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# **Enzymatic Polishing and Reactive Dyeing** of Cotton Fabric in One Bath

#### Abstract

Cellulase has the ability to remove fuzz-fibres from cellulosic textiles, which endows fabric with a glabrous appearance and soft handle. In this paper, the combined uses of bio-polishing and reactive dyeing in one bath were carried out, aiming at shortening the treatment time and reducing the overall consumption of water and energy as well. The properties of cotton fabrics before and after the combined treatments were investigated, including the colour strength, colour fastnesses and tearing strength. The results indicated that the colour fixing agent of soda had noticeable inhibiting effects on cellulase activity. Meanwhile reactive dyes might react with cellulase proteins under alkaline conditions, resulting in decelerating influences on the dye-fibre covalent reactions. The acceptable one-bath process consisted of bio-polishing and dyeing of cotton in neutral solution, followed by dye fixation under alkaline conditions. Compared to polishing and dyeing in two baths, the one-bath method endowed fabric with a bit lower colour strength.

Key words: cotton fabric, neutral cellulase, reactive dye, one-bath method, colour strength.

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### Introduction

Cellulase has the ability to degrade cellulosic fuzz-fibres of cotton, flax and viscose by hydrolysing the repeating unit of β-1,4-glycosidic bonds in cellulose molecules [1, 2] and has been used for enzymatic treatments of different cellulosic textiles. During the bio-scouring of cotton fabrics, the combined use of cellulase and pectinase might enhance the efficacy of impurity removal and impart satisfactory wettability to fabric without severe environmental pollutions [3]. As for the bio-finishing of cellulosic textiles, countless literatures have reported that cellulase could endow fabrics with a smooth appearance, fading effects and better hand feeling. It has been widely used for lyocell polishing, denim washing and eliminating the prickle of linen [4 - 6]. More recently, the enzymatic polishing of carded cotton fabrics with cellulases has been increasingly used instead of the singeing method, owing to its mild treating condition of low temperature. Conventional bio-polishing treatments have been commonly performed separately before or after the dyeing procedure, thus a long incubating time has been necessary for the dyeing-polishing combined treatment, which undoubtedly increases the additional manufacture cost. On the other hand, some investigators have also reported that the efficiency of the cellulase catalytic reaction is easily affected by the dye class, dye structure and its reactive property during the polishing of dved cellulosic textiles [7, 8]. Choe et al. [9] found that a high concentration of the pre-existing dyes in fibres, especially

with planar structures, had decelerating influences on the bio-polishing of cotton fabrics; however, the inhibition mechanisms have not been clearly explained up to now. Thus it follows that the bio-polishing method and interaction mechanism between cellulase and dyes should be further considered despite decades of cellulase research and commercial applications.

To develop an efficient process for the dyeing and polishing of cotton fabrics, the combined use of cellulase treatment and reactive dyeing was designed and investigated. Reactive dyes were able to react with the hydroxyl groups of cellulosic substrates and create cross-links between the dyes and fibres. The dyeing procedure usually consisted of two distinct phases: firstly dyeing was conducted in neutral solution and sodium salts were used to promote the dye exhaustion, and then an appropriate amount of alkali was added to the dyeing bath to accelerate the reaction between the reactive dyes and hydroxyl groups of cellulose. Bifunctional reactive dye containing two potential reactive groups gave high degrees of dye fixation [10], and its dyeing condition was similar to the treatment condition of neutral cellulase, i.e., approximately 60 °C and a near-neutral pH condition. Accordingly it was possible to make the combined use of the bio-polishing and dyeing of cotton fabric simultaneously in one bath, which might reduce the negative effects of inhibition on cellulases, undoubtedly shorten the incubating time and save on the overall consumption of water and energy as well. Although some reports discussed

the optimisations of one bath for bio-polishing and dyeing [11, 12], the effects of reactive dyes on enzyme proteins of cellulase were not considered in detail.

The present work analysed the impacts of the dyeing agents of salt and alkali on the enzyme activity of cellulase, and was the first to demonstrate the interactivity between cellulase and bifunctional reactive dye during the one bath bio-polishing and dyeing of cotton fabrics. The objective of this study was to extend the utility of a short process for the dyeing and polishing of cotton fabrics.

### Experimental

### Materials

Neutral cellulase SUKACell-N1000 from microorganisms was purchased from Sukahan Bio-Technology Company (Weifang, China). The incubating condition recommended for the cellulase was 55 - 60 °C and pH 6.5-7.0. Reactive Red M-3BE (C.I. Reactive Red 195), with monochlorotriazine and vinyl sulphone as reactive groups, was kindly supplied by Shanghai Colva Dyestuff Co., Ltd. (China). Carded cotton knitted fabrics (160 g/m²) were scoured and bleached beforehand. Other chemicals used in this work were analytical grade.

## Cellulase activity and protein concentration determinations

The enzyme activity of cellulase was measured spectropotometrically using a filter paper strip as a substrate according to modified procedure [13]. After incubating the cellulase with additives

at 60 °C for 40 min, the residual enzyme activity was determined using a 2802 S UV/Vis spectrophