



FIRST REPORT OF BIO-POLISHING OF JUTE FIBERS USING CELLULASE PURIFIED FROM A NOVEL ISOLATE OF *TRICHODERMA LONGIBRACHIATUM* IN IRAQ

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Abstract:

The Bio-polishing technique of jute fibers was achieved for the first time in Iraq by using cellulase purified from a novel Iraqi isolate *Trichoderma longibrachiatum* which recorded first in Iraq in NCBI site as AB1. Enzymatic treatment of jute fibers was carried out through the released reducing sugars and percentage of loss in weight of jute fibers were determined, then morphological and structural characteristic were examined by using X-ray diffraction, FT-IR spectroscopy and scanning electron microscope.

Enzymatic treatment was achieved by incubating 1200mg of jute fibers with gradual concentrations of purified cellulase enzyme (33, 50 and 100%) for 1-6 hours at 40°C (the optimum temperature of enzyme activity and stability). Results showed that the maximum release of reducing sugars has been achieved after three hours of incubation with the enzyme, where the concentration of released reducing sugars was 1.50, 0.89 and 0.69 mg/ml after the treatment with the enzyme concentrations of 100, 50 and 33% respectively.

The results of enzymatic treatment also indicated a significant decrease in ($P < 0.05$) in the weights of jute fibers after enzymatic treatment for three hours at a temperature of 40 ° C in all enzymatic concentrations. As the percentage of weight loss reached 26.5% after treatment with the concentrated enzyme 100% due to the presence of a higher concentration of the enzyme relative to the fixed amount of jute fibers (1200 mg), compared to the loss rate of 19.18% and 15.78% after the treatment with cellulase enzyme at a concentration of 50% and 33%, respectively. Structural and morphological characteristics of enzymatically treated jute fibers were studied. Texture analysis by using scanning electron microscope and X-ray diffraction showed that bio-polishing caused the removal of the protruding hairs and micro fibrils, and loosened fibers to give a smoother product compared with the control sample.

These results were confirmed by studying the absorbed spectra of infra red which refers to the peak area of hydroxyl groups (-OH), methyl groups (-CH₂) and carbonyl groups (-C=O) were higher in the cellulosic structure of fibers compare with the peak area of the same groups in control samples.

Finally, The Bio-polishing technique has demonstrated, through these criteria used above, that jute fibers have been softened, biologically smoothed and it is considered a cheap, safe and environmentally friendly method instead of using undesired chemicals method that may cause harm to the environment and factory workers.

Keywords: Novel Iraqi isolate, *Trichoderma longibrachiatum*, Cellulase, Bio-polishing technique, jute fibers, First report, Iraq.



INTRODUCTION:

Jute is one of the oldest and most widely used cotton fabrics in the world. It belongs to the genus *Cortichorus* in the basswood family, Tiliaceae. Jute is being known as Golden Fiber due to its golden and silky shine. Jute fibers consist of lignin (12-14%), cellulose (58-63%), hemicellulose (21- 24%),nitrogenous matter (0.8-1.5%), traces of pigments, inorganic matter (0.6-1.2%) and fats and waxes (0.4-0.8 %), which made it the most widely used in various applications such as garment linings, household items, furniture upholstery, automotive carpeting, automobile sound absorption materials, carpet underlays, building materials for insulation and roofing felt, and low-end blankets (Liew *et al.*, 2017). Previously, smoothing of the jute fiber with chemicals materials was used in the textile industries in factories, where it causes damage to the environment. In addition, it causes skin sensitivity and respiratory infections for workers in the factories due to the dispersal of fine bristles and harsh prickly from the surface of the jute fabric (Kdraduman *et al.*, 2012). Therefore, it is necessary to reduce these shortcomings by using an environmentally friendly biological method instead of chemical methods that are harmful and polluting to humans and the environment, otherwise it is bio-polishing technique. Cellulase enzyme was used in the bio-polishing of jute fabric in particular by removing cellulosic impurities and hair protrusions from the surfaces of jute fabrics and giving the fibers a smooth texture and thus improving the appearance and softness of jute textiles. The aim of this study is to use the bio-polishing method of jute fabric treated enzymatically with cellulase by investigating the morphological and structural characteristics of jute fiber. This method is considered for the first time in Iraq by using purified cellulase enzyme from novel strain *Trichoderma longibrachiatum* and registered for the first time in Iraq is under the symbol AB1.

MATERIALS AND METHODS:

Fungal source and Jute fiber

Trichoderma longibrachiatum AB1 strain was used in the production of cellulase and published earlier (Hamdan and Jasim, 2021). Jute fibers were obtained from local market in Iraq.

Cellulase production and Purification

The cellulase enzyme production by Smf and SSF using *Trichoderma longibrachiatum* AB1 strain and purification this enzyme according to researchers respectively (Hamdan and Jasim, 2018A; Hamdan and Jasim, 2018B).

Bio-polishing application

The enzymatic treatment of jute fiber was carried out with cellulase enzyme purified from novel strain *T. Longibrachiatum* as assigned AB1 as follows:

1. Jute fibers were first physically cleaned by hand to remove seed fragments and debris impurities.
2. Cleaned jute fibers were then boiled for 30 min. in water bath.
3. After cooling, fibers were rinsed with deionized DW, then dried at 50°C.
4. A weight of 1200 mg of dried jute fibers was soaked in 5ml of purified cellulase enzyme at three concentrations (100%, 50%, and 33%) in test tubes, and incubated at 40°C for six hours.
5. After incubation, test tubes were incubated in boiling water bath for 5 minutes to inactivate enzyme activity. After cooling to room temperature, reducing sugars were determined in incubation mixture as described in (I).
6. Enzyme solution was decanted from test tubes, and the jute fibers in each tube were rinsed thoroughly with distilled water, then air dried even weight stability.

I. Reducing sugars liberation

The estimating of the reducing sugars which liberated from jute fibers (i.e all monosaccharides such as fructose, glucose and galactose), was carried out according to the method described by Otajevwo and Aluyi, (2011) and as follows

A. Preparation of glucose standard curve

For preparation of glucose standard curve, glucose stock solution was prepared first by dissolving 1g of glucose in 10 ml of D.W., then serial concentrations were prepared for determining reducing sugars. Absorbance was measured at 540 nm after the addition of 3,5-DNS reagent and boiling for 5 min for each glucose concentration as indicated in table (1). Standard curve representing the relationship between glucose concentrations and absorbance was plotted as shown in figure (1).

Table (1): Concentration and absorbance of glucose solution for determining the reducing sugars.

Tube No.	Volume of Glucose stock soln.(ml)	Volume of DW(ml)	Final vol.(ml)	Final glucose conc. (mg/ml)	ABS (540nm)
1	0	10	10	0	0
2	0.3	9.7	10	3	0.06
3	0.6	9.4	10	6	0.141
4	0.9	9.1	10	9	0.197
5	1.2	8.8	10	12	0.264
6	1.5	8.5	10	15	0.315
7	1.8	8.2	10	18	0.38
8	2.1	7.9	10	21	0.44
9	2.4	7.6	10	24	0.505
10	2.7	7.3	10	27	0.556
11	3.0	7.0	10	30	0.61

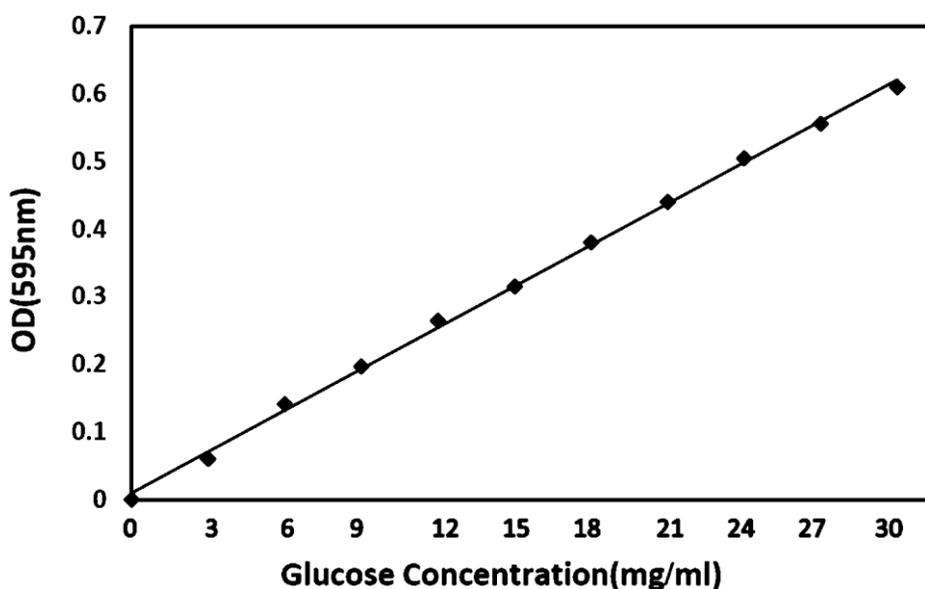


Figure (1): Glucose Standard curve for determining reducing sugars.

B- Estimation of reducing sugars

The concentration of reducing sugars released from the enzymatic treatment of jute fiber was estimated by 0.5 ml of the supernatant to 1.5 ml distilled water in a test tubes. Then 1.5ml dinitrosalicylic acid (DNS) reagent was added to each tube and then test tubes were heated in boiling water bath for 10 minutes. after heating, test tubes were left to cool at room temperature to stop the reaction, and color development, which is temperature sensitive.

After cooling, 21.5 ml D.W was added to each test tube, mixed well and the absorbance was measured at 540nm. The concentrations of free reducing sugars were estimated according to the same steps followed in paragraph (A) by using glucose standard curve (Fig. 1) (Fu *et al.*, 2012).

II. Weight loss %

At the end of the enzyme treatment, the jute fiber were washed, dried at 40 °C for 72 h, followed by



weight loss determination. The weight loss of the samples were calculated as follows:

$$\text{Weight loss (\%)} = [(W_0 - W_1) / W_0] \times 100$$

where W_0 was the original weight of jute before enzymatic treatment and W_1 was the residual weight of the jute after treatment (Fu *et al.*, 2012).

III. Investigation the morphological and structural properties of enzymatically treated jute fabric

1. FT-IR Spectral Analysis

Fourier transform infrared (FTIR) spectroscopy is a promising tool for the rapid, non invasive, and multiparameter analysis of samples. The method was used for identifying the constituents of lignocellulosic samples (Jute fiber) and to investigate the structural changes in untreated and treated enzyme of jute fiber. Samples were oven-dried at 105 °C for 4 h, mixed with KBr, and pressed under vacuum to form the pellets. Transmittance was measured over a range from 4000 to 500 cm^{-1} with the spectral resolution of 4 cm^{-1} and 64 scans per sample (Bagewadi *et al.*, 2016).

2. X-Ray Diffraction (XRD) analysis

The properties of the jute fibers and non-enzymatically treated jute fibers, were studied. The powder fibers were examined between angles (2θ) 5° to 70° to obtain the equatorial reflections using X-ray diffractometer (XRD) Bruker AXS D8 with CuK α radiation (λ - 1.5418Å). The generator was utilized at 40KV, 30mA at a scanning level of 1.2° / 1min.

3. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to study the morphological and physical changes of the fiber structure of jute fiber which untreated and treated enzyme. The lyophilized samples were mounted on aluminum stubs, and sputter-coated with a gold layer and the SEM images were taken at different magnifications (Bagewadi *et al.*, 2016).

RESULTS AND DISCUSSION:

Application of cellulase in bio- polishing application

1. Liberation of reducing sugar

Cellulase purified from the novel strain *T.longibrachiatum* was used in Bio- polishing application.

The results showed that the time of treatment (1-6) hr, ratio of jute fiber to cellulase conc.(w/v) (1200) mg /100%, 50%, 33% and incubation temperature 40°C significantly influenced on the reducing sugar and found that after 2hr enzyme reaction, The optimum reducing sugar was optimum at 3hr in treatments of reducing sugar at different concentrations of cellulase.

In table(2) indicated that the maximum of reducing sugars liberated from the jute fibers was after three hours of incubation with the enzyme (100%) which reported of 1.50 mg/ml compared to 0.89 and 0.69 mg/ml after treatment with enzyme concentrations 50% and 33%, respectively. This indicates the occurrence of breakdown of the cellulose chains in the structure of the fibers.

Hrs	Reducing sugars (mg.ml ⁻¹) at different concentrations of cellulase from 1200mg of jute fiber at 40°C			
	Cellulase Conc. 100%	Cellulase Conc. 50%	Cellulase Conc. 33%	Av. Cell.conc.
1	0.90	0.50	0.33	0.576
2	1.10	0.69	0.51	0.766
3	1.50	0.89	0.69	1.026
4	1.25	0.76	0.56	0.856
5	0.76	0.45	0.31	0.506
6	0.55	0.35	0.20	0.366
Av. Hr.	1.01	0.606	0.433	

LSD 0.05, C= 0.00696 , H= 0.00984 C*H=0.01705



Table(2): Effect of time (hr) on the yield of reducing sugars and jute fibers to cellulase conc.(1200mg/100,50,33%) on the biopolishing of jute fiber by cellulase purified from the novel strain of *Trichoderma longibrachiatum* AB1 at 40°C.

2. Weight loss (%) of jute fiber

Weight of jute fibers was measured after treatment with different concentrations of purified cellulase for 6 hours at 40°C. The results showed that the enzymatic treatment has a significant effect on the weight loss by hydrolyzing cellulosic chains. It was observed that the change in the concentration of the cellulase enzyme is significant in the weight loss rate with the highest percentage of 26.5% with 100% cellulase concentration compared to 19.18 and 15.78% with concentration of cellulase which reported of 50% and 33% respectively, due to the complete removal of the microfibrils, water extractable material and other impurities from the jute fiber (i.e elimination of lignin and hemicellulose). The weight loss of jute fibers after enzymatic treatment changed the quality of the texture (smooth texture) which applied in the various textile, household and industrial industries.

3. FT-IR

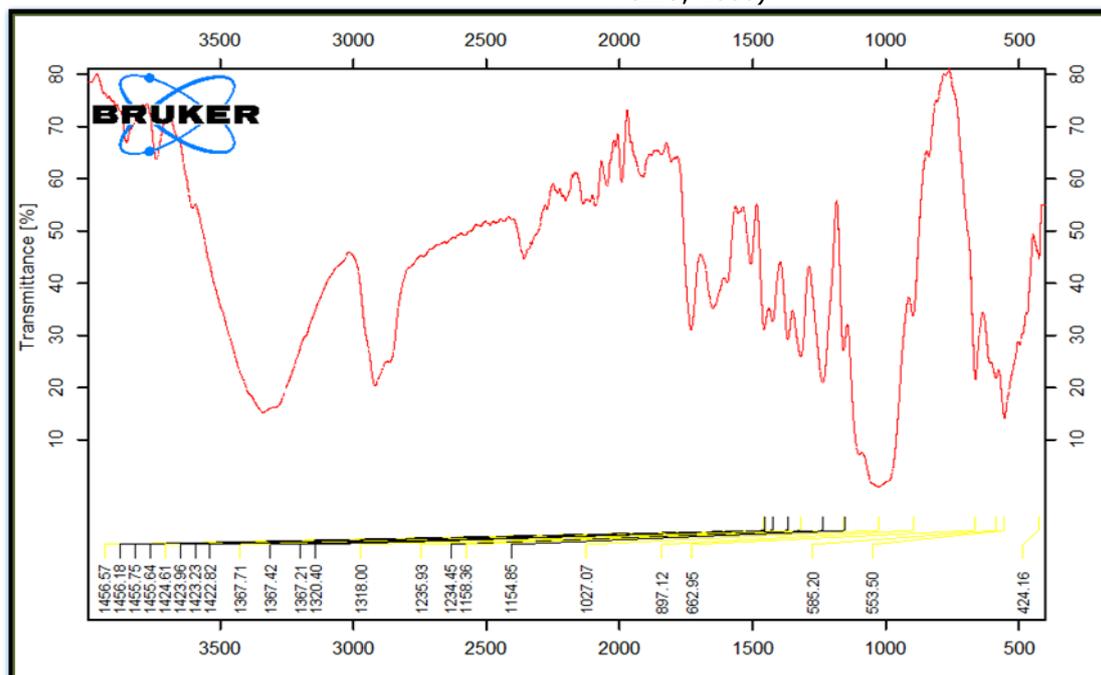
The treatment temperature 40°C was shown in figure (2) and (3), The peak 1730cm⁻¹ in untreated fiber was attributed to C=O stretching of the carbonyl and acetyl groups in the 4-O-methyl-glucanoacetyl

xylan component of hemicellulose in the jute fiber whereas in treated fiber this peak disappeared which confirmed that cellulase enzyme and functional group reaction of this peak were effective (Amantes *et al.*, 2017; Moran *et al.*, 2008).

