPO4, MgSO4, pH and CuSO4 at 2 levels and 8 runs. The significant factors identified were Peptone @10 g-1; pH 4 and MgSO4@ 0.8 g-1 for *Phoma herbarum*(Table-3). The higher enzyme activities, were recorded as 7.5(U/ml) from *Phoma herbarum* followed by *Aspergillus niger* 7.0(U/ml) (Table-2). Higher production of pectinase enzyme by *Phoma* species was also reported by earlier workers(Madhu *et al*., 2015). The study also highlights the potentiality of *A. niger* as a good source of pectinase producing fungi. Similar results for abundant availability with high secondary metabolite production by *A.niger* strain was also reported by Piccoli-valle *et al* (2001).

Banana fibres were degummed with different concentration of fungal based enzymes as described (Jacob and Prema, 2008). The degumming time required for the present method is only six hr with enzymatic treatment solving the time taking problem of chemical treatment and natural retting to a great extent. The enzymatic treatment has proven to be useful for banana fiber treatment, achieving an improvement in terms of cleanliness and fibrillation.

The chemical treatment shows poor response in comparison to enzymatic treatment. Among the chemical banana fiber degumming treatment the Commercial soda(10%) shows good response with moderate Tenacity (131.953/den) and Toughness (1.570 g/den) which was followed by NaOH treatment(Table-4). Further enzymatic retting process continued for optimization of quality banana fibre extraction. While in bio-enzymatic treatment enzyme produce from *Phoma herbarum* and *Aspergillus niger* showed higher degumming response at 45ºC with a treatment duration of 6h.

It was also observed that after 6h treatment maintaining the banana fiber for long duration treatments (24 h, 48 h) it did not provide any better results probably due to enzyme deactivation. Among the bio-enzymatic treatment durations the 6 h was recorded as the optimal duration of treatment to obtain a textile grade banana fiber.

The chemical treated fibers can be seen as rough and flatten, although pithy material was removed. Treatment with sodium hydroxide reduced the size of the fiber into the smaller bundles. The primary wall of the ultimate fibres ruptured due to tremendous swelling, and they appears like squeezed straws.

The enzyme synthesised by *Phoma herbarum* (ENZ-5/2h-treatment) shows better results with higher Tenacity (225.155/den) and Toughness (2.206g/den) which was followed by *Aspergillus niger* (ENZ-1/2h treatment) with Tenacity (147.851 /den) and toughness (2.669 g/den). The enzyme synthesized by *Aspergillus niger* shows better results with Tenacity(147.851 g/den) and Toughness (2.669 g/den) which was followed by the *A. fumigatus* (Tenacity 124.908 g/den and Toughness- 2.307 g/den(Table-5). The results indicate that clean banana fiber production based on bio processing by using fungal originated pectinase enzyme can be employed in fiber extraction and degumming process instead of utilizing harsh chemicals like surfactants, alkali chlorite, hypochlorite based bleaching etc.

Scanning electron microscope (SEM) analysis was carried out to evaluate fiber matrix interfaces and analyze the structure of the fractured surfaces of banana fiber. The Scanning SEM photographs of both raw, chemically and enzymatically treated banana fibre surface study reveal that Laccase enzyme treated banana fiber surface are smoother in comparison to the chemical treated and control fibers (Plate- 5).

Banana fiber can also be spun to produce yarns, and blend of banana fiber with other natural fibers (Jute) and the ratio of banana fiber with Jute fiber at 75:25 recorded to be best among the other blending ratio studied.

Table 1: Primary screening isolates: Clear zone to colony diameter ratio

Sl no.	Species name	Fungal colony(diameter) (mm)	Zone of hydrolysis (mm)	REA (mm)
1.	*Aspergillus sclerotiorum*	13	42	2.23
2.	*Phoma herbarum*	10	46	3.6
3.	*Penicillium citrinum*	12	37	2.08
4.	*Aspergillus niger*	7	29	3.14
5.	*Fusarium oxysporum*	12	45	2.75
6.	*Aspergillus tamarii*	12	30	1.5
7.	*Paecelomyces lilacinus*	13	38	1.28
8.	*Penicillium purpurogenum*	11	39	2.54
9.	*Verticillium lecanii*	24	35	0.45
10.	*Fusarium solani*	9	29	2.22
11.	*Penicillium chrysogenum*	20	67	2.35
12.	*Paecelomyces lilacinus*	12	33	1.75
13.	*Penicillium glabrum*	14	52	2.71
14.	*Trichoderma reesei*	16	28	0.75
15.	*Penicillium pinophyllium*	18	53	1.94
16.	*Trichoderma viridae*	23	48	1.08
17.	*Fusarium sp*	43	50	0.34

Table 2: Pectinase activity of different fungal isolates grown under submerged fermentation at 28° C for 4 days at 150 rpm

Secondary screening: pectinase activity (Unit/ml).

Sl No	Isolates Number	Isolates	Enzyme activity*
1	Pct1	*Aspergillus sclerotiorum*	3.8
2	Pct5	*Phoma herbarum*	7.5
3	Pct6	*Penicillium citrinum*	5.2
4	Pct8	*Aspergillus niger*	7.0
5	Pct10	*Fusarium oxysporum*	5.2
6	Pct12	*Aspergillus tamarii*	1.5
7	Pct13	*Paecelomyces lilacinus*	1.0
8	Pct14	*Penicillium purpurogenum*	3.0
9	Pct21	*Verticillium lecanii*	0.03
10	Pct27	*Fusarium solani*	2.2
11	Pct29	*Penicillium chrysogenum*	4.2
12	Pct34	*Penicillium glabrum*	2.0
13	Pct37	*Trichoderma reesei*	0.01
14	Pct38	*Penicillium pinophyllium*	1.9

- Values are mean of 3 replicates

Secondary screening: pectinase activity (Unit/ml).

The *Phoma herbarum* shows higher pectinase enzyme activity (7.5 Unit/ml) which was followed by the *Aspergillus niger* (7.0 Unit/ml), *Penicillium citrinum* (5.2 Unit/ml) and *Fusarium oxysporum* (5.2 Unit/ml).

Table 3: Optimization of fungal based pectinase production

The optimization of pectinase production by submerged fermentation was carried out using Plackett-Burman Design using 6 variables, 2 levels and 8 runs.

6 variable, 2 levels, 8 runs

	Glucose	Peptone	K_2HPO_4	$MgSO_4$	pH	$CuSO_4$
Unit	g^{-1}	g^{-1}	g^{-1}	g^{-1}	scale	g^{-1}
High(+)	10	10	1	0.8	4	$30*10^{-4}$
Low(-)	1	0.5	0.1	0.2	8	$0.5*10^{-4}$

Run	Variables					
	Glucose (X1)	Peptone (X2)	K2HPO4 (X3)	MgSO4(X4)	pH(X5)	CuSO4(X6)
1	+	-	-	+	-	+
2	+	+	-	-	+	-
3	+	+	+	-	-	+
4	-	+	+	+	-	-

5	+	-	+	+	+	-
6	-	+	-	+	+	+
7	-	-	+	-	+	+
8	-	-	-	-	-	-

		FACTORS						Pectinase activity (m eq NaOH)				
RUN		X1	X2	X3	X4	X5	X6	R1	R2	R3	avg	
	1	1	-1	-1	1	-1	1	3.2	3	3.2	3.133	
	2	1	1	-1	-1	1	-1	6.3	6.4	6.4	6.367	
	3	1	1	1	-1	-1	1	2.9	2.4	2.8	2.700	
	4	-1	1	1	1	-1	-1	4.6	4.6	4.8	4.667	
	5	1	-1	1	1	1	-1	4	4.2	4	4.067	
	6	-1	1	-1	1	1	1	9.6	9.6	9.5	9.567	
	7	-1	-1	1	-1	1	1	3	3.2	3.2	3.133	
	8	-1	-1	-1	-1	-1	-1	0.9	1	0.9	0.933	
Effects		-0.508	3.008	-1.358	2.075	2.925	0.625		103.7			∑Y
Coefficients		-0.254	1.504	-0.679	1.038	1.463	0.313		448.070			∑(Y)2
									594.77			∑Y2
SS	∑SS	df	MS	F calculated								
SSX1	1.182	1	1.182	0.574	AT 95% CONFIDENCE LEVEL. ALL FACTORS WITH $F_{calculated}>F_{critical}$ ARE THE SIGNIFICANT			FX1,critical				4.45
SSX2	41.413	1	41.413	20.103				FX2,critical				4.45
SSX3	8.443	1	8.443	4.099				FX3,critical				4.45
SSX4	19.703	1	19.703	9.564				FX4,critical				4.45
SSX5	39.151	1	39.151	19.005				FX5,critical				4.45
SSX6	1.788	1	1.788	0.868				FX6,critical				4.45
SSTOTAL	146.670							FX7,critical				4.45
SSERROR	35.020											
dfError	17				**Findings : Peptone @10 g-l; pH@4 and $MgSO_4$ @ 0.8 g-l for** *Phoma herbarum*							
MSError	2.060											

The optimization of pectinase production by submerged fermentation was carried out using Plackett-Burman Design. Plackett-Burman Design were followed for optimization of pectinase production by submerged fermentation using 6 variables such as Glucose, Peptone, K2HPO4, MgSO4, pH and CuSO4 at 2 levels and 8 runs. The significant factors identified were Peptone @10 g-l; pH@4 and MgSO4 @ 0.8 g-l for *Phoma herbarum*.

Table 4: Tensil properties of banana fiber treated with different chemical treatments

Sl No	Treatment	Load at Maximum(g)	Strain at Maximum(%)	Modulus (Aut Yong) (g/den)	Tenacity at Break (g/den)	Tenacity at Maximum	Toughness
0	CHEM-0	283.527	2.247	88.504	79.603	79.603	1.189
1	CHEM-1(24 h)	175.259	1.933	2860.088	120.074	120.074	1.270
2	CHEM-1(48 h)	238.173	2.268	3075.420	68.049	68.049	0.768
3	CHEM-2(24 h)	461.836	3.400	4175.708	131.953	131.953	1.570
4	CHEM-2(48 h)	308.937	2.533	3859.822	88.268	88.268	1.074

Among the chemical degumming treatment of banana fiber the Commercial soda Na2CO3(10%) (CHEM-2/24h treatment) shows good response with moderate Tenacity (131.953/den) and Toughness (1.570 g/den) which was followed by NaOH treatment with Tenacity (120.074/den) and Toughness (1.270 g/den).

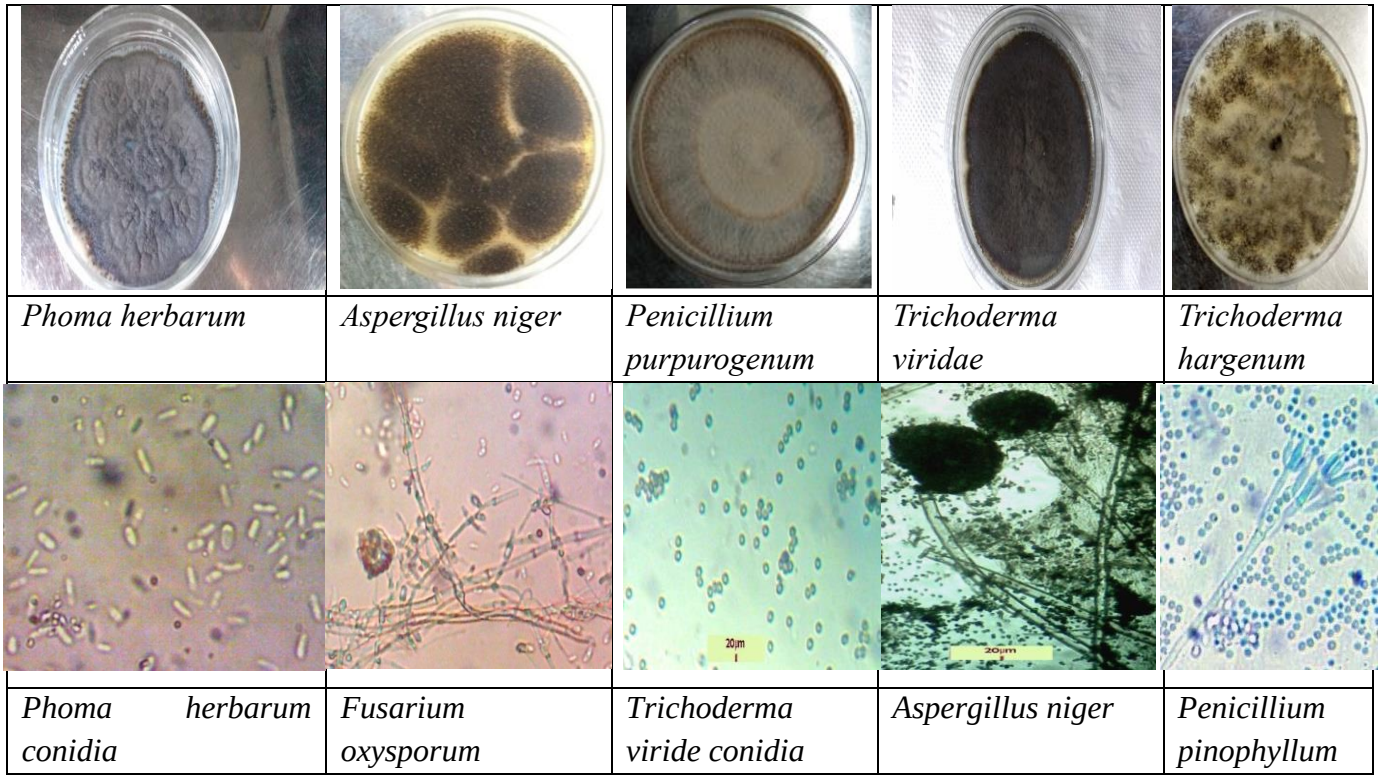
Table 5: Tensile properties of banana fiber treated with different mycogenic enzyme treatments

