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Effect of biopolishing on structural degradation and physical properties of cellulose

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Abstract: Cellulase treatment on cotton fabric is ecofriendly way of biopolishing. In the present work, biopolishing of cotton fabric using free and immobilized cellulase under various treatment conditions have been carried out using Box-Behnken design and the physical properties like pilling grade, abrasion resistance and flexural rigidity were analyzed. The degradation of cotton fabric minimized and similar results were obtained for pilling and abrasion resistance of free and immobilized cellulase treatment. Higher flexural rigidity is the result of immobilized cellulase treated fabric when compared with the free cellulase. The degradation of amorphous region improves the crystallinity index of the treated samples. This shows that the amorphous region were more prone to enzymatic attack than the crystalline regions. The enzymatic treatment increase the accessible surface area of cotton fibre and it can be measured by Methylene blue absorption value. Methylene blue absorption of the fabric was more for free cellulase than the immobilized cellulase treated fabric. The change in hydrogen bond by the treatment was measured and analyzed by the Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR).

Keywords: Cotton fabric, biopolishing, cellulase, immobilization, ATR-FTIR

INTRODUCTION

Biopolishing of fabric is a finishing process in textile industry and it is an advanced technique whereas the traditional finishing treatment is achieved by using harsh chemicals. Later in 1980s harsh chemical was replaced by enzymes in order to reduce water, chemicals, energy and environmental pollution.[1] Biopolishing is one of the methods to improve the surface properties of cotton fabrics by minimizing fuzz, pilling and improving lustre of the fabric.[2,3] Cellulase comprises of endoglucanases (EnG), exoglucanases (ExG) and β-glycosidase (BG) which acts synergistically to hydrolyze cellulose by degrading of f β- (1, 4)

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linkages and releases glucose as the end product.[4,5] Cellulose is a linear unbranched polymer and it has highly oriented molecular structure with intermolecular chain bonding.[6,7] Mechanism of cellulase action on cellulose as initially endoglucanase randomly attacks cellulose chains and cleave the β-1,4-linkage between the glucose units creating new cellulosic chain ends, exoglucanase splits at the reducing and non reducing ends of cellulose with a release of cellobiose, and finally *β*-glucosidase hydrolysis cellobiose into glucose,[8] mechanism of cellulase action on cellulose is shown in Fig. 1. Immature and short fibres are protruding on the surface of the yarn and fabrics which leads to pills formation and reduction of lustre. Protruding fibers on the surface of fabrics are removed by hydrolysis of cellulose by cellulase. The process parameters of cellulase treatment such as activity, concentration of cellulase and treatment temperature and time influences the surface modification and other mechanical properties of the fabrics.[3,9,10] The rate of degradation of cellulose is normally assessed by weight loss of the fabric after cellulase treatment. During biopolishing process, the enzyme molecule penetrate inside the structure of cellulose due to its smaller dimension such as 60 Å and it breaks the molecular chain [11] results severe strength loss and weight loss of the fabric.[12] The weight loss and tensile strength loss can be minimized by increasing the size of the enzyme molecule, so that the enzyme action is limited to the surface. The increase in size of cellulase enzyme molecule can be attained by immobilization techniques.[13] Bioaffinity immobilization allows more deposition of enzymes on the small surface, has higher catalytic activity, enhanced stability when compared with enzyme immobilized using covalent linkage.[14] In bioaffinity immobilization, , Concanavalin A acts as affinity ligand for enzyme. In our previous work commercial cellulase enzyme has been immobilized on Concanavalin A layered calcium alginate bead.[15] In the present work, the comparison of effect of biopolishing of cotton fabrics with free cellulase and immobilized cellulase were studied, the hydrolysis of cellulose leads to decrease in O-H stretching which was analyzed using ATR-FTIR spectroscopy. Cellulase can be more easily digest the amorphous region than the highly ordered crystalline region of cellulose. It also enlarges the pore radius and increase in accessible surface area of the fibre leads to increase in methylene absorption values.[16]

EXPERIMENTAL

Materials

Commercial liquid cellulase with activity of 60 IU/mL was purchased from United Alacrity, Chennai. Cotton fabric with the following specifications was used; warp yarn count 2/30 Ne, Ends per inch 100, weft yarn count 40 Ne, picks per inch 45, fabric thickness of 0.3 mm with the fabric weight per unit area of 212 g/m^2. Sodium alginate, calcium chloride, starch and methylene blue were of analytical grade.