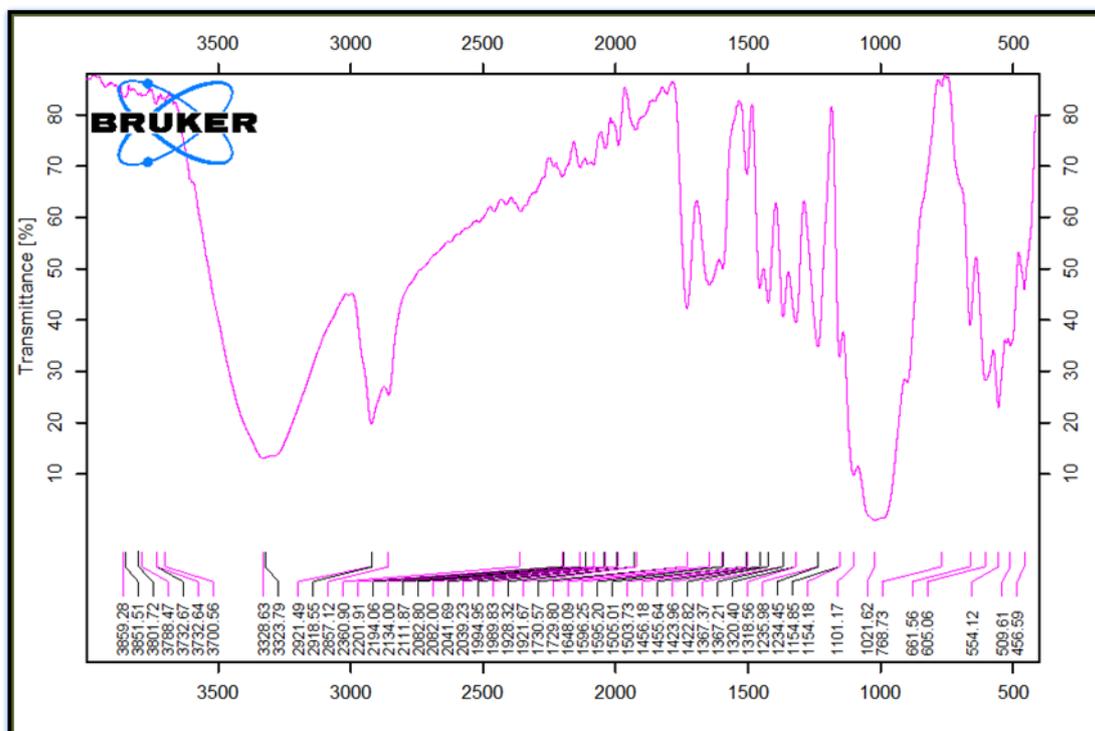
The aromatic ring of lignin stretch was observed in 1600-1500cm⁻¹ range with aromatic C=C bending in untreated fiber while in treated fiber showed lower band intensity at this wave length which signifies delignification effect (Viera *et al.*, 2007). The peaks between 1250-1200 cm⁻¹ range attributed to the C-O stretching of aryl ethers, and phenolic of lignin-derived compounds and C-O stretching of pyranone rings and guaiacyl monomers in untreated fiber whereas in treated fiber was diminished of this range of peaks which represented that lignin was decreased (Sukmawan *et al.*, 2016; Rosa *et al.*, 2012).

In peak of 1101.17cm⁻¹ represented the hemicelluloses in untreated fiber (Mwaikambo and Ansell, 2002) while disappeared in treated fiber which indicated successful interaction of cellulase with jute fiber that may result in removal of impurities from fiber surface.

A peak founded around 897.12cm⁻¹ was associated with C-O stretching and β-glucosidic linkages of the glucose ring of cellulose which increased to high wave number after treatment of cellulase in treated fiber because removal of Syringyl-Guaiacyl-Hydroxyphenyl (SGH) units of lignin (Buranov and Mazza, 2008).