Sl No	Treatment	Load at Maximum(g)	Strain at Maximum(%)	Modulus (Aut Yong) (g/den)	Tenacity at Break (g/den)	Tenacity at Maximum	Toughness
0	ENZ-0	280.211	2.360	86.296	80.060	80.060	1.005
1	ENZ-2(2h)	117.868	2.268	1652.190	33.676	33.676	0.369
2	ENZ-4(2h)	522.474	3.400	5384.899	149.278	149.278	2.252
3	ENZ-2(2h)	359.200	3.400	3328.410	102.654	102.654	1.663
4	ENZ-2(4h)	127.821	2.205	1961.277	36.968	36.968	0.388
5	ENZ-1(4h)	222.926	2.533	2992.540	63.693	63.693	0.785
6	ENZ-3(4h)	382.269	3.333	3766.668	114.917	114.917	1.827
7	ENZ-3(4h)	172.266	3.002	1889.971	49.219	49.219	0.695
8	ENZ-3(4h)	172.266	3.002	1889.971	49.219	49.219	0.695
9	ENZ-4(4h)	250.447	3.267	2987.253	71.556	71.556	1.035
10	ENZ-5(6h)	704.925	4.135	5256.159	201.407	201.407	3.974
11	ENZ-5(6h)	568.044	3.397	4002.586	225.155	225.155	2.206
12	ENZ-1(6h)	424.233	3.601	3553.020	121.209	121.209	2.112
13	ENZ-1(6h)	420.219	4.354	2902.574	147.851	147.851	2.669
14	ENZ-3(6h)	402.208	3.933	3772.698	124.908	124.908	2.307
15	ENZ-2(8h)	397.273	2.287	2091.796	87.679	87.679	1.348
16	ENZ-3(8h)	402.208	3.333	3766.668	114.917	114.917	1.827
17	ENZ-3(8h)	357.233	3.074	2806.797	122.768	122.768	1.697

18	ENZ-3(10h)	398.288	4.574	1869.907	117.703	117.703	1.397
19	ENZ-4(10h)	322.674	3.548	1381.872	99.203	99.203	1.151
20	ENZ-2(10h)	316.247	3.433	1348.591	87.372	87.372	1.696
21	ENZ-4(12h)	146.628	2.468	2384.809	68.618	68.618	0.789
22	ENZ-4(24h)	243.200	2.860	2976.273	63.811	63.811	1.263
23	ENZ-5(48h)	237.926	2.538	1889.874	74.528	74.528	0.696

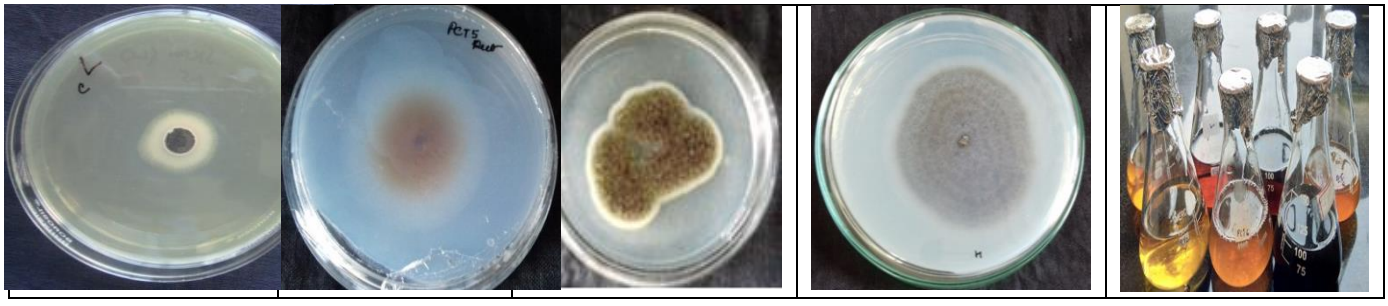
In compression to chemical treatment the enzymatic treatment shows better response for degumming, firmness and quality of banana fibers. The enzyme synthesised by *Phoma herbarum* shows better results with higher Tenacity(149.278 g/den) and Toughness(2.252g/den) followed by *Aspergillus niger* with Tenacity(147.851 g/den) and Toughness(2.669g/den), and *A. fumigatus* with Tenacity(124.908g/den), Toughness(2.307 g/den).

