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Use of uni-enzyme on cotton knitted fabric and its comparison with commercial formulation

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This research compares the effectivity of a neutral cellulase isolated from *Bacillus licheniformis* KM999221 with a commercial biofinishing agent. The results clearly show the efficacy of this enzyme and its potential to perform over and above a commercial biofinishing formulation. The application of this neutral cellulase would not only be soft on the environment but also fabrics friendly in terms of fabric handle, bursting strength and minimal fabric pilling. This study helps in successfully optimizing the biopolishing of cotton fabrics using a uni/single system, thus paving the way for the use of more such enzyme to be tapped as potential biofinishing agents.

Keywords: *Bacillus licheniformis,* Biopolishing, Cellulase, Cotton knitted fabric, Uni-enzyme

1 Introduction

Biotechnology has played a crucial role in production of natural fibres with highly improved and modified properties, in addition to providing opportunities for development of absolutely new polymeric materials[1]. Apart from economic considerations, the usefulness of a fibre for commercial purposes is determined by properties, such as weight loss, bursting strength, pilling grade, dyeability and various other surface properties. Most textile fibres (natural or synthetic) are slender, flexible and relatively strong[2]. Enzymatic applications in textile processing have been initiated long ago; a concoction of enzymes, like cellulases, proteases and amylases have been commercially used for biofinishing of textiles since the last decade as a sustainable eco-friendly alternative to toxic chemical agents**.** Microorganisms like *Bacillus sp*, *Aspergillus sp*, *Trichoderma viride*, *Trichoderma reesei and* have been used for commercial production of cellulases. The stability of these enzymes in regard to temperature, *p*H and other treatment conditions however a crucial limitation for their commercial use is as biofinishing agents. Literature cites development as well as application of a variety of methods that promote the stability as well as efficacy of these enzymes through immobilization and / or chemical modifications Biopolishing agents more specifically, neutral cellulases, have been preferred not only because they are soft on the environment, but also on the fabric, since they do not leave any residues on the processed fabrics[3]. Polishing (removal of the surface hairiness) of fabrics by biological agents/enzymes results not only in lesser energy consumption and reduction of toxic environmental effluent load but also enhances the fabric properties in terms of fabric handle, minimal fabric pilling etc . Due to the removal of hairiness of the textile fibre, however the textile materials are known to lose weight. These weights vary from fabric to fabric and are termed as fabric loss[4].

The present study attempts to enhance the biopolishing of a knitted cotton fabric through the use of a potent neutral cellulase. The highlight/ special feature of this enzyme includes the fact that it has been isolated from *Bacillus licheniformis* KM999221, an inhabitant of damaged archival papers. A uni/ single enzyme system has been proposed to be used in order to minimize residues due to preservatives/ silicon used in commercial biofinishing agents. Cotton knitted fabrics are generally popular because of their flexibility and ability to adapt as well as stretching them to a particular shape when worn and because of their general comfortable wear[5]. This is unique `study that successfully optimizes biopolishing of cotton fabric using a uni/single enzyme system free of any chemicals or additives that could potentially affect the fabrics, paving the way for use of more such enzymes to be tapped as potential biofinishing agents.

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2 Materials and Methods

2.1 Materials

The cotton knitted fabrics (17g/piece, 30*30 cm) and biofinishing agent were procured from Indian Council of Agricultural Research (ICAR)-CIRCOT, Mumbai. Neutral cellulase was purified from *Bacillus licheniformis* KM999221 in the Department of Biotechnology, University of Mumbai, India. All chemicals used were of analytical grade. All standard chemicals including sodium hydroxide, sodium silicate, kieralon wetting agent, pottasium dihydrogen phosphate, dipotasium hydrogen phosphate, carboxymethyl cellulose (CMC), ammonium sulphate, magnesium sulphate, sodium chloride, bovine serum albumin,3, 5-dinitrosalicylic acid (DNS) reagentwere procured from Merck, Pvt.Ltd (India). Yeast extract was procured from Himedia, Pvt. Ltd (India) and Nova cronturquoise blue dyeing agent was procured from Huntsman, Pvt. Ltd (India). Rice husk was collected directly from field, Pune, Maharashtra, India.