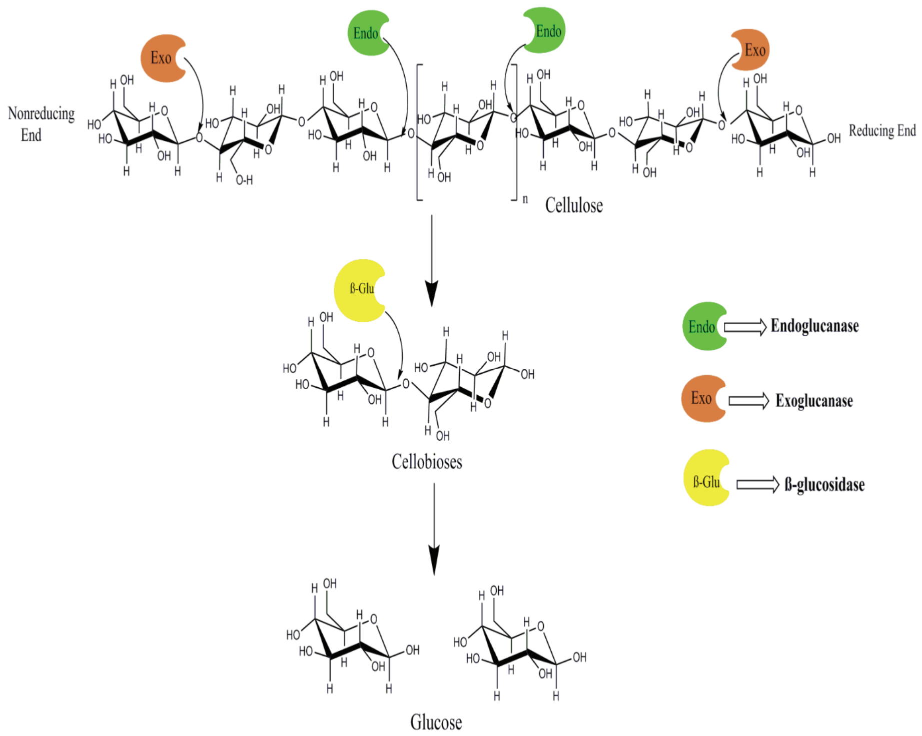

Fig. 1. Mechanism of cellulase on cellulose.

Immobilization of cellulase on concanavalin A layered calcium alginate beads

Concanavalin A (Con A), a lectin carbohydrate binding protein was extracted from Jack Bean (*Canavalia ensiformis)*, 5.0 g of jack bean was overnight soaked and homogenized using 50 mL of 0.1 M tris buffer with pH 6.2. The residue was centrifuged at 12000 x g for 20 min and supernatant is the source of Con A. Calcium alginate/starch bead was prepared using syringe drop method. Sodium alginate, 0.25 g and starch, 0.25 g was mixed homogenously with 10 mL of distilled water. The mixture was taken in a syringe and added drop by drop to the 200 mM calcium chloride solution and the formation of beads was observed. The calcium alginate - starch beads were incubated with 10 mL of Concanavalin A solution for overnight and the Concanavalin A layered beads were then washed for 3 – 4 times using 0.1 M sodium acetate buffer with the pH of 5.5 and the beads were then incubated with cellulase for 12 h at room temperature. Beads were dried and stored for further use.[15] Mechanism of immobilization was shown in Fig. 2.

Determination of activity of free and immobilized cellulase

The activity of cellulase was measured using UV spectrophotometer at 540 nm by DNS method. One unit of Cellulase activity is defined as the amount of enzyme that produces 1µmole of glucose in one minute. The enzyme substrate mixture incubated for 10 min at 50 °C and reaction stopped by DNS reagent. The reaction mixture were kept for boiling for 5min, then cooled immediately for color stabilization. The amount of reducing sugar is measured based on the intensity of color developed by DNS solution and OD measured at 540 nm.

