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Biofinishing of Jute fibers using *Aspergillus nidulans* SU04 cellulase

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Abstract. The enzyme cellulase of higher activity (49.82 U/ml) from *Aspergillus nidulans* SU04 was applied for the biofinishing of jute fibers in this study. Application of cellulase in fabric biofinishing is investigated by treating the jute fibers with partially purified cellulase and studying the enhancement of fiber brightness, smoothness and weight loss. The biofinishing conditions including treatment time, fiber to enzyme ratio (concentration), agitation rate and temperature are optimized for a smooth fiber surface. The results of the scanning electron microscope (SEM) analysis, X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) favored maximum surface finishing at a jute fiber concentration of 16% (w/v) at 45^{0}C and pH 5.0 at 17th h of the treatment.

Keywords. Cellulase, *Aspergillus nidulans,* Jute fiber, Biofinishing, Scanning electron microscope, X-ray diffraction, Fourier transform infrared spectroscopy.

1. Introduction

In the textile industry, there is a need for novel cellulases that are active at neutral and alkaline pH values, have short reacting time, do not compromise the strength of fabric, and have good finishing properties. The ability of certain fungal species to decompose the cellulosic biomass into glucose, which in turn can be converted into valuable chemicals, has made cellulases as one of the most important commodity [1]. Cellulases are inducible enzymes which are synthesized by microorganisms during their growth on cellulosic materials [2]. Cellulase production in fungi is found to be extra cellular and has three components such as endoglucanase(endo-1, 4- β-D-glucanase, EC 3.2.1.4), exoglucanase (exo-1, 4-β-D-glucanase, EC 3.2.1.91)and β- glucosidases(1, 4- β-D-glucosidase, EC 3.2.1.21). The main application of these enzymes is in textile, paper and pulp, food, animal feed, fuel and chemical industry. These industries entail highly stable enzymes, competent to outshine at extreme conditions of pH and temperatures. Cellulase treatment of fibers is an environmental friendly way of improving the property of fabrics, their desirable appearance and soft handle [3].

Success of jute biofinishing is influenced by pH, temperature, substrate concentration, enzyme concentration time and mechanical action [4]. Endoglucanases are key enzymes in biofinishing. Studies conducted to evaluate the best component of cellulase for high performance in biofinishing was favorable towards endoglucanase [5]. Commercial cellulases for biofinishing originate from *Trichoderma reesei* and *Humicola insolens* [6]. *Trichoderma reesei* cellulases action on cotton fibers and yarn decreased hairiness and increased yarn evenness [7]. The crude cellulase extract obtained from *Aspergillus nidulans* strain was partially characterized and studies were made aiming at its possible application in jute fiber treatment. The assessment of jute fiber was performed for its effectiveness with respect to fiber brightness, smoothness and weight loss.

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2. Materials, Methods, Results and Discussion

2.1. Enzyme and Jute fiber

Aspergillus nidulans SU04 was used in the production of cellulase [8]. The clarified crude enzyme preparation was used in enzyme assays and jute fiber treatment. Jute fibers were obtained from Sorbead mills, Gujarat.

2.2. Analytical techniques and cellulase treatment

Assay for Cellulases and reducing sugar were carried out according to IUPAC recommendation [9,10,11]. Glucose in the culture supernatant was analyzed by using UV Visible spectrophotometer (Hitachi Model: 100-40 Spectrophotometer) at 540 nm. The jute fiber was soaked thoroughly in 100 ml enzyme preparation (49.82 U/ml) and incubated at 50°C for 24h. Control samples consisted of fibers treated in an identical manner but without enzymes. Treated fibers were tested for their brightness, smoothness and weight loss. X-ray diffraction analysis was carried out to examine the structural properties of jute samples (Miniflex Model, Rigaku, Japan). Surface morphology was analyzed using Scanning electron microscope (Philips XL30 SEM). FTIR spectrum was recorded in the region of 400-4000 cm^{-1} (Nicolet 20DXB spectrometer). Brightness index was measured on (Photovolt reflection meter: model 670), provided with tristimulus filters of known reflectance value (93%) with reference to magnesium oxide as 100% brightness value. Microprocesser controlled smoothness tester (Automatic bekk: model 58-05-00) is used for the determination of smoothness of the fiber matrix.

2.3. Biofinishing experiments on jute fiber

The fibers were cleaned to remove impurities and boiled in water at 100°C for 30 min.

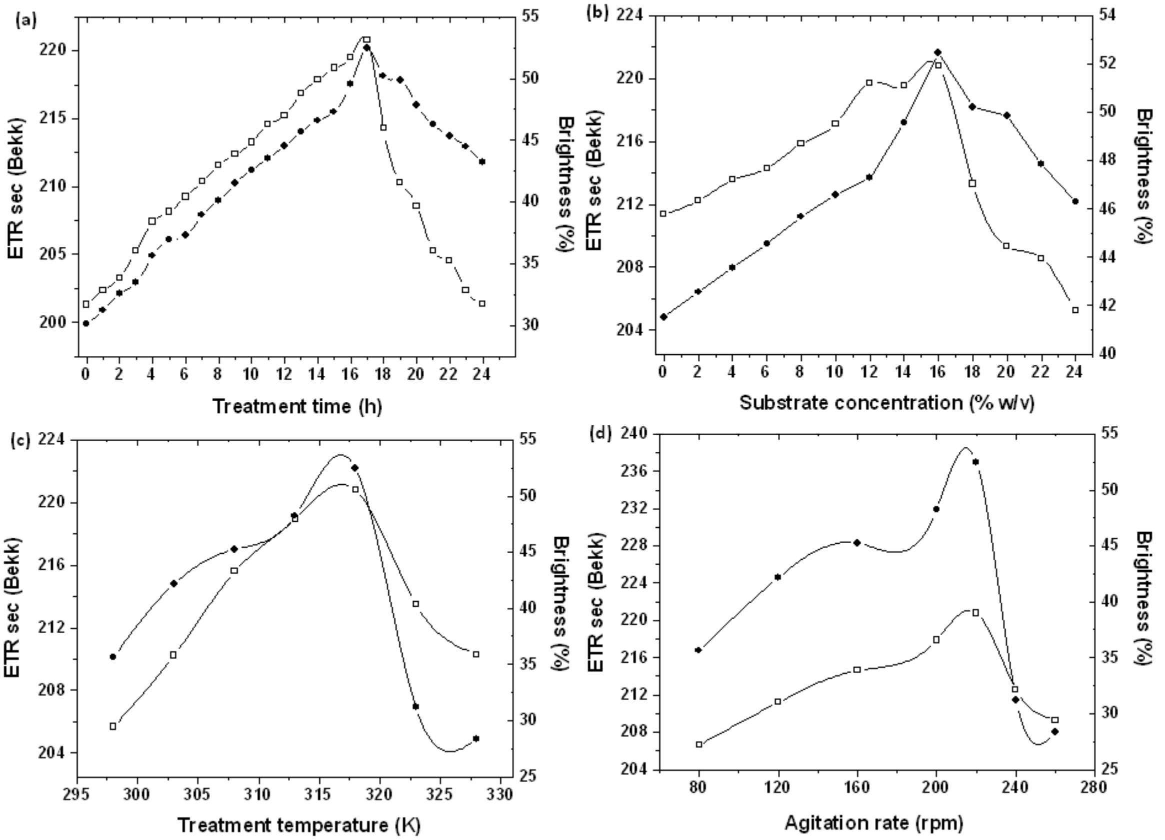

Fig. 1: Effect of **(a)** time, (**b**) substrate concentration, (**c**) temperature and (d) agitation rate on smoothness (□) and brightness % (●) of Jute fibers.