The *p*H was monitored using pH/conductivity meter-CpHC (Contech, Mumbai, India). Hot air oven (Meta lab, India) was used for determination of moisture content of the fabric. Infra colour dyeing machine (R. B. Electronics and Engineering Pvt Ltd, India) was used for dyeing of knitted fabrics. Washing fastness tester (R.B. Electronics and Engineering Pvt Ltd, India) was used for biopolishing of knitted fabrics. McSparr bursting tester was used for the analysis of bursting strength of fabrics. Pilling tester instrument (Ameet industries, India) was used for the pilling analysis. Dye ability of knitted fabrics was analysed by Reflectance spectrophotometer with D65light using colour control software. Surface morphology of fabric was analysed by scanning electron microscopy (Quanta 250, 80Pa, 15KV, Image J (software for modification) software.

2.2 Methods

2.2.1 Partial Purification and Activity Determination of Neutral Cellulase

Bacillus licheniformis KM999221 was isolated from archival documents as reported by Jacob and Mane[6]. For cellulase enzyme induction *Bacillus licheniformis* KM999221 were inoculated in sterile basal salt medium (BSM) ,containing 0.1% KH_2PO_4, K_2HPO_4, 0.04% $MgSO_4$, 0.005% NaCl, 0.0001 % $FeSO_4$ and 0.5% yeast extract supplemented with 1% sieved rice husk powder as an enzyme inducer. The media was incubated at 37°C for 72 h, and the bacterial mass was then harvested by centrifugation at 10,000 rpm for 20min. The supernatant was subjected to 30% & 60% ammonium sulphate precipitation and refrigerated overnight to complete the precipitation. The precipitate was then separated by centrifugation at 10,000 rpm for 20min and subsequently suspended in 500µL of 20mM sodium phosphate buffer (*p*H 7.0). This was then dialyzed against the same buffer for 12h at 4°C and protein content determined by Bradford's assay. Bovine serum albumin (BSA) was used as a standard and enzyme activity was assayed by DNS method[7-10].

2.3 Comparative Analysis of Partially Purified Cellulase Enzyme and Commercial Enzyme

All the experiments were performed in triplicates and controls maintained wherever necessary. Prior to biopolishing of the cotton knitted fabric, combined scouring-bleaching analysis was performed using optimized concentration of H_2O_2 (7.0g/L)/ sodium hydroxide (3.0g/L), sodium silicate (2.0g/L) and kieralon OL as a wetting agent (0.1 g/L). The experiment was performed in a dyeing machine, keeping material-to-liquor ratio as 1:20 at 98°C for 60min. After scouring and bleaching process, the knitted fabric was washed with three volumes of hot water (70-80°C) and two volumes of cold water (25°C) at the room temperature (27 °C)[11,12] .

2.4 Evaluation of Treated Fabrics

2.4.1 Weight Loss

Samples were prepared according to ASTM D-3776-961. Moisture content of the fabric was determined to calculate the actual weight of the samples before and after the treatment using glass weighing bottles with air tight cover. The weighed samples along with the bottles were dried at 105˚C for 4 h in hot air oven. The bottles were then cooled to 27 °C at room temperature (27 °C) and kept in desiccator for 30 min and weighed again till constant weight was obtained. Loss in weight of treated fabrics was measured using the following equation [13]:

$$\text{Weight loss \%} = \frac{(\text{Fabric weight before biopolishing} - \text{Fabric weight after biopolishing})}{\text{Fabric weight before biopolishing}} \times 100$$

2.4.2 Biopolishing of Cotton Fabric with Cellulase

Biopolishing of the cotton knitted fabric was carried out using both NC with CBA. The knitted fabrics were treated with three different concentrations of biofinishing agents, viz. 2.0%, 4.0%, and 6.0% (w/v) on-weight of fabric (owf) basis at 40°C for 45min. The speed of the machine was maintained as 50 rpm and the material –to-liquor ratio as 1:20. The

fabrics were then boiled in water for 5min to inactivate the enzymes and subsequently rinsed with hot (60-70ºC) water followed by cold (25ºC) water washing three times at room temperature (27 ºC) The fabrics were then dried in air for 18h and later subjected for characterization [11-14].

