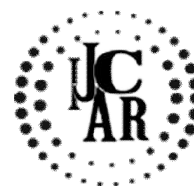
International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 10; Issue 04 (A); April 2021; Page No.24122-24126
DOI: http://dx.doi.org/10.24327/ijcar.2021.24126.4782



Research Article

PROCESS OPTIMIZATION FOR BIO-DEGUMMING AND SURFACE MODIFICATION OF NATURAL BANANA FIBRE

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A R T I C L E I N F O

Article History:
Received 4th January, 2021
Received in revised form 25th February, 2021
Accepted 18th March, 2021
Published online 28th April, 2021

Key words:

Phoma herbarum, Aspergillus niger, Pectinase, Degumming, tensile strength.

A B S T R A C T

Banana generates considerably high amount of plant biomass which could be used for various purposes. Banana pseudostem fibre is a lignocellulosic material, from which good quality fibers can be extracted. Banana fibres are extracted using either alkali or acid treatment for degumming which are neither eco-friendly nor user friendly. Pectinase are a group of enzymes which is able to break down pectic polysaccharides of plant tissue. Pectinolytic enzymes catalyzing the degradation of pectic substances are of great industrial importance (Sapunova *et al*., 1995), (Bajpai, 1999). Since 40% of the dry weight of plant cambium cells is comprised of pectin so pectinases are believed to play a leading role in the processing of bast fibres (Bajpai, 1999). Thus, application of pectinolytic enzymes would loosen the pseudostem tissue thereby facilitating extraction of banana fibres efficiently. In the present study 17 different fungal strains were isolated from soil samples of which only 9 strains found as positive for pectinase activity. Among the strains studied *Phoma herbarum* and *Aspergillius niger* showed higher enzymatic production on pectin plate assay. The optimization of pectinase production by submerged fermentation was carried out using Plackett-Burman Design. The highest enzyme activities, were recorded as 7.5 (U/ml) from *Phoma herbarum* followed by *Aspergillius niger* 7.0 (U/ml).
The study also showed the immense potentiality of *Phoma herbarum* followed by *Aspergillius niger* for effective degumming and extraction of banana fibre from pseudostem. Banana fibres treated with enzymes from *Phoma herbarum* showed higher (tenacity: 137.893 g/den) and Toughness (2.366 g/den) in comparison to *Aspergillus niger* treated fibre (tenacity: 64.660 g/den), Toughness (0.891 g/den).

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INTRODUCTION

Bananas are produced in large quantities in tropical and subtropical areas. World production of Musa is estimated at 102 million MT of which about 68% has been classified as bananas and 32% as plantains (FAO, 2012). India is the largest producer of banana contributing to 27 % of the world production (Vellaichamy and Gaonkar, 2017; Mohapatra *et al*., 2010). More than fifty million tonnes of banana pseudostem is generated after harvesting of banana plant from which 3.9 million tonnes of fibre could be extracted (Chauhan and Sharma *et al*., 2014). Banana plant is one of the valuable bio resource which generates huge quantities of biomass most of which goes as waste due to non-availability of suitable technology for its commercial utilization. Banana pseudostem fibre is a lignocellulosic material mainly consists of polysaccharides with cellulose microfibrils (50-60%) embedded with hemicelluloses (25-30%), lignin (12-18 %), pectin (3-5%), water soluble material (2-3 %), wax and fat (3-5 %) and ash (1-1.5 %) (Mohiuddin *et al.,* 2014).

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From the pseudo stem of banana good quality fibers can be extracted along with other plant components with bioconversion potential. Relatively high tensile strength and stiffness of banana fiber make it attractive as new and renewable biomaterials for textiles and other industries. In comparison to the synthetic fibers the natural fibers are with added advantages for their stiffness, readily availability, low cost, and with renewable properties. Fibres undergo degumming to reduce the plants' glue-like gum content and render them clean and spinnable. There are different methods of degumming like chemical, mechanical and biological for removal of heavily coated, non-cellulosic gummy material from the cellulosic part of plant fibers. So, there is an urgent need to find a simple process to enhance the possibility of waste-less utilization of banana pseudostem for higher quality yield of fibres. Presence of adherent pith in the fibreduring mechanical extraction results in inferior quality of the product. Pectinases are believed to play a leading role in the processing of bast and leaf fibers, since 40% of the dry weight of plant cambium cells is comprised of pectin (Bajpai, 1999). Pectinolytic enzymes are used in commercial fibre extraction from biological materials such as remie (Bruhlmann *et al*.,

1994), cotton (Etters, 1999; Li and Hardin, 1998 a; Karapinar and Sariisik, 2004), hemp (Li *et al*., 2013) and reported good quality of fibres and yarn, besides non-polluting the environment. Application of pectinolytic enzymes would loosen the pseudo stem tissue thereby facilitates extraction of banana fibres efficiently. Therefore, exploration of pectinolytic enzymes from native fungal strains would be helpful for eco-friendly extraction of quality banana fibre.