Figure(2): FT-IR spectral of untreated jute fiber 40°C

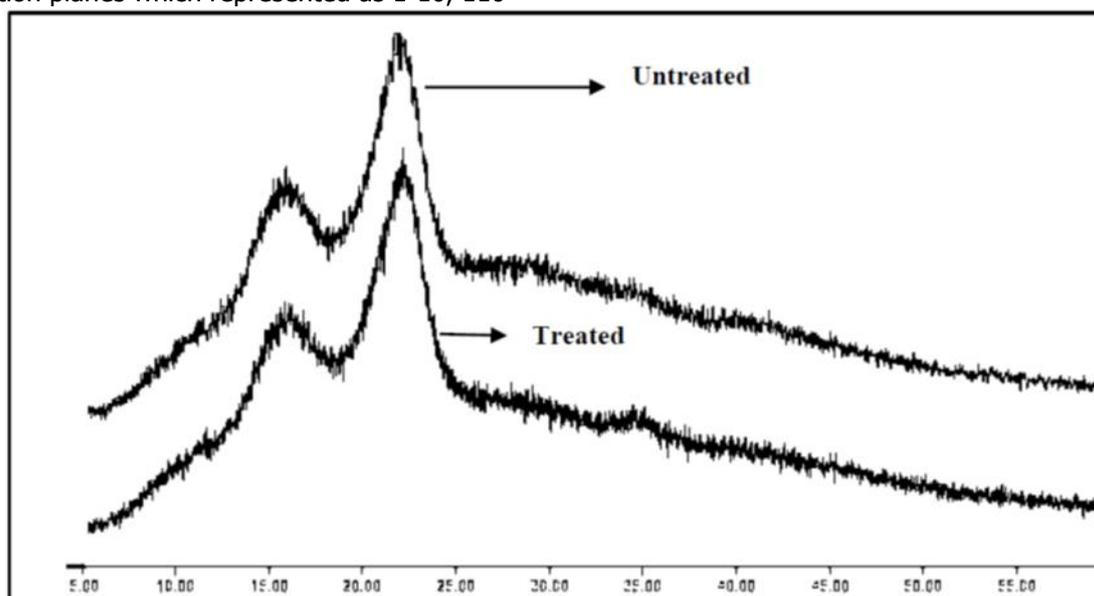


Figure(3): FT-IR spectral of treated jute fiber 40°C.

4. XRD diffraction patterns

In figure(4) which investigated the structural variations using the X-ray diffraction patterns. The results indicated an increase in the intensity of the crystallization planes which represented as 1 10, 110

and 200 reflections in the enzymatically treated fibers, due to removing of the amorphous contents lignin and hemicellulose which changed the nature of the fibers to a more crystalline (more softer) .



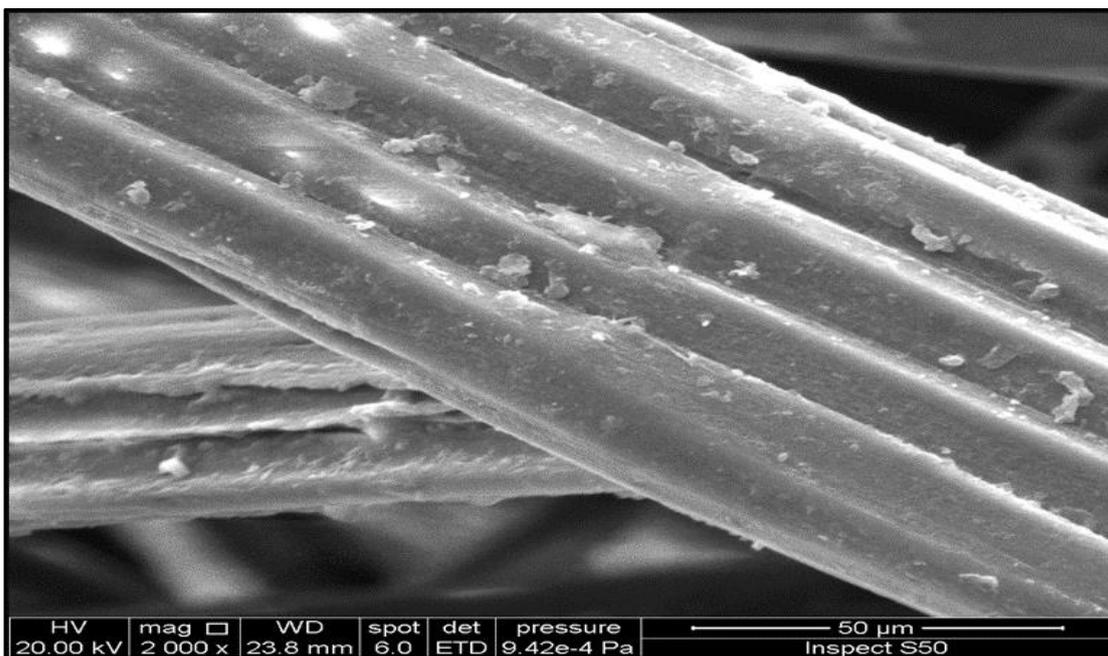
Figure(4): XRD pattern of untreated jute and cellulase treated jute fibers.