Plate 1: Photographs and micrographs of few fungal isolates for extraction and degumming of banana fiber



Phoma herbarum	*Aspergillus niger*	*Penicillium purpurogenum*	*Trichoderma viridae*	*Trichoderma hargenum*
Phoma herbarum conidia	*Fusarium oxysporum*	*Trichoderma viride conidia*	*Aspergillus niger*	*Penicillium pinophyllum*

Plate 2: Pectinase enzyme producing fungal isolates (a –d), extracellular pectinase production in submerged fermentation(e)



Penicillium chrysogenum	*Phoma herbarum*	*Aspergillus niger*	*Aspergillus sclerotiorum*	Submerged fermentation

Plate 3: Laccase enzyme producing isolates

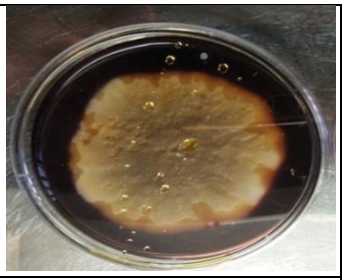
			
Aspergillus niger	*Penicillium citrinum*	*Phoma herbarum*	*Fusarium oxysporum*

Plate 4: Processing for degumming and surface modification of banana fiber

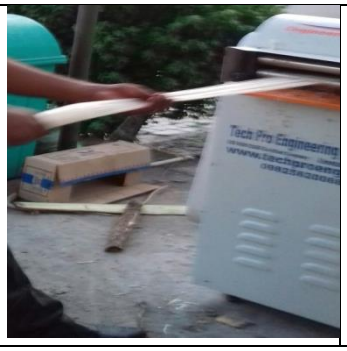
		 
Banana pseudostem processing	Mechanical extraction of banana fiber	Extracted banana fiber
		
Degumming and surface modification of banana fiber with Pactinase and laccase enzyme		Control

Plate 5: SEM photographs of both raw, chemically and enzymatically treated banana fibre surface

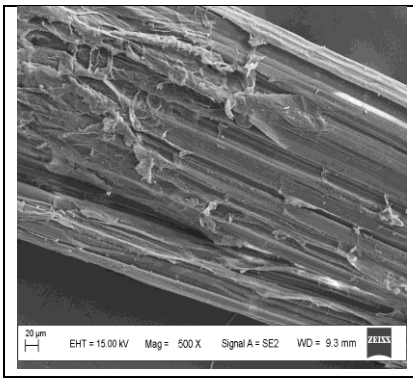
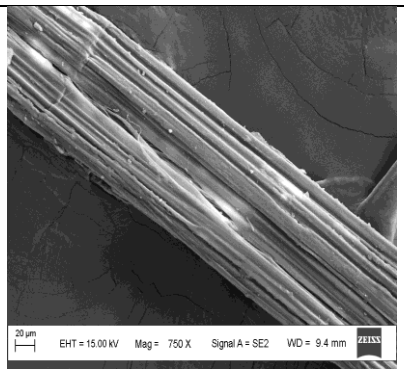
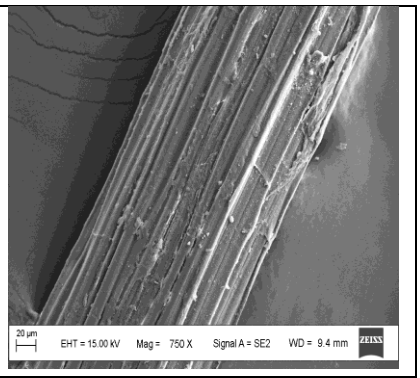
		
a) Untreated banana fibre surface	b) Laccase enzyme Treated banana fibre	c) Chemically treated (NaOH) banana fiber

Plate 6: Blending of surface modified banana fibre with other natural fiber (Jute)

		
a) Blended fibre of Banana : Jute (25:75)	b) Blended fibre of Banana : Jute (50:50)	c) Blended fibre of Banana : Jute (75:25)

Conclusion:

The present findings includes production of clean banana fiber through bio processing with mycogenic enzymes. The findings also established that the Fungal originated pectinase enzyme can be employed in banana fiber extraction and degumming process instead of utilizing harsh chemicals like surfactants, alkali chlorite, hypocholorite based bleaching etc. It can also be concluded from the present investigation that, banana fibre which is till now being wastes after harvesting of fruits, showed good potential for using value-added textiles. If the technology is adopted/implemented large number of poor farmers would get additional remuneration with very minor additional inputs.

