Biopolishing of cotton fabric with fungal cellulase and its effect on the morphology of cotton fibres

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Attempt has been made to analyse structural changes in cotton fibres occurred during biopolishing using cellulases obtained from *Trichoderma reesei*. Cellulase hydrolysis results in weight loss of the samples, which, in turn, results in the splitting of fibres and removal of surface irregularities of the fibres as revealed by SEM images. Degree of crystallinity is not influenced by the biopolishing process due to random hydrolysis of the cellulase enzymes on cotton fibres. Lateral order of the crystallites, measured between (101) and (10$\bar{1}$) peaks of the x-ray diffraction reduces from 0.662 to 0.667 on account of the hydrolysis though the crystallite thickness measured perpendicular to (002) plane remains unchanged. FTIR results reveal the increased -OH bending, CH_2 in-plane bending, and C-H vibrations of the cellulose chains in the biopolished cotton samples using cellulase.

Keywords: Biopolishing, Cellulase, Cotton, Crystallinity, Crystallite size, Lateral order, Reducing sugar

1 Introduction

Biopolishing of cotton fabrics, using cellulases, is aimed to remove cellulosic impurities, individual and loose fibre ends that protrude from fabric surfaces to provide an enhanced appearance and handle of the fabrics. Spiral fissures, helical cleavages and transverse fissures are often observed in the cotton fibres after prolonged hydrolysis by cellulases[1]. Weight loss values ranging from 1.7% to 19.7% with proportionate loss in the breaking strength have been reported in the biopolished samples[1-4]. Moisture regain of the biopolished samples increases marginally from 6.00% to 6.10% under high agitation levels during the enzyme hydrolysis, mainly due to fibrillation in the fibres[4-6]. Affinity of cellulases to the cotton depends on degree of crystallinity, conditions of the substrates and components of cellulase present in the reaction system. Though random hydrolysis of cellulose in the cotton fibres does not affect the degree of crystallinity, different results have been reported in the past[6-10]. The present study has been carried out to analyse structural changes of the cotton fibres occured during biopolishing of cotton fibres, using cellulases obtained from *Trichoderma reesei,* a fungal source for cellulose production.

2 Materials and Methods

2.1 Cellulase Production using Submerged Fermentation

Sub-cultures of *Trichoderma reesei* MTCC 162, obtained from the Institute of Microbial Technology, Chandigarh, was used for the production of cellulase enzyme. Culture media of submerged fermentation[11] for cellulase production was prepared using peptone (0.25 g/L), yeast extract (0.10 g/L), potassium dihydrogen phosphate (2.00 g/L), ammonium sulphate (1.40 g/L), urea (0.3 g/L), magnesium sulphate (0.30 g/L), calcium chloride (0.30 g/L), with carboxy methyl cellulose and powdered bleached cotton linters as the carbon source. Trace elements comprising (per 100 mL) ferrous sulphate (50 mg), manganese sulphate (15.6 mg), zinc sulphate (33.4 mg) and cobalt chloride (14 mg) were added at 10 mL per 100 mL of the ferment. The medium was autoclaved and after cooling to the room temperature, it was inoculated with the spore suspensions of *Trichoderma reesei*. The flask was placed on an orbital shaker (150 rpm) for the fungal growth and cellulase production for 10 days. The crude cellulase was filtered and the filtrate was centrifuged at 7000 rpm for 10 min at 4°C. The protein content[12] and enzyme activity were measured as per the standard methods.

Fungal growth in the media was measured at different stages[13, 14] by weighing the mat weight.

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2.2 Measurement of Cellulase Activity

Half millilitre of cellulase enzyme, diluted in citrate buffer of *p*H 5.5 and 0.5 mL of 1% carboxy methyl cellulose were mixed and incubated at 45°, 50°, 55° and 60°C for 15 min. To that mixture, 0.5 mL of 3, 5-dinitrosalicylic acid (DNSA) was added, boiled for 15 min before adding 1 mL of sodium potassium tartarate and the contents were cooled. Colour change at the wavelength (λ_{max}) of 540 nm was measured, subtracted from that of enzyme blank and translated into glucose values using the standard curve[15].

2.3 Determination of Enzyme Activity

Effect of temperature on enzyme activity and temperature profile were determined by measuring reducing sugar from the samples incubated at 30-70°C for 30 min. Similarly, the effect of *p*H on enzyme activity was also measured by incubating the cellulase enzyme with cellulose for 30 min using acetate buffer of *p*H 3.0 - 5.9 and phosphate buffer of *p*H 6.0 - 7.0.