MATERIAL AND METHODS

Isolation of fungal isolates

Fungal strains were isolated from partially decomposed banana pseduostem, garden soil samples nearby the root zone and soil samples collected from garbage site by inoculating inPDA media by serial dilution method as to get pure culture. Pure fungal culture was established by single spore isolation methods and the isolated pure strains were maintained at 4ºC in slants for further study.

Screening of fungal strains for the Pectinase Enzyme Production

Pectinase Screening Agar Medium (PSAM) was used for the selective growth of the fungal strains which release pectin. PSAM contained 0.3g/100ml (NH4)2HPO4, 0.2 g/100ml KH2PO4, 0.3 g/100 ml K2HPO4, 0.01g/100 ml MgSO4, 2.5 g/100 ml Agar, 1 g/100 ml Pectin with pH 4.5. After the preparation and sterilization of the media, 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. After incubation the plates were screened for the identification of Zones of hydrolysis indicating the positive result for Pectinase production. Fungal utilization of Pectin was detected by flooding the culture plates with freshly prepared Iodine-Potassium iodide solution (Hawksworth *et al*., 1983). After treating with Iodine-Potassium iodide solution results in development of colour to the medium containing pectin and demarcation of pectin degradation by forming a translucent halo in the region where pectin get degraded. Simultaneously, a set of control plate having the media without pectin or any other carbon source was also maintained. A clear halo zone around the colonies indicates the ability of an isolate to produce pectinase (Beg *et al*., 2000). The diameter of the fungal colony and the total zone of enzyme activity including the growth diameter was measured in each case. On the basis of this 'Relative Enzyme Activity' (REA) was calculated. The fungal isolates that gave biggest zone of inhibition were selected as the best producer of pectinase enzyme.

Production of pectic enzymes by submerged fermentation

The submerged fermentation test was performed with the pectinase positive isolates selected from primary screening. Broth media contained (g/L) $(NH_4)2SO_4$ 2; K_2HPO_4 2; KH_2PO_4 2; yeast extract: 3 and pectin: 5 (pH 7.0) was studied for Pectinase production. The screened potent strains with pectinase activity were inoculated into 100 mL of the broth and incubated for 24 h at 30°C and 175 rpm in a rotary shaker.

Pectin Enzyme Assay

The liquid enzyme mixture and the mycelia mat was separated by filtering through Whatman No 1 filter paper and then centrifuged at 10,000 rpm for 15 minutes. This crude enzyme extract was used for measuring pectinase activity.

Pectin esterase activity was measured by increase in free Carboxyl group by titrating against NaOH in the presence of a pH indicator like Phenolphthalein. For assaying pectin esterase activity 20ml of 1% pectin was dissolved in 0.15M NaCl (pH - 7) and 4ml of crude enzyme extract was taken in a beaker and incubated for 1 hour. After incubation, the solution was titrated against 0.02 N NaOH to reach PH 7 using Phenolphthalein as indicator (colour change from colourless to pink) the heated crude enzyme extract was used as control.

Pectin esterase activity = Vs – V b (Normality of NaOH) ×100/Vt

Where, Vs- Volume of NaOH used to titer sample (ml), Vb- Volume of NaOH used to titer blank (ml), V - Volume of incubation mixture (ml), t – Reaction time (min). Pectin esterase activity will be expressed as milli equivalents of NaOH consumed min $^{-1}$ ml $^{-1}$of crude enzyme extract under the assay conditions.

Plackett-Burman Design were followed for optimization of pectinase production by submerged fermentation using 6 variables such as Glucose, Peptone, K_2HPO_4, $MgSO_4$, pH and $CuSO_4$ at 2 levels and 8 runs.

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 3-5 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. All the sets of fibres were evaluated for physical strength.

Evaluation of physical strength: To ascertain the effect of mycogenic pectinase enzyme treatment on the quality of extracted banana fibre the physical strength properties were studied for both enzymatic treatment and control (without enzyme) by using standard Universal Testing Machine (UTM), Instrone methods. A total of three sets of fibres were evaluated for each of the test parameters. Scanning Electron Microscopic (SEM) study were performed to study the bio-enzymatic treated, chemically treated and untreated banana fibresafter degumming at Indian Institute of Technology (IIT), Guwahati.

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 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.	Pct33	*Paecelomyces lilacinus*	1.0
13.	Pct34	*Penicillium glabrum*	2.0
14.	Pct37	*Trichoderma reesei*	0.01
15.	Pct38	*Penicillium pinophyllium*	1.9

Table 3 The optimization of pectinase production by submerged fermentation was carried out using Plackett-Burman Design 6 variables, 2 levels, 8 runs

	Glucose	Peptone	K_2HPO_4	$MgSO_4$	pH	$CuSO_4$
Unit	g-l	g-l	g-l	g-l	scale	g-l
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	-	-	-	-	-	-

RESULTS AND DISCUSSION

The potentiality of microorganisms as a source of industrially relevant enzymes stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms (Jayani *et al*., 2005). In this present study the pectinase producing fungal isolates were screened out by disc- plate method and submerged fermentation method. The study is in agreement with earlier workers for isolation of native strains of fungi for pectinase production (Reddy and Sreeramulu, 2012; Hitha and Girija, 2014).

A total of 17 fungal strains were isolated from banana pseudo stem and soil samples on the medium containing pectin as the sole carbon source (Table 1). These fungal strains were further tested for pectin hydrolysis by plate assay at pH5. Development of clear zone around the colony indicated pectin degradation. Depending upon the zone of clearance around the colony they were classified as good pectinase producers (0.5-1 cm); if the halos were ≤0.5 cm they were considered poor pectinase producers, while non-pectinolytic strains showed no zone of clearance.

Among the 17 fungal strains studied only 9 strains were found as positive for pectinase production (Table-2). Phoma herbarum and Aspergillius niger showed higher enzymatic production on pectin plate assay. The optimization of pectinase production by submerged fermentation was carried out using Plackett-Burman Design following optimization of pectinase production by submerged fermentation using 6 variables such as Glucose, Peptone, K 2 HPO 4 , MgSO 4 , pH and CuSO 4 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.

FACTORS								Pectinase activity (m eqNaOH)				
		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	
RUN	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		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	AT 95% CONFIDENCE LEVEL. ALL FACTORS WITH $F_{calculated}>F_{critical}$ ARE THE SIGNIFICANT	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						
MSError	2.060				**Findings : Peptone @10** g-l**; pH@4 and $MgSO_4$ @ 0.8 g-l for** *Phomaherbarum*		

Table 4 Tensile properties of Banana fibre after different method of extraction

Sl. No	Treatments	Load at maximum(g)	% Strain at maximum (g)	Modulus(Aut Young)(g/den)	Tenacity at break(g/den)	Toughness (g/den)
1.	*Aspergillus niger*	226.310	2.853	2704.910	64.660	0.891
2.	*Phoma herbarum*	482.626	3.574	4257.933	137.893	2.366
3.	Manual extraction	280.211	2.360	86.296	80.060	1.005
4.	Chemical extraction	224.939	3.799	1776.224	64.268	1.261