Immobilized enzyme beads were pre-incubated with the substrate for desired time interval and the beads alone removed and DNS solution added to the reaction mixture and activity was measured as mentioned above. The formula for determining cellulase activity was measured as Activity of cellulase (µmol / mL. min) = 1000 W/ MVt where W is the amount of glucose produced, M is the molecular weight of glucose, V is the volume of sample, t is the reaction time.

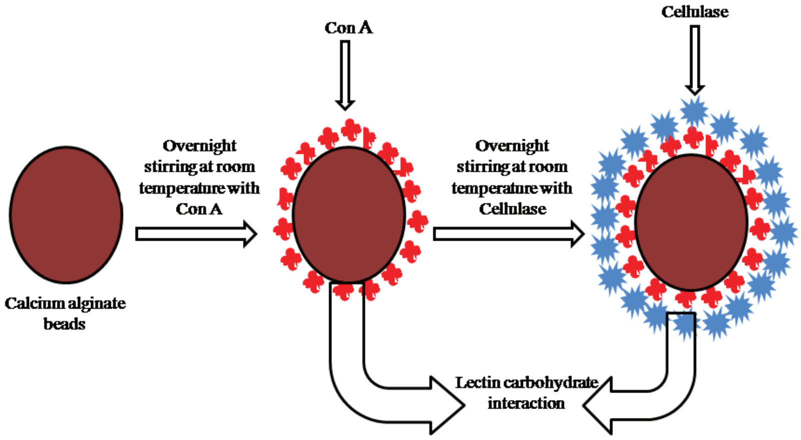

Fig. 2. Mechanism of immobilization process.

Biopolishing of cotton fabric using free and immobilized cellulase

Enzymatic treatment of cotton fabric was carried out using both free and immobilized cellulase using infrared dyeing machine (Infracolour DC4 F/R-SP). For biopolishing, three concentrations of cellulase such as 1, 2, and 3 % on-weight of fabric (owf), treatment temperature as 50, 55, and 60 °C and time 40, 55 and 70 min were used as per the experimental plan shown in Table I. Immobilized cellulase beads were taken based on their activity which will give the concentration of 1, 2 and 3 % (owf) with respect to the free enzyme. The dyeing machine speed in rpm was maintained as 50 and M:L ratio was 1:10. After the treatment, the cotton fabric was rinsed and dried for further characterization. The biopolishing process was terminated by hot treatment at 80 °C for 20 min. Optimization of the biopolishing treatment conditions and its effects on the fabric physio-mechanical properties requires number of experiments. Hence, Response Surface Methodology (RSM) has been selected to design the experiment. Response surface methodology is a set of techniques that confine in designing a set of experiments that will yield more reliable measurements. Under the RSM, Box-Behnken method has been used in this study to design the experimental plan and fifteen trails have been conducted.

TABLE I. Variables and their levels used in Experiments

Variable	Process parameter	Level		
		-1	0	+1
X_1	Enzyme Concentration, %	1	2	3
X_2	Treatment Temperature, °C	50	55	60
X_3	Treatment Time, min	40	55	70

ATR-FTIR Analysis

ATR-FTIR spectrum for the samples was obtained by Perkin Elmer - Spectrum FTIR Spectrometer with the ATR diamond accessory. The absorbance was measured for the samples from the region 4000-450 cm^{-1}.

XRD Analysis

The crystalline nature of control, free, and immobilized cellulase treated fabrics was analyzed using X-ray diffractometer (Bruker Company, Germany). The angles scanned were 10–80° at 4°per min. The crystallinity index of the fabric was calculated by the "Segal method" using the height ratio between the intensity of the crystalline peak and total intensity.[17]

$$CrI = 100\frac{I_{002} - I_{\mathrm{am}}}{I_{002}} \tag{1}$$

where I_{002} is the peak intensity from the (002) lattice plane and I_{am} is the peak intensity from the amorphous region.