2.4 Biopolishing of Cotton Samples

The crude cellulase enzyme was used to study the effect on biopolishing of cotton fabric samples. The fabric samples (20 cm × 20 cm) were introduced in the flask containing cellulase in the appropriate buffer system and then incubated in a shaker (120 strokes/min). Three levels (1, 2 & 3) of cellulase concentrations (6, 8, 10 mL/L), treatment duration (40, 50, 60 min), *p*H (4.5, 5.0, 5.5) and temperature (45, 50, 55°C) conditions were used as the design parameters with L9 orthogonal array, suggested by Taguchi method of experimental design, for optimization of design parameters[16] in biopolishing treatment. Mechanical agitations given by the shaker bath was enhanced by addition of 10 glass balls (each weighing 1 g). Weight loss values after biopolishing treatment were determined in terms of signal-to-noise ratio, larger-the-better S/N (θ) = 10 $\log_{10}$ [(1/yi^2)/n], where θ is the independent variable; *y*, the observation; and *n*, the number of observations.

2.5 Weight Loss

Weight loss of the samples after the cellulase treatment was calculated as the ratio of difference in weights before and after the treatment, to the original weight. Before weighing, every sample was allowed to reach equilibrium under the standard conditions with a relative humidity of 65% ± 2% at 25 ±2° C.

2.6 Surface Morphology

Surface morphology of fibres was assessed using scanning electron microscope (SEM, Jeol 6390) with a small piece of cellulase treated sample and untreated fabric sample. The micrographs were taken at ×1000 magnification level to assess the surface modifications due to enzyme hydrolysis.

2.7 Degree of Crystallinity, Crystallite Size and Lateral Order

Yarns drawn from the fabrics were cut into small pieces for the purpose of measuring the degree of crystallinity using wide angle x-ray diffraction. The powder samples were scanned between the angles (2θ) 10° and 45° to obtain the equatorial reflections, using Bruker AXS D8 with Cu Kα ($\lambda - 1.5418$ Å) radiation. Crystallinity index (CrI) of the samples was calculated using the following equation:

$$\text{Crystalline index (CrI)} = I_{002} - I_{Am}/I_{002} \qquad ... (1)$$

where I_{002} & I_{Am} indicate the x-ray diffraction intensities of (002) reflections and the amorphous region of the sample respectively[17,18]. Crystallite size (τ) of the fibres sample was calculated using the following Scherrer equation:

$$\tau = K\lambda/\beta \cos\theta \qquad ... (2)$$

where λ is the wavelength of the x-ray used; K, the shape factor; and β, the half-width maximum of the equatorial reflections[19]. Lateral order, also known as crystalline order or micelle perfection, of the samples was calculated using the following equation:

$$\text{Lateral order} = I_{101} - I_{min}/I_{101} \qquad ... (3)$$

where I_{101} and I_{min} are the diffraction intensities of (101) reflections and minimum intensity in the region between (101) and $(10\bar{1})$ reflections[18, 20] respectively.

2.8 FTIR Spectrum

To ascertain the functional groups present in the cotton samples, FTIR spectra were obtained using Shimadzu FTIR 8400S Spectrophotometer. Twenty scans were carried out for every specimen to reduce the influence of the noises and the spectra were obtained in the wave number range 4000 - 400 cm^{-1}.

3 Results and Discussion

3.1 Fungal Growth and Enzyme Production

Growth of *Trichoderma reesei* in the incubation bath followed the typical growth curve of the fungi with the initial slow growth, followed by logarithmic growth phase and finally decline phase. Akin to the

fungal growth, the protein content in the medium also increases steadily during the incubation period (Fig. 1), marginally lagging behind the growth curve, till 7th day post-inoculation and then decreasing steeply to the 60% level of the maximum, perhaps due to decrease in the nutrients present in the culture medium and decline in the fungal growth.

Trend of the cellulase activity of enzyme and the protein content in the incubation broth is appeared similar to each other throughout the incubation period. There is a marked decrease observed in the cellulase activity compared to protein content (Fig. 1b) from day 6 of post inoculation, though there is no such difference at the beginning of the incubation.