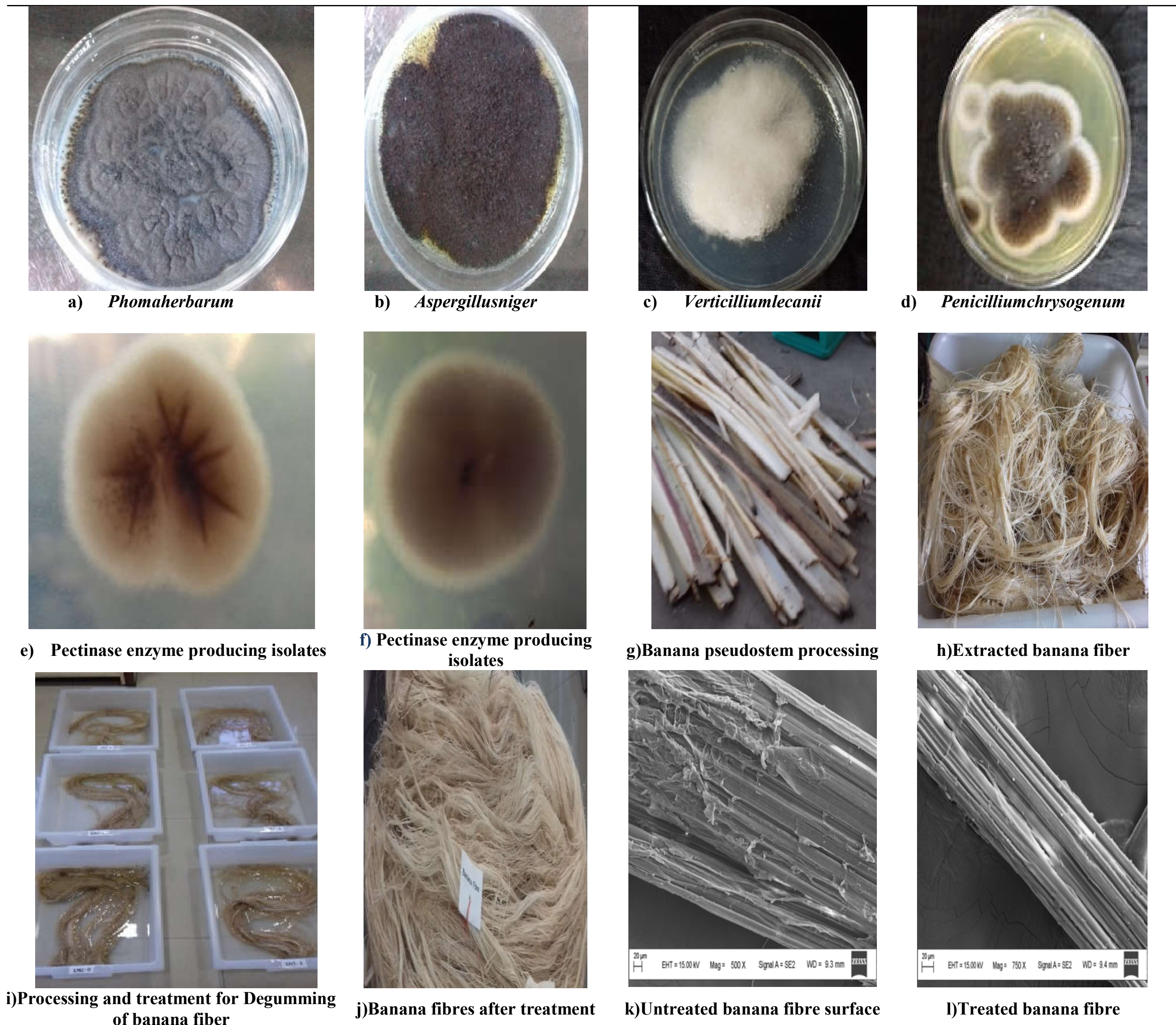

Fig a, b, c, d Different fungal isolates; e, f: Pectinase enzyme producing fungal isolates; g- Banana pseudostem processing; h- Extracted banana fibres; i- Processing and treatment for degumming of banana fibres; j: Banana fibres after treatment; k, l : SEM photographs of banana fibres

The higher enzyme activities, were recorded as 7.5 (U/ml) from Phoma herbarum followed by Aspergillius niger 7.0 (U/ml). Higher production of pectinase enzyme by Phoma species was also reported by earlier workers (Madhu *et al*., 2015). The study also highlighted 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).

The present findings with pectinase enzymes accomplished positive results for banana fibre extraction and degumming. In comparison to the control the biologically synthesized pectinase treatment showed improvement in physical properties of banana fibres.

Physical properties of banana fibre like Young's modulus and tensile strength decreased with increasing fibre diameter. The enzyme synthesised by Phoma herbarum (ENZ-5/2h-treatment) shows better results with higher Tenacity (137.893g/den) and Toughness (1.005g/den) which was followed by Aspergillus niger (ENZ-4/2h treatment) with Tenacity (64.660 g/den) and Toughness (2.366 g/den). Moderate response is observed for the manually extracted banana fibres with Tenacity (80.060g /den) and Toughness (1.261g/den) but with abundant pith. Whereas, NaOH treated fibres reduced the size of the fibres into the smaller bundles with Tenacity (64.268 g /den) and Toughness (1.261g/den) (Table- 4).

Scanning electron microscopic (SEM) study showed the surface of raw banana fibres differs in smoothness and roughness than the treated banana fibres Plate 8 (a,b,c). In case of enzyme treatment, the fibres become smooth and pithy material was removed. However, the chemical treated fibres can be seen as rough and flatten, although pithy material was removed.

The results indicate that smooth and clean banana fibre production based on bio processing by using fungal originated pectinase enzyme can be used in fibre extraction and

degumming process instead of utilizing harsh chemicals like surfactants, Sodium hydroxide, alkali chlorite, hypocholorite based bleaching etc. Thus, the mycogenic enzymes like pectinase able to enhance natural banana fibre yields with characteristic smooth texture without damaging the cellulosic structure of the fibres. Thus, enzymatic treatment has proven to be useful for banana fibre treatment, achieving an improvement in terms of cleanliness and fibrillation which can be spun to produce yarn or blended with other natural fibres.

Acknowledgement

The authors would like to acknowledge the Department of Biotechnology (DBT), Govt of India for the financial assistance (Ref: BCIL/NER-BPMC/2018/245, 26/03/2018). The authors also like to acknowledge Director General, TERI for inspiration and support.

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How to cite this article:

Indrani Sarma, A.C. Deka (2021) 'Process Optimization for Bio-Degumming and Surface Modification of Natural Banana Fibre ', *International Journal of Current Advanced Research,* 10(04), pp. 24122-24126.
DOI: http://dx.doi.org/10.24327/ijcar.2021.24126.4782

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