Pilling grade

Pilling grade and Abrasion resistance was determined using Martindale abrasion and pilling tester. The pilling grade (1000 rubbing / cycles) of cotton fabric was measured as per the ASTM standard D 4970. The degree of pilling grade was evaluated visually by comparing the tested sample with the standard rating scale from 5 to 1 (no pilling to very severe pilling).

Abrasion resistance

The abrasion resistance was measured as per the ASTM standard D 4966 using the Martindale abrasion and pilling tester. The test fabrics were subjected to the rubbing motion against emery sheet as abrading over the area of the test fabric. The end point is the breakage of two or more threads.

Bending length and flexural rigidity

Bending length (stiffness) was measured using Shirley stiffness tester with fabric of length of 15.2 cm x 2.5 cm as per the ASTM standard D 1388. The fabric was placed in the horizontal platform and moved slowly till the end projects and reaches the datum line which is having the angle of 41.5°. The length of the projected sample is measured from the scale mark. Flexural rigidity is the measure of stiffness related to the handle of the fibre, it can be determined from the equation 2.

$$G = WC^2 \tag{2}$$

where, G is flexural rigidity, W is grams per square centimeter of the fabric, C the bending length of the fabric.

Methylene blue absorption test

The degradation of cellulose was measured by methylene blue absorption test.[18] 0.2 g of cotton was put in 25 mL of methylene blue solution and the mixture was kept at 25 °C for 24 h and the speed was maintained as 180 rpm. After the 24 h the methylene blue solution concentration was measured using UV spectrophotometer at 660 nm.[19] The methylene blue absorption value can be calculated as the difference between initial absorption and after absorption by the cotton fabric.

RESULTS AND DISCUSSION

ATR-FTIR analysis

The ATR-FTIR is a very useful technique in structural elucidation, the infrared spectra was measured by absorbance mode. The FTIR spectra for the controlled and enzyme treated samples were shown in the Fig. 3. The hydrolytic attack of cellulase on cellulose will modify the structural and functional properties of the cotton fabric. Hydrolysis of cellulose affects the hydrogen bonding, hence the spectrum between 3600 to 3000 cm^{-1} provide the information about the functional changes that occurred during biopolishing. After biopolishing, the absorption bands in the region 3600 to 3000 cm^{-1} can be attributed to the O-H stretching, shows a few changes in the position of absorption bands and slight change in the intensity of the peaks., it has been reduced marginally based on the concentration of the enzyme. Higher the enzyme concentration such as 3% reduces the intensity of the absorbance of FTIR specta has been noted in the results. Compared to samples treated with free cellulase, the immobilized cellulase treated samples have higher intensity of the absorbance. The total crystallinity index (*TCI*)[20] A_{1372}/A_{2900} and lateral order of index (LOI)[21] A_{1437}/A_{899} of the controlled and enzyme treated samples were used to evaluate the infrared crystallinity. Hydrogen bonding intensity (HBI)[22] A_{3308}/A_{1330} was used to measure degree of intermolecular regularity and the results were shown in Table II. The crystallinity indices have been increased after the enzymatic treatment due to the degradation of amorphous region of the cellulose and hydrogen bonding index has been decreased after the enzymatic treatment due to breakage of hydrogen bonds when compared with the control fabric.