2.4.3 Pilling Test

Pilling of fabrics was determined according to ASTM D-4970-05. The knitted cotton fabric was mounted on polyurethane tubes and tumbled in a cork lined box. After 18000 resolution (60 resolutions/min), appearance of the samples was evaluated by visual assessment using photographic standards of different grades, ranging from 1 to 5. The observed resistance to pilling was reported using rating scale, pilling resistance from 1(very severe pilling) to 5(no pilling). Rating was given based on the surface appearance of the sample[15-17].

2.4.4 Bursting Strength

Bursting strength of a fabric was measured using Mcsparr bursting tester, UK with pneumatic loading arrangement. The test sample was clamped on the rubber mould and uniform pressure (0 -14 kg/cm^2) was applied until the test piece was torn. Samples were set on the diaphragm, automatic bursting strength tester was used, and time, distortion, pressure and flow rate to burst the knitted fabric material were measured[18].

2.4.5 Dyeing and Determination of Colour Strength

The dye bath was prepared using1% of novacronturquoise,60 g/L sodium chloride and 15 g/L sodium carbonate at a material-to-liquor ratio of 1:20. The sample was introduced in the dye bath at 80 °C and dyeing was continued at this temperature for 30 min. The dyed sample was rinsed thoroughly in cold water (25ºC) followed by hot water (70-80ºC) and then sun dried at an ambient temperature (30ºC). The dyed knitted fabric was tested in a reflectance spectrophotometer with D65 light using colour control software interfaced with IBM PC. The percentage colour strength of the samples was read by the instrument [19,20].

2.4.6 Surface Morphology of Biopolished Fabric

To analyse the surface morphology, the treated and control samples were observed under the SEM using wide range of magnifications available, Large area of the fabric was first visualised at low magnification and then selected areas at increasing magnification until surface detail of a single fibre could be observed. The micrographs were taken to assess the modifications occurring on the surface of individual fibres [21].

3 Results and Discussion

3.1 Partial Enzyme Purification

The supernatant was precipitated by ammonium sulphate, dialysed against 20mM sodium phosphate buffer (*p*H 7.0) for 12 h at 4°C and finally used as a source of enzyme for the further work. Specific activity of NC is found to be 450U/mg and that of CBA is found to be 500U/mg with protein content of 180mg/mL for NC and 300mg/mL for CBA.

3.2 Weight Loss of Knitted Fabrics in Enzymatic Treatment

Weight loss of cotton knitted fabric is an important factor for industrial treatment. The weight loss of NC and CBA treated knitted cotton fabric is shown in Fig. 1. Weight loss is found within the range of 0.17-0.30%. The enzyme concentration (2.0%, 4.0% and 6.0%) is applied separately on the bleached knitted cotton fabric for 45 min at 40ºC. The weight loss is found to be 0.17% at 4.0% concentration. In the case of CBA, weight loss percentage is found more as compared to NC, as evident in Fig 1.

3.3 Pilling Grade

Pilling grade of NC treated fabrics has been expressed in Fig 2. The finding clearly indicates that the enzyme significantly improves the pilling resistance of the treated fabric sample. Literature reports that the pilling resistance rating of bleached fabrics is in between 3 and 4 under 4500 cycle operation in pilling test. NC at 4% concentration shows no change in pilling testing (rating of 5). Under 1800 cycle of operation, samples 1, 3, 4, 6 show a rating of 4-5. This pilling performance clearly indicates that the NC significantly improves the pilling resistance of the treated fabric samples. When friction is applied on the treated fabric during the pilling test, the tendency of pill formation is significantly reduced and majority causes a rating of 5, i.e. no change

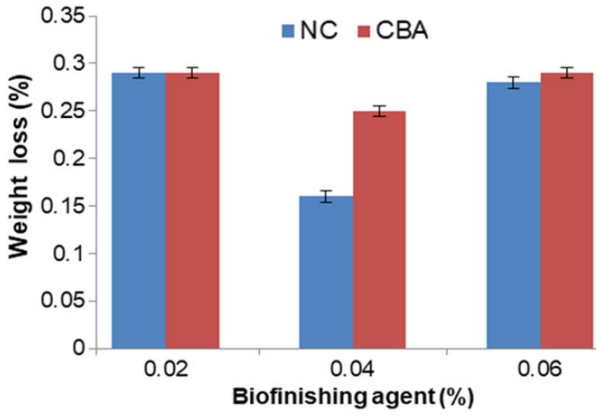

Fig. 1 — Weight loss of knitted fabrics in enzymatic treatment

at the surface. A good de-pilling effect is also observed due to mechanical agitation which accelerates the cellulase action.

3.4 Bursting Strength

Bursting strength of the fabric is improved after biopolishing with enzymes. Bursting strength of NC at 4% concentration (w/v) is found to be high with (608.012 kPas) as compared to that of CBA (Fig. 3). Moreover, heavy bio-polishing will degrade cellulose causing fall bursting strength. Therefore, to maintain standard bursting strength in the finishing processes, proper chemical concentration, *p*H, temperature and time of treatment for optimum production and good quality fabrics must be controlled by setting up all the parameters.

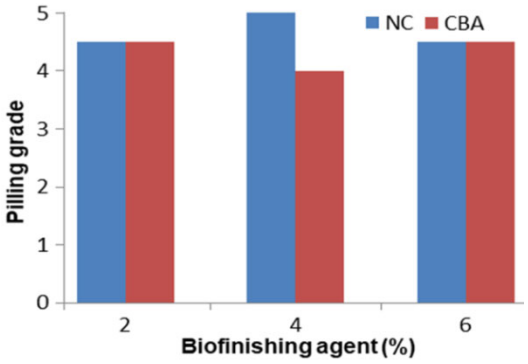

Fig. 2 — Pilling grade of knitted cotton fabrics treated with NC and CBA

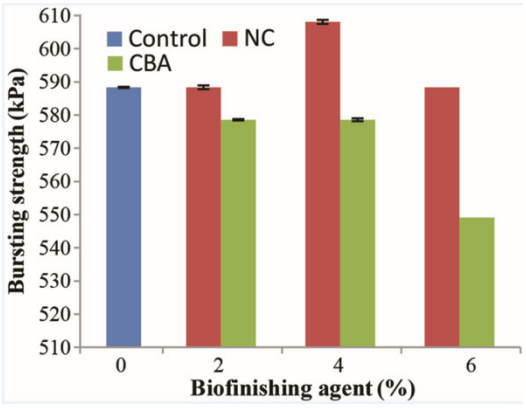

Fig. 3 — Bursting strength of biopolished cotton knitted fabrics using NC and CBA