Reference:

1. Acuna-Argulles, M., Viniergra-Ganzalez G., Favela- Torres E.1994. Effect of water activity on exopectinase production by *Aspergillus niger* CH_4 on solid state fermentation. *Biotechnol. Lett*. 16: 23-28.
2. Aly A.S, Moustafa A.B, Hebeish A. 2004. Bio-technological treatment of cellulosic textiles. *Journal*

of Cleaner Production. Volume 12, Issue 7, September 2004, Pages 697-705

3. Aly, A. S., Sayed, S. H. M. and Zahran, M. K. 2010. 'One-step process for enzymatic desizing and bioscouring of cotton fabrics', *Journal of Natural Fibers*, 7:2, pp. 71–92.
4. Balakrishnan S, Wickrama singhe GLD and Wijayapala S U .2019. Investigation on improving banana fiber fineness for textile application.Volume 89: 21-22
5. Balakrishnan S., Wickramasinghe G., Wijayapala U. S. 2021. Eco-Friendly softening for banana Fibers from Sri Lankan banana cultivar: Influence on physical and chemical Properties on banana Fibers. *Journal of Natural Fibers*. Vol 19(14) Pages 9177-9189
6. Banat, Ibrahim M., Nigam, Poonam, Singh, Datel, Marchant, Roger.1996. Microbial decolorization of textile-dye containing effluents: A review. *Bioresource Technology***.** Volume 58, Issue 3, December, Pages 217-227
7. Barreto A.C.H., Costa M.M., Sombra A.S.B., Rosa D.S., Nascimento R.F., Mazzeto S.E. *et al*. 2010. Chemically modified banana fibre: structure, dielectrical properties and biodegradability, *Journal of Polymer and Environment.* 18: 523-531.
8. Beg Q. K., Bhushan, B. Kapoor, M., and Hoondal G. S. 2000. "Production and characterization of thermostable xylanase and pectinase from *Streptomyces* sp. QG-11-3," *Journal of Industrial Microbiology and Biotechnology*, 24(6): 396–402
9. Bhat, M.K. Cellulases and related enzymes in biotechnology.2000. *Biotechnology Advances*. 18(5):355-383.
10. Bhatnagar, R., G. Gupta, and S. Yadav. 2015. A review on composition and properties of banana fibers. *International Journal of Scientific & Engineering*. 6 (5): 49–52.
11. Cavaco-Paulo, A. and Gubitz, G.M. 2003. Textile Processing with Enzymes. Woodhead Publishing Limited.
12. Deka, B., P. Deka, and R. Borgohain. 2014. Exploration of banana fibre as low cost eco-friendly waste management. *Asian Journal of Biological Sciences*. 9 (1):129–30.
13. Dyk J.S. van, Sakka M., Sakka H., Pletschke B.I.2010. Identification of endoglucanases, xylanases, pectinases and mannoses in multi-enzyme complex of *bacillus licheniformis* SVD1, *Enzyme and Microbial Technology*. 47: 112-118.
14. Elayaperumal, T. A Preliminary Study for Improving the Banana Fibre Fineness using Various Chemical Treatments, *The Global Journal of Researches in Engineering*, 2016.16(3): 16-22.
15. Felgueiras,C, Azola, N.G, Gonclaves C. (2021). Trends on the Cellulose-Based Textiles: Raw Materials and Technologies. Frontiers in Bioengineering and Biote. DOI: 10.3389/fbioe.2021.608826 Bhatnagar, R., G. Gupta, and S. Yadav. 2015. A review on composition and properties of banana fibers. *International Journal of Scientific & Engineering* 6 (5):49–52.
16. Goswami,T.; Kalita, D. and Rao, P.G.2008.Grease proof paper from banana (*Musa paradisiaca* L.) pulp fiber, *Indian Journal of Chemical Technology*. 15: 457-461.
17. Gulzar T, Farooq T., Kiran S, Ahma I, Hameed A. 2019. 1- Green Chemistry in the wet processing of textiles. *The impact and prospect of green chemistry for textile techonology*. 1-20
18. Gupta, U. S., M. Dhamarikar, A. Dharkar, S. Chaturvedi, A. Kumrawat, N. Giri, S. Tiwari, and R. Namdeo. 2021. *Plasma modification of natural fiber*: A review. Materials Today: Proceedings 43:451–57. doi:10.1016/j.matpr.2020.11.973.
19. Hawksworth, D.L., Sutton, B.C. and Ainsworth G.C. 1983. Ainsworth and Bisby's Dictionary of the