After removal from the boiling water, the fibers were rinsed with deionized water and air-dried. The treatment of the jute fibers with crude CMCase resulted in an increase in the yield of reducing sugars from 2nd h till the 17th h after which the release of reducing sugars decreased. The results of the effect of enzymatic hydrolysis on jute fiber were studied at substrate concentration from 2% to 24% (w/v). Minimum loss in weight, maximum brightness and maximum surface smoothness were achieved at substrate concentration of 16% jute fiber. The cellulase treatment of jute fibre was carried out between 25°C (298 K) and 55°C (328 K). The temperature of 45°C was found to be optimum for the treatment of jute fibers with the cellulase. The agitation rate of 200 rpm was found to be optimum for the treatment of jute fibers with the cellulase obtained from *Aspergillus nidulans*. The ETR values observed after the smoothness test was 230.2 sec (Bekk) at 50.66 kPa. At 48.00 kPa, the ETR value for 16% jute fiber treated for 17 h at 45°C was observed to be 220.78 sec (Bekk) confirming the smoothness of the enzyme treated fiber matrix. In the test for brightness and whiteness, the voltmeter of Photovolt reflection meter was set according to manual and 30 readings were taken randomly. The averages were considered as appropriate brightness. The brightness property of jute fiber was enhanced to some extent by the enzymatic treatment. The maximum brightness of jute fiber was recorded as 52.46 % for 16% (w/v) jute fiber treated for 17 h at 45°C. Result indicates bleaching effect of crude enzyme preparation from *Aspergillus nidulans* on jute fibers. The ETR values and brightness values (%) upon various treatment times (2 - 24 h), substrate concentrations (2-24% w/v), temperatures 25°C (298 K) - 55°C (328 K) and agitation rate (80-260 rpm) were shown in Fig. 1a-d. The change in the morphology of the fiber mat during the treatment shows the development of smoothened surface possibly due to the accumulation of the enzyme in the fiber matrix and subsequent removal of the unwanted micro fibrils. After 17th h of operation, a linear reduction in weight change was seen possibly due to the roughening of the fiber surface by the formation of cavities and stripping off of the fiber wall. SEM images provide a qualitative confirmation of these results. At the 17th h of the treatment (Fig. 2a-c), more peculiar surface features appear. Surfaces become more regular and form a continuous Cover. Large regions can be seen where miniature fibrils and platelet-like structures protrude from the surface. Endoglucanase acts on the amorphous region of the cellulose, forming the protruding hairs and loosens it. The mechanical action of stirring during the treatment in turn removes the loosened fibers to give a final finished product. The weight loss less than 0.5% during the treatment may be only due to the removal of surface fibrils, water extractable material, and other impurities from the jute fiber.

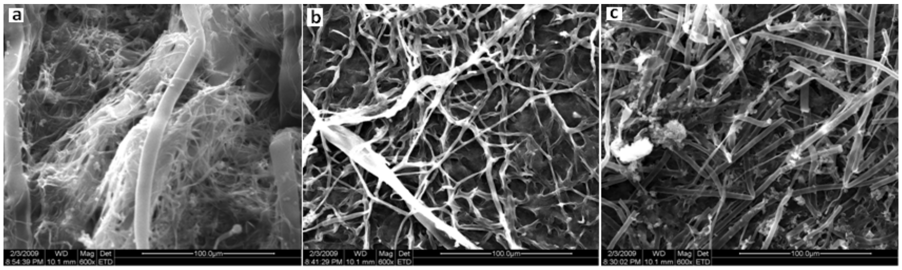

Fig. 2: SEM micrographs of (a) untreated jute fibers (100µm) (b) jute fibers treated for 15 h, 16% (w/v), 45°C, pH 5.0, 49.82 U/ml cellulase (100µm) (c) jute fibers treated for 17 h,16% (w/v),45°C, pH 5.0, 49.82 U/ml cellulase (100µm).

Loss in weight of the fiber is directly related to the degradation of crystalline cellulose and amorphous cellulose [12]. Since, amorphous cellulose is less structured and easily accessible, loss in weight may be attributed to the degradative action of endoglucanase on amorphous cellulose causing the removal of the extra microfibrils [13, 14].