3.5 Determination of Colour Strength

The results of the colour strength (*K/S*) values of cotton knitted fabrics dyed after cellulase treatment are represented in Table 1. The result shows the positive effect of biopolishing treatment on K/S values, which improves for all treatments. This may be due to the removal of protruding fibres and decreasing the scattering coefficient, which depends on degree of polymerization, ratio of amorphous to crystalline regions, swell-ability, accessibility, chemical reactivity, surface morphology and affinity for dyes. From Table 1 it is observed that the knitted fabrics show high *K/S* value in NC treated fabrics as compared to that in CBA treated sample. Surface depth of the colour and *K/S* value of the dyed samples are determined on a spectrophotometer. The *K/S* value of dyed material is found directly proportional to the amount of dye present in the material. As the temperature increases upto 80°C, the molecular structure becomes open, which facilitates the dye uptake and hence the higher *K/S* value is obtained. The results show that *K/S* value of NC dyed fabrics is high (16.53), whereas that of CBA treated fabrics is low in the range of 12 -15 (Table 1). The declining value of *K/S* for knitted cotton fabrics means that this concentration has less impact. Manonmani *et al.*[2] show that the dyeing of cotton knitted fabric with natural dye extracted from *Acacia catechu* has good depth of dyeing (3.1) when dyed along with mordant, whereas the control fabric dyed with the extract alone shows less *K/S* value (1.9). Enzyme type used in biopolishing can also change the dyeability of the fabrics. The dyeability of cellulosic fibre is related to the morphological structure of the fibre. The amorphous structure of the fibre has great importance on the dye uptake.

Table 1—Dyeing ability of knitted fabrics

Samples	L	A	B	C	H	*K/S*	Wavelength, nm
Control	55.922	-32.660	-27.294	42.563	219.869	15.1549	670
NC 2.0 %	57.054	-32.455	-26.922	42.168	219.660	13.8845	670
NC 4.0 %	55.323	-33.086	-27.675	43.135	219.895	16.5335	670
NC 6.0 %	56.655	-32.588	-27.423	42.591	220.065	14.2882	670
CBA 2.0%	56.098	-32.949	-27.490	42.911	219.823	15.6650	670
CBA 4.0%	56.309	-32.738	-27.253	42.597	219.760	15.3073	670
CBA 6.0 %	58.367	-32.452	-26.506	41.901	219.225	12.7131	670

L–Lightness, A–red/green coordinate, B–yellow/blue coordinate, *K* and *S* are the absorption coefficient and scattering coefficients respectively.

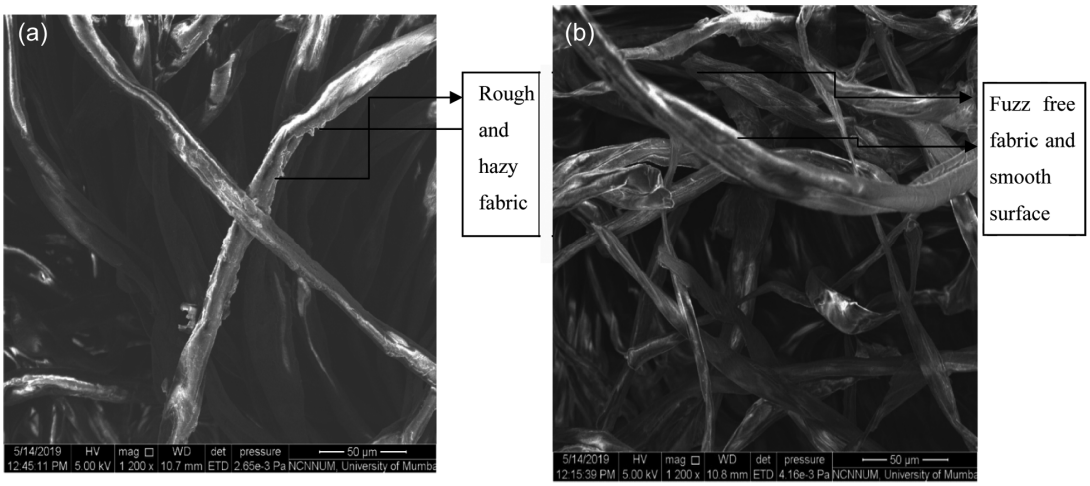

Fig 4 — Scanning electron microscopy of control sample (a) and enzyme- treated sample (b).