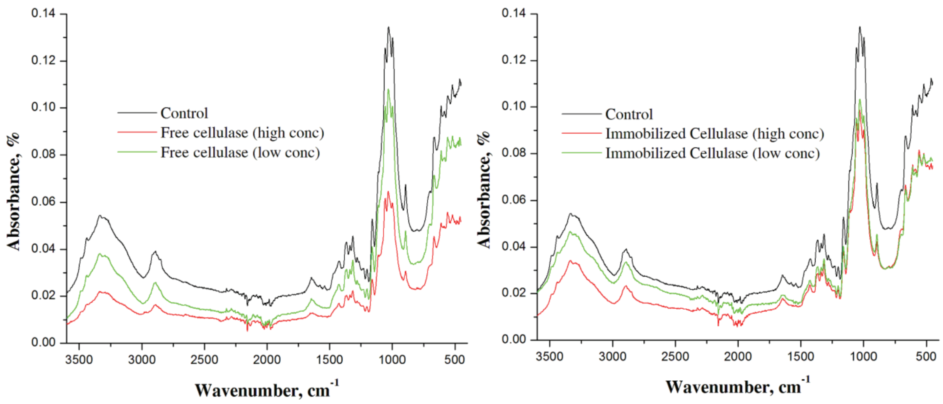

Fig. 3. ATR-FTIR spectra of a) control fabric, fabric treated with low and high concentration of free cellulase and b) control fabric, fabric treated with low and high concentration of immobilized cellulase.

TABLE II. Crystallinity indices of cotton fabric before and after enzymatic treatment with free and immobilized cellulase

Sample	LOI (A_{1437}/ A_{899})	TCI (A_{1372}/A_{2900})	HBI (A_{3308}/A_{1330})
Control	0.50	0.76	1.32
Free cellulase (low conc)	0.53	1.0	1.08
Free cellulase (high conc)	0.55	1.133	1.06
Immobilized cellulase (low conc)	0.525	0.911	1.137
Immobilized cellulase (high conc)	0.531	1.130	1.130

XRD analysis

The crystallinity index of the cellulose was calculated before and after treatments from the XRD spectra. The effects of biopolishing on cellulose with respect to the low and high concentrations of cellulase are shown in Fig S-1 of the Supplementary material to this paper. The derived crystallinity index values for all samples are given in Table III. Increase in the crystallinity index by biopolishing was noted and it was due to the degradation of the amorphous regions. The hydrolytic attack is more prone to amorphous region, than the crystalline region and it leads to increase in crystallinity.[13, 23] The crystallinity index (CrI) of immobilized cellulase treated fabric was lower than the fabric treated with free cellulase, because the immobilized cellulase action is limited to the surface whereas the free enzyme penetrates in to the inner structure of the fabric. For untreated fabric the CrI value is 60.28 %, whereas the fabric treated with minimum and maximum concentrations with free cellulase was 64.19 and 80.87 % respectively. When the fabric was treated with minimum and maximum concentrations of immobilized cellulase gives the CrI value as 61.91 and 68.42 % respectively.

Methylene blue absorption

Enzymatic polishing of cotton fabric increases accessible surface area and pore radius of the cotton fibre.[16,24] The ability of cellulose to absorb low molecular weight compounds reveals the morphology, amount of crystalline and amorphous regions, and the microfibrilar structure.[25] Hence, its fibre accessibility can be detected using methylene blue absorption. The methylene blue solution was absorbed by the cotton fabric when it was immersed. The methylene blue absorption value was calculated by measuring the difference between the concentration of methylene blue solution before and after absorption by the fabric. Higher the degradation of cellulose increases the affinity of methylene blue dye than the untreated sample and the results were given in Table III. The cellulase enzymatic treatment increases the Methylene blue absorption value and it is higher for free cellulase treated fabric than the immobilized cellulase treated fabric. The increase in cellulase concentration, increases the fibre accessibility by biopolishing results in higher MBA values.

TABLE III. Crystallinity index value and methylene blue absorption value for the samples

Concen-tration, %	Tempe-rature, °C	Time, min	Crystallinity index, %, free cellulase	Crystallinity index, %, immobilized cellulase	Methylene blue absorption (OD), free cellulase	Methylene blue absorption (OD), immobilized cellulase
1	50	55	64.19	61.91	0.353	0.110
	55	40	65.90	62.69	0.395	0.160
	55	70	75.50	62.44	0.445	0.178
	60	55	65.47	62.28	0.385	0.116
2	50	40	67.42	62.81	0.419	0.170
	50	70	75.82	65.31	0.462	0.203
	55	55	69.42	63.56	0.445	0.179
	55	55	69.87	62.97	0.434	0.181
	55	55	68.27	63.72	0.439	0.177
	60	40	65.72	62.34	0.404	0.148
	60	70	75.88	67.37	0.478	0.216
3	50	55	74.46	62.58	0.420	0.162
	55	40	75.92	64.64	0.450	0.188
	55	70	80.87	68.42	0.482	0.245
	60	55	75.40	64.03	0.431	0.191