Activity measurement of cellulase enzyme, using DNSA method, at various temperatures and pH levels, shows a typical 'bell' shape curve [Figs 2 (a) and (b)]. Maximum activity of cellulase at pH 5.0 is found to be 786 U/mL/min, which is reduced to ~ 50% at pH 3 (367 U/mL/min) and pH 8 (382 U/mL/min). However, temperature change of ± 5°C does not result
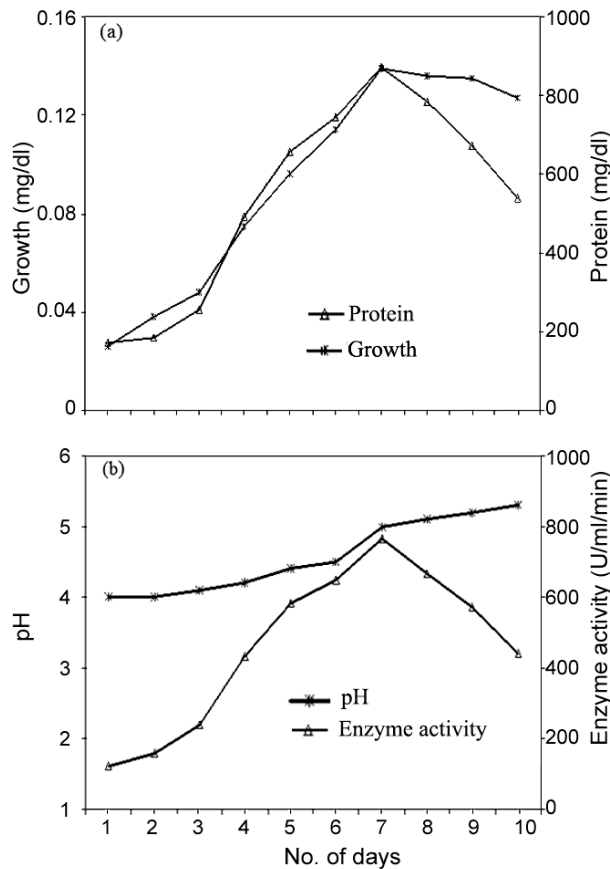
in significant changes in cellulase activity, with the retention of ~ 95% activity levels, though there is ~ 55% and ~ 75% reduction observed in the cellulase activity at 70° C and 35° C respectively. Hydrolytic activity of cellulase on cellulose measured by DNSA method shows the maximum release of reducing sugar (643 μg/mL) of cellulase at 50°C, comparable to the values reported earlier[9,21].

3.2 Weight Loss

All the design factors used in the Taguchi experimental set-up appear to have a strong influence on weight loss values of fabrics in cellulase hydrolysis. The highest weight loss value, in the given set of experiments, is found to be 7.1 % and the least weight loss value is 3.3%. Highest signal-to-noise ratio (Table 1) is observed for the samples treated with the highest level of concentration (10 mL/L or 7860 Units of cellulases) and temperature (55° C). This is in confirmity with the earlier results[4, 22].

3.3 Surface Morphology of Biopolished Samples

Scanning electron microscopic observations reveal the fuzz-free fabric surface, in the case of cellulase biopolished samples compared to the untreated
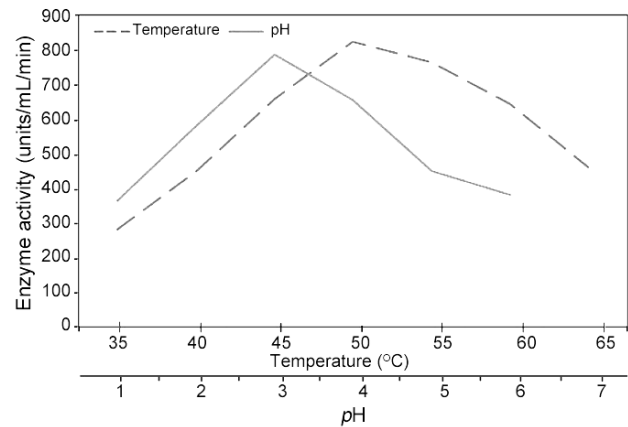
Fig. 1—(a) Growth of *T. reesei* and (b) cellulase activity in culture medium

Fig. 2—Thermal and pH activity of cellulase

Table 1—Effect of design parameters on signal-to-noise ratio of weight loss values