5-Fiber topography/SEM images

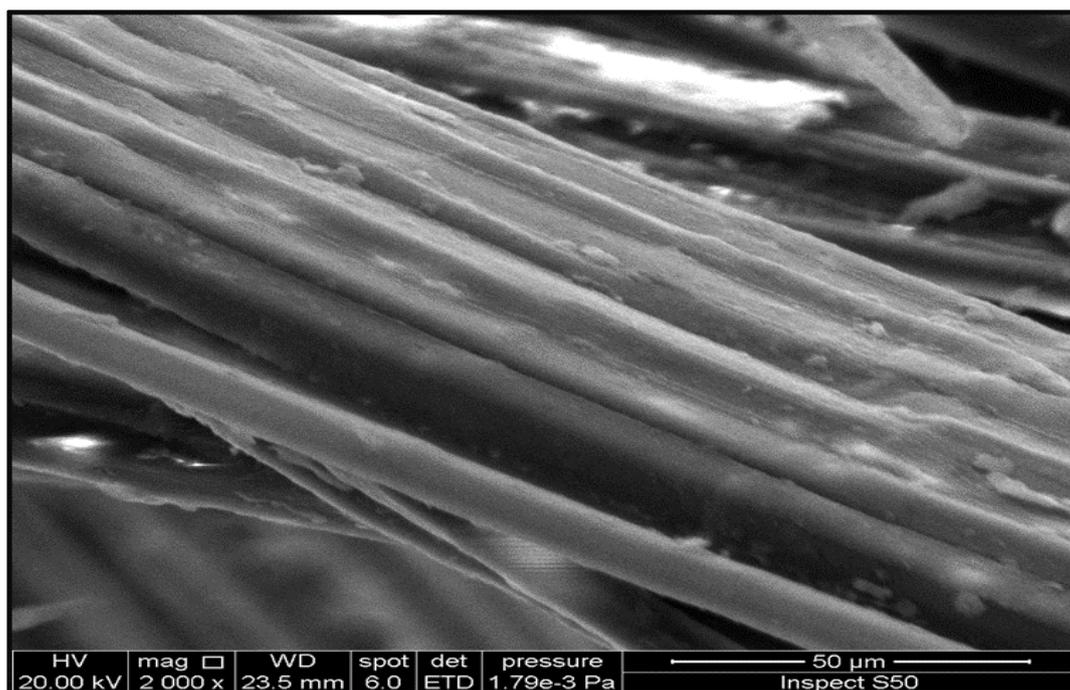
Samples of the jute fibers were analyzed under the scanning electron microscope (SEM) to check the changes in fiber surface appearance due to the cellulase treatment.

The change in the morphology of the fiber during the treatment showed the development of smoothened surface and more softer due to the

accumulation of the enzyme in the fiber matrix and subsequent removal of the unwanted micro fibrils and adhesive substances like pectin and hemicelluloses which become more regular of the surface and form a more continuous cover of the fiber surface. Therefore, cellulase enzyme was effective in biopolishing application to improve the quality of jute blended fabrics.



Figure(5): SEM images of untreated jute fiber at 40°C.



Figure(6): SEM images of treated jute fiber at 40°C.



CONCLUSIONS:

These are pioneer findings of the results are promising towards developing advance bio-polishing technique. Through these criteria used above, This technique reported for the first time and proved that the jute fibers were smoothed using cellulase enzyme from novel strain *Trichoderma longibrachiatum* AB1. Additionally, it suggest that the aforementioned technology as alternative method for fabric bio-smoothing rather than the undesired chemical method.

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