Pilling grade

Biopolishing of fabrics has improved the pilling grade when compared with control fabrics and the results were given in Table IV. Cotton fabric treated with the free cellulase enzyme improves the pilling grade in the range of 4-5, while the untreated cotton fabric showed the pilling grade of 3. Immobilized cellulase treated cotton fabric showed pilling grade of 5. The free enzyme penetrates the structure of the yarn and the fabric, and degrades the cellulose results migration of free ends of fibres on the surface of fabric. The fibres protruding on surface was removed due to surface contact of immobilized enzymatic beads. For the sample 11 and 14, with the concentrations of 2 % and 3 % of free enzyme with the temperature as60 and 55 °C and the treatment time of 70 min results the pilling grade 4. This might be the raise of fibers on surface of the fabric due to structural damage. With the same treatment time and concentration of the cellulase, the fabric when treated with the immobilized cellulase shows higher pilling grade and it is due to the immobilized cellulase action only limited on the surface of the fabric.

Abrasion resistance

The loss of weight of the fabric due to abrasion was given in Table IV and Fig. S-2a, S-2b, S-3a and S-3b. The mass loss of the fabric by abrasion is due to the removal of protruding fibres on surface by the enzymatic treatment, which contributes major proportion in mass loss. At higher enzyme concentration and higher temperature, the maximum proportions of protruding fibres are removed by biopolishing process. Hence, when the abrasion resistance of fabric is observed with respect to mass loss as shown in Fig. S-2a, lower the mass loss at higher concentrations and temperature due to the fibres exposed to abrade is poor when compared to controlled samples. From Fig. S-2b, at 2.5 % enzyme concentration with treatment time of 50 min, the mass loss was decreased after that there was an increase in mass loss. This might be due to the rise of fibres on the surface of the fabric by the hydrolytic attack when higher enzyme concentration and prolonged treatment time was used to treat the fabric. Fig. S-3a and S-3b shows the mass loss by abrasion of the biopolished fabric with immobilized cellulase. Linearity of variables on the graph was noted, which shows the increase in enzyme concentration, temperature and time, decrease in the mass loss due to the surface activity of immobilized cellulase. Cotton fabric treated with free cellulase has higher mass loss when the fabric treated with immobilized cellulase. The maximum mass loss of the cotton fabric treated with free and immobilized cellulase was 0.841 g and 0.693 g respectively, while for the untreated cotton fabric the mass loss was 1.49 g. Increase in the enzyme concentration results the decrease of mass loss for both free and immobilized cellulase treatment due to the increase of CrI of the fabric. There was slight increase in the mass loss by the free cellulase treatment.

TABLE IV. Physical properties of free and immobilized cellulase treated fabrics

Concen-tration, %	Tem-per-ature, °C	Time, min	Pilling grade of free cel-lulase	Pilling grade of immobil-ized cel-lulase	Abrasion resistance (mass loss), g of free cellulase	Abrasion resistance (mass loss), g of immo-bilized cellulase	Flexural rigidity, g cm of free cel-lulase	Flexural rigidity, g cm of immo-bilized cellulase
1	50	55	5	5	0.84	0.69	326	347
1	55	40	5	5	0.83	0.67	196	359
1	55	70	5	5	0.73	0.68	97.0	294
1	60	55	5	5	0.77	0.69	279	311
2	50	40	5	5	0.76	0.64	167	387
2	50	70	5	5	0.74	0.64	74.9	245
2	55	55	5	5	0.73	0.62	85.6	299
2	55	55	5	5	0.73	0.61	77.6	284
2	55	55	5	5	0.73	0.60	97.6	250
2	60	40	5	5	0.70	0.59	224	283
2	60	70	4	5	0.69	0.59	63.8	289
3	50	55	5	5	0.66	0.59	159	309
3	55	40	5	5	0.64	0.57	98.9	276
3	55	70	4	5	0.76	0.56	53.2	225
3	60	55	5	5	0.73	0.57	133	311