2.4. X-ray diffraction pattern

Fig.3a shows an XRD pattern of jute samples with 2θ verses intensity having several peaks of cellulose indicating random orientation for the crystalline nature. Measured interplanar distances agreed with the values reported for cellulose in the literature [15]. The XRD of α – cellulose, untreated jute (UTJ), pretreated jute (PTJ) and cellulase treated jute (CTJ) prove decline in the crystallinity of samples in the order of α - cellulose standard, UTJ, PTJ and CTJ.

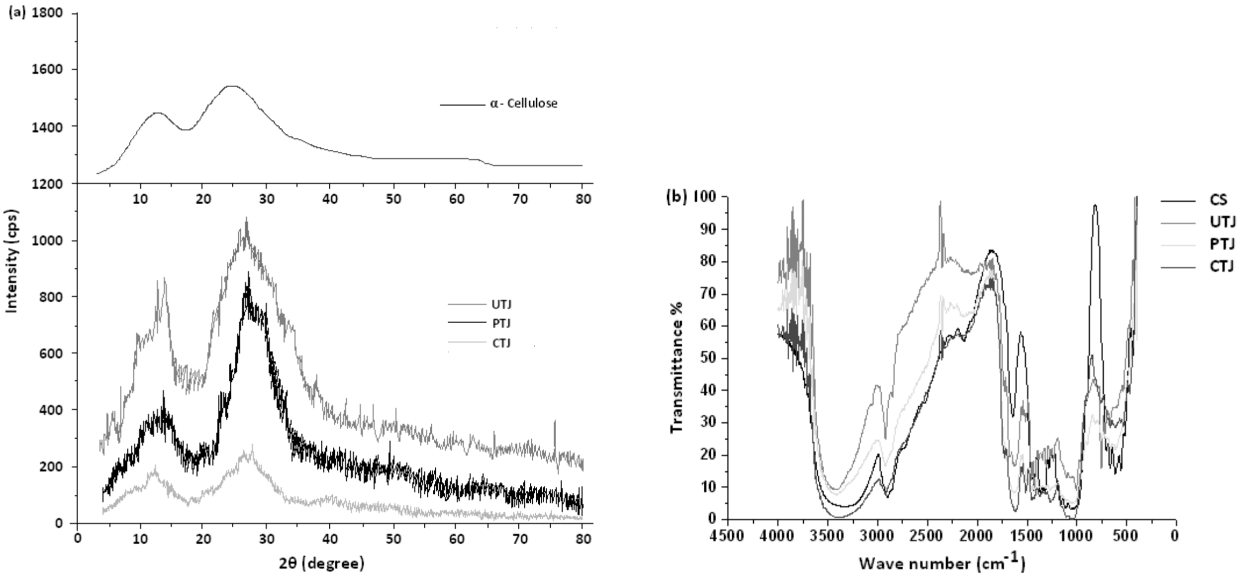

Fig. 3: (a) XRD pattern and (b) FTIR spectra of α-cellulose, untreated jute (UTJ), pretreated jute (PTJ) and cellulase treated jute (CTJ) fibers.

2.5. FTIR spectra

The Fourier transform infrared spectra of jute shows peaks corresponding to cellulose [16,17] and lignin[18]. The lignocellulosic composition of the jute could be identified from the presence of peak between 1600 cm^{-1} and 1000 cm^{-1}. The FTIR spectra of the jute samples were shown in Fig. 3b. Band intensities of lignin peaks were predominant in the untreated jute 1238 cm^{-1}. The band intensities of all the cellulose peaks of cellulase treated samples were lower than that of alkali samples, proving the hydrolysis effect of cellulase.

3. Conclusion

The present study demonstrates the magnitude of cellulase produced by *Aspergillus nidulans* and its potential for textile finishing processes. Cellulase treatment on 16% (w/v) jute fiber for 16 h at 45^{0}C showed good biofinishing effect. *Aspergillus nidulans* cellulase, rich in endoglucanase appears to be exceptional for biofinishing operations.

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