fungi Kew: Commonwealth Mycological Institute.
20. Jacob, N.; Prema, P. 2006. Influence of mode of fermentation on polygalacturonase production by a novel strain of *Streptomyces lydicus*. *Food Technol. Biotechnol*. 44: 263-267.
21. Justin-Smith, N.G., Junior Virgo, and Buchanan, V.E.2008."Potential of Jamaican banana, coconut coir andvbagasse fibers as composit materials,"*Material Charactirization*. 59(9): 1273-1278.
22. Kaur A, Varghese L. M., Battan B, Mahajan R. 2020. Bio-degumming of banana fibers using eco-friendly crude xylano-pectinolytic enzymes. Preparative Biochemistry & Biotechnology 50(5):1-8. DOI: 10.1080/10826068.2019.1710713
23. Kulkarni G., Satyanarayana K. G., Rohatgi P. K. and Vijayan K., Mechanical properties of banana fibres (*Musa sepientum*), *Journal of Material Science*. 1983.(18): 2290-2296. https://doi.org/10.1007/BF00541832
24. Khan MA, Wahid, A, Ahmed M. 2020. World Cotton Production and Consumption: An Overview In book: Cotton Production and Uses. Springer Nature Singapore Pte Ltd.10.1007/978-981-15-1472-2-1
25. Khan, G.M.A. and M.S. Alam. 2012."Thermal Characterization of Chemically Treated Coconut Husk Fibre". *Indian Journal of Fibre and Textile Research*. 37:20 – 26
26. Lenindy D. Soraisham, Nabnita Gogoi, Leena Mishra & Gautam Basu .2021.: Extraction and Evaluation of Properties of Indian Banana Fibre (*MusaDomestica* Var. *Balbisiana*, BB Group) and Its Processing with Ramie, *Journal of Natural Fibers*, DOI: 10.1080/15440478.2021.1897728
27. Madhu, Amit , Chakraborty, J.N. 2017.Developments in application of enzymes for textile processing. *Journal of Cleaner Production***.** 145, 1: 114-133
28. Madhu, H N, Pushkar R., Manasa, V., Anand *et al*. 2015. The Potential of Hydrolytic Enzymes from *Phoma exigua* for Fruit Juice Clarification. *International Journal of Pharmacognosy and Phytochemical Research.*7(4): 750-757
29. Madhu and Chakraborty(2017). Developments in Application of Enzymes for Textile Processing. Joural of cleaner production.145. DOI: 10.1016/j.jclepro.2017.01.013
30. Mira Chares Subash &Muthiah Perumalsamy.2022. Enzymatic Degumming of Banana Fibers by Enterobacter cloacae PMC04 for Fineness Improvisation: An Eco-friendly Approach. *Journal of Natural Fibers*. Volume 19 (15). https://doi.org/10.1080/15440478.2022.2048937
31. Mira SubashMuthiah Perumalsamy .2022.Green degumming of banana pseudostem fibers for yarn manufacturing in textile industries. Biomass Conversion and Biorefinery. DOI: 10.1007/s13399-022-02850-1
32. Mohapatra, D., S. Mishra and N. Sutar, 2010. Banana and its by-product utilisation: An overview. *J. Sci. Ind. Res*., 69: 323-329.
33. Mohiuddin A.K.M., Saha M.K., Hossain M.S., Ferdoushi A.(2014) Usefulness of Banana (Musa paradisiaca) wastes in Manufacturing of Bio-Products : A Review, 12(1): 148-158.
34. Mukhopadhyay S., Talwade G. Vijay, Dhake R., J.D., Pegoretti A.2006. Some studies on banana fibers, International Conference on Advances in Fibrous Materials, Nonwoven and Technical Textiles, Coimbatore, India.
35. Oliveira, F. R., L. Erkens, R. Fangueiro, and A. P. Souto. 2012. Surface modification of banana fibers by DBD plasma treatment. Plasma Chemistry and Plasma Processing 32 (2):259–73. doi:10.1007/s11090-012-9354-3.
36. Ortega, Z., M. Morón , M.D. Monzón, P. Badalló and R. Paz, 2016. Production of banana fiber yarns