Flexural rigidity

The stiffness of the fabric in terms of flexural rigidity was measured and the results ae given in Table IV. Fig. S-4a shows the effect of temperature and concentration on flexural rigidity of fabric treated with free cellulase enzyme. Flexural rigidity of untreated fabric is 392.62 g cm. Increase in enzyme concentration and treatment temperature increases the rate of hydrolytic attack of enzyme on cellulose leads to reduction of flexural rigidity. Fig. S-4b shows the effect of enzyme concentration and treatment time, with increase in treatment time of the biopolishing process at higher cellulase concentration, the rigidity of the fabric decreases, but after 2.5 % concentration of cellulase with treatment time of 50 min flexural rigidity decreases. Figures S-5a and S-5b shows the flexural rigidity of the immobilized cellulase treated fabrics and the flexural rigidity of the immobilized cellulase treated fabric was greater than the free cellulase treated fabrics. The enzymatic treatment of cotton fabric considerably reduces the flexural rigidity, i.e., due to the hydrolysis of cellulose which causes the decrease in the modulus of the fibre constituents of the fabric.[26] But the immobilized cellulase treated fabric shows higher flexural rigidity than with the fabric treated with free cellulase.

CONCLUSION

Biopolishing of cotton fabric results structural degradation of cellulose which weakens the fibre due to breaking of chain length and it changes the physical properties. The crystallinity index has been increased for the free and immobilized cellulase treatment due to the hydrolytic attack of the enzyme at amorphous region. CrI values are less for the immobilized cellulase due to the hydrolytic attack of immobilized cellulase is limited on the surface of the fabric whereas the free enzyme penetrates to the inner structure of the fabric. The total crystallinity index and lateral order index of the free cellulase enzyme treated fabrics were higher due to the more structural degradation of cellulose when compared to control and immobilized enzyme treated fabrics. The rate of reduction of hydrogen bond intensity (HBI) is higher for free cellulase treated fabrics. Methylene blue absorption values show the fibre accessibility is more in the free cellulase treatment for fabrics. The pilling grade of the fabric has been improved after the treatment with both free and immobilized cellulase when compared with the controlled fabric. Abrasion resistance of the fabric shows higher mass loss in untreated fabric, whereas after treatment the mass loss has been reduced. The flexural rigidity was more in the immobilized cellulase treated fabric than the free cellulase treated. Hence, biopolishing of cotton with free and immobilized enzyme modifies the structural and physical properties of the fabric and immobilized enzyme treatment retain the physical properties with improved surface modification.

SUPPLEMENTARY MATERIAL

XRD spectra and additional presentations of the data are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

УТИЦАЈ БИОПОЛИРАЊА НА ДЕГРАДАЦИЈУ И ФИЗИЧКА СВОЈСТВА ЦЕЛУЛОЗЕ

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Биополирање је прихватљив начин обраде памучне тканине помоћу ензима целулазе са аспекта заштите животне средине. У овом раду, су приказани резултати биополирања памучне тканине помоћу слободних и имобилисаних целулаза, изведени под различитим условима коришћењем Box-Behnken експерименталног дизајна. Затим су анализирана њихова физичка својстава, као што су оцена отпорности на пилинг, отпорност на хабање и чврстоћа при савијању. Деградација памучне тканине је била минимална, а слични резултати су добијени за оцену отпорности на пилинг и отпорности на абразију при примени слободне и имобилисане целулазе. Више вредности чврстоће при савијању тканине су добијене при третирању имобилисаном у поређењу са слободном целулазом. Деградација аморфне области узорака је утицала на повећање степена кристалиничности третираних узорака, што указује да је аморфна фаза подложнија ензимском нападу у односу на кристалну фазу. Дејством ензима се повећава развијеност и доступност површине памучних влакана, што је потврђено апсорпцијом метиленског-плавог. Тканина третирана слободном целулазом је више апсорбовала метиленског-плавог у односу на тканину третирану имобилисаним ензимом. Промена у интензитету водоничних веза након третирања ензимима је мерена и анализирана помоћу Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR).

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