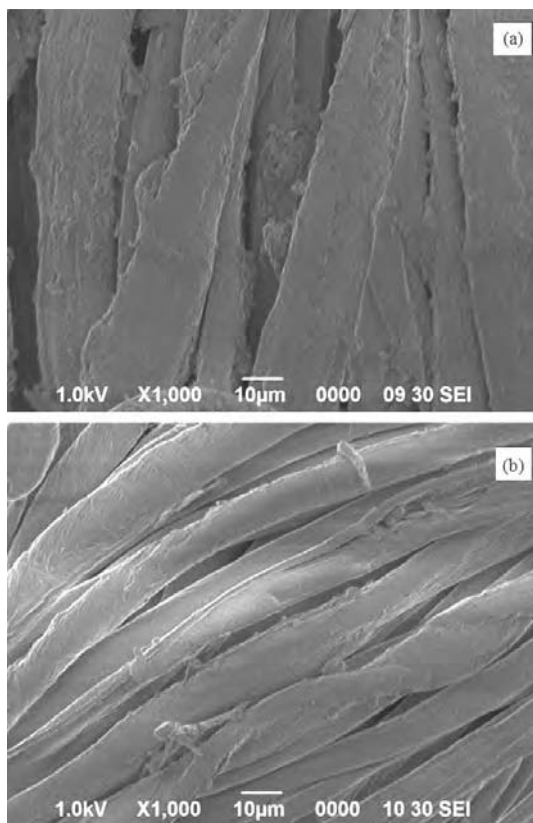| Exp. run	Conc. mL/L	Time min	pH	Temp. °C	Signal-to-noise ratio, dB
1	1	1	1	1	12.68
2	1	2	2	2	12.77
3	1	3	3	3	12.86
4	2	1	2	3	12.65
5	2	2	3	1	12.18
6	2	3	1	2	13.08
7	3	1	3	2	11.71
8	3	2	1	3	16.39
9	3	3	2	1	15.68

Fig. 3—SEM images of (a) scoured and (b) biopolished Samples

samples (Fig. 3). Prominent cracks in the surface of the fibres and splitting of surface near the centre of fibre along the fibre axis are observed in the biopolished fibres. Surface boundaries become sharper in the cellulase hydrolysed fibres compared to untreated fabric samples, which show hazy boundaries on magnification.

3.4 Crystallinity, Crystallite Size and Crystalline Order

High degree of lateral and longitudinal order in the cotton cellulose makes the fibres less susceptible for cellulase hydrolysis. Hydrolysis of cotton cellulose in the standing bath, using cellulases, does not affect the prominent x-ray diffraction intensities like (101), $(10\bar{1})$, (002), (021) and (040). The degree of crystallinity of raw cotton (84 %) does not change significantly after biopolishing of the cotton fabrics (84.6%) in spite of the considerable amount of weight loss. However, higher degree of crystallinity has also been reported for a short duration hydrolysis[23]. Biopolished cotton samples do not show any change in the crystallite size (44.6 Å) compared to original fibre (44.3 Å), measured perpendicular to (002) plane. The lateral order of crystalline region measured between (101) and $(10\bar{1})$ diffraction intensities show a marked reduction compared to raw cotton samples (0.692) after biopolishing (0.667), which is obviously due to preferential hydrolysis in these crystalline planes that results in changes in the crystalline perfection due to splitting of fibrils.

3.5 FTIR Analysis

It is a much surprising fact that virtually no published research papers are available about the FTIR assessment of biopolished samples, for which assessment in terms of physical properties is in vogue[24]. FTIR fingerprint of biopolished sample was found to be entirely different compared to that of raw cotton fibres. The absorbance of the samples in the entire spectra is found to be less with more transmittance in the range 4000 – 3000 cm^{-1}. Peaks in the 900 – 800 cm^{-1} become minor from the major ones compared to the raw cotton, due to reduction in the $C_1 – O – C_4$ stretch and C-O stretch, by hydrolysis of cellulose molecules.

The FTIR peaks in the range 3600 – 3500 cm^{-1} (hydrogen bonded O-H stretch) reduced marginally due to hydrolysis of cellulose molecules. O-H in-plane bending of primary hydroxyl groups (1045 cm^{-1}), prevalent among the cellulosics chains, becomes more pronounced in the biopolishing. Peaks at 1200 – 1100 cm^{-1} become pronounced due to CH_2 in-plane bending, C-H vibrations of the cellulosics chains.

4 Conclusion

Cellulase produced by *T. reesei* exhibits the optimum activity in the acidic *p*H at 50° C. Weight loss of the biopolished samples shows wide variation, depending on the process conditions employed during the treatment i.e. concentration of cellulase and temperature conditions to a larger extent. Degree of crystallinity and crystallite size measured perpendicular to (002) plane do not register any significant change in the fibres. However, splitting of fibres and reduction in the lateral order are observed on account of the cellulose hydrolysis together with removal of surface irregularities that are present in the fibres. FTIR results reveal an increased -OH bending, CH_2 in-plane bending, C-H vibrations of the cellulose chains with reduction in the $C_1 – O – C_4$ and C-O stretch.

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