3.6 Surface Morphology of Biopolished Fabric

The SEM photographs of control sample and enzyme- treated sample are depicted in Fig. 4. Scanning electron microscopic observations reveal the fuzz-free fabric surface in case of biopolished sample as compared to control. It is seen clearly in Fig. 4(b) that the fibrils in the peripheral cells are opened up in case of enzyme treated sample. This could be due to the removal of irregular cemented cells in fibre. Further, from the SEM photograph, it can also be seen that the surface becomes even in case of enzyme-treated fibre as compared to control, and this is reflected in the smoothness of the surface as sensed while handling of the fibre. The bio-polishing treatment is affected by the cellulase treatment. The change in the morphology of the fibre during the treatment shows the development of smoothened surface microscopic appearance possibly due to the accumulation of the enzyme in the fibre matrix and subsequent removal of the unwanted micro fibrils. The ridges that are present in the control fibre samples cease to exist in the case of cellulase-treated fibres. Surface boundaries become sharper and smoother in the cellulase hydrolysed fibres as compared to untreated fabric samples, which show hazy boundaries on magnification. In the biosoftening process, some weight loss is observed, which however does not yet indicate any fibre damage.

4 Conclusion

This study explores compares the influence of partially purified enzyme with a commercial biofinishing agent on the physical properties of cotton knitted fabric. The protein content is determined by Bradford method and it is found to be higher in commercial biofinishing agents. The fabric treated with lab cellulase shows enhanced bursting strength, dyeability as well as surface morphology. It also minimizes the fabric weight loss and hence dyeability of the treated fabrics is improved.

References

1 Khas H, *Indian J Fibre Text Res,* 26(2001) 206.
2 van Dam J E G, *Proceedings of the Symposium on Natural Fibers*, (2009) 3.
3 Kumari A, *Int J Life Sci,* 9(2014)1355.
4 Ullah S, *Textiles Clothing Sustainability*, 9 (2015) 1.
5 Sitotaw D B, *J Eng*, (2018) 1-9.
6 Jacob S M, Bhagwat A M & Kelkar Mane V, *Int Biodeterioration Biodegradation*, 104(2015)46.
7 Geng W, Cao M, Song C, Xie H, Liu L, Yang C & Wang S, *J Bacteriology,* 193(2011)3393.
8 Ratnakomala S, Fahrurrozi & Yopi, *IOP Conf Ser Earth Environ Sci,* 7(2019)251.
9 Olajuyigbe F M & Ogunyewo O A, *Biocatal Agric Biotechnol* , 7(2016)110.
10 Dutta S Deb, Tarafder M, Islam R & Datta B, *Biocatalysis Agricult Biotechnol*, 14 (2018)279.
11 Uddin M G J, *Innov Dev Strategy,* 4(2010)18.
12 Mukthy A A & Hoque S M A, *Eur Sci J*, 10(2014) 375-389.
13 Uddin Mohammad Gias, *Text Clothing Sustainability,* 1(2015)9.
14 Ali H, Hashem M, Shaker N, Ramadan M, El-Sadek B & Hady M A, *Res J Text Apparel,*16(2012) 57.
15 Kumar V S, Meenakshisundaram S & Selvakumar N, *J Text Inst,* 99(2008) 339.
16 Noreen H, Zia M A, Ali S & Hussain T, *Indian J Biotechnol,*13(2014) 108.
17 Kotb R M & Eladwi M M T, *Int J Recent Sci Res*, 7(2016)9772.
18 Biswas H R P K, Mitra B K & Rakesh S R, *Int J Current Eng Technol*, 4 (2014)4242.
19 Ren Y, Gong J, Wang F, Li Z, Zhang J, Fu R & Lou J, *Dyes Pigm,*134(2016) 334.
20 Xiao H, Zhao T, Li C H & Li M Y, *J Cleaner Production,*165(2017)1499.
21 Zuber M, Zia, K M, Bhatti I A, Ali Z, Arshad M U & Saif M J, *Int J Biol Macromole*, 51(2012)743.