for technical textile reinforced composites. Materials, 9(5): 370.
37. Ortega, Z., M. Morón, M. D. Monzón, P. Badalló, and R. Paz. 2016. Production of banana fiber yarns for technical textile reinforced composites. Materials 9 (5):370–79. doi:10.3390/ma9050370.
38. P. Peças , H. Carvalho , H. Salman and M. Leite, Natural Fibre Composites and Their Applications: 2018. A Review, *Journal Composite Science*. 2(4): 66. https://doi.org/10.3390/jcs2040066
39. Pappu, A., V. Patil, S. Jain, A. Mahindrakar, R. Haque and V.K. Thakur, 2015. Advances in industrial prospective of cellulosic macromolecules enriched banana biofibre resources: A review. *Intl. J. Biol. Macromolecules*, 79: 449-458.
40. Pappu, A., V. Patil, S. Jain, A. Mahindrakar, R. Haque and V.K. Thakur, 2015. Advances in industrial prospective of cellulosic macromolecules enriched banana biofibre resources: A review. *Intl. J. Biol. Macromolecules*, 79: 449-458.
41. Paramasivam Suresh Kumar, Panneerselvam Divya, Sundaram Durgadevi, Nallappagounder Karur Shiva &Uma Subbaraya. Extraction, Characterization and Enzymatic Degumming of Banana Fiber.2020. *Journal of Natural Fibers*. Volume 19(4):1333-1342.https://doi.org/10.1080/15440478.2020.1764456
42. Piccoli-valle, R.H, Passos, F.M.L., Passos, F.J.V., Silva, D.O.2001. Production of pectin lyase by *Penicillium griseoroseum* in bioreactors in the absence of inducer. *Braz. J. Microbiol*. 32:135-140
43. Preethi, P. and G. Balakrishna Murthy, 2013. Physical and chemical properties of banana fibre extracted from commercial banana cultivars grown in Tamilnadu state. *Agrotechnol*., 11: 1-3.
44. Priyadarshan, S. K. and S. K. Ghosh.2021. "Statistical and artificial neural network technique for prediction of performance in AlSi10Mg-MWCNT based composite materials," *Materials Chemistry and Physics*, vol. 273, article 125136, 2021.
45. Rahman M D, Polay H D, Billah MM, Nabi M. N. U.2020.Bio-based textile processing through the application of enzymes for environmental sustainability. *International Journal of Technology Management and Sustainable Development*. 19(1):87-106
46. Shabir Sadaf, Faheem, Muhammad, Ali Naeem *et al*.2020. Periphytic biofilm: An innovative approach for biodegradation of microplastics. *The Science of the Total Environment*. Vol. 293.
47. Sheikh J and Bramhecha I, .2019. Multi-functionalization of linen fabric using a combination of chitosan, silver nanoparticles and *Tamarindus indica* L. seed coat extract. *Cellulose*. 26: 8895-8905. https://doi.org/10.1007/s10570-019-02684-7
48. Shroff, Ankita. 2015. Bio–softening of Banana Fiber for Nonwoven Application. *International Journal of Scientific Research*. Vol.4. issue 4.
49. Soraisham L.D., Gogoi N., Mishra L., Basu G.J. 2021. Extraction and Evaluation of Properties of Indian Banana Fibre (*Musa Domestica* Var. Balbisiana, BB group) and its processing with ramie; pp. 1–12
50. Susheel Kalia, Kamini Thakur, Annamaria Celli, Marjorie A. Kiechel Caroline I, Schauer.2013. Surface modification of plant fibers using environment friendly methods for their application in polymer composites, textile industry and antimicrobial activities: A review. *Journal of Environmental Chemical Engineering*. Pg. 9-112
51. Twebaze, C., M. Zhang, X. Zhuang, M. Kimani, G. Zheng, and Z. Wang. 2022. Banana fiber degumming by alkali treatment and ultrasonic methods. *Journal of Natural Fibers*. 19:1–13. doi:10.1080/15440478.2022.2079581.
52. Vajpayee, M., M. Singh, L. Ledwani, R. Prakash, and S. K. Nema. 2020. Investigation of

antimicrobial activity of DBD air plasma-treated banana fabric coated with natural leaf extracts. ACS Omega 5 (30):19034–49. doi:10.1021/acsomega. 0c02380.

53. Vigneswaran, C., V. Pavithra, V. Gayathri, and K. Mythili. 2015. Banana fiber: Scope and value added product development. *Journal of Textile Apparel, Technology Management* .9 (2):1–7.
54. Vishnu Vardhini, K.J. and Murugan, R. 2017. Effect of Laccase and Xylanase Enzyme Treatment on Chemical and Mechanical Properties of Banana Fiber. *Journal of Natural Fibers*. 14(2): 217-227
55. Wu X, Yuan X, Zhao J, Ji D., Guo H., Yao W., Li Xiaoping, Zhang L.(2023) Study on the effects of different pectinase/cellulase ratios and pretreatment times on the preparation of nanocellulose by ultrasound-assisted bio-enzyme heat treatment, RSC Adv. 13(8): 5149–5157.
56. Xuehong Ren, Gisela Buschle-Diller.2007. Oxidoreductases for modification of linen fibers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 299(1–3, 15):15-21
57. Yu Cao, Huimin Tan.2002. Effects of cellulase on the modification of cellulose. *Carbohydrate Research*. 337(14): 1291-1296
58. Zhang J, Henriksson G, Johansson G. 2000. Polygalacturonase is the key component in the enzymatic retting of flax. *J Biotechnol*. 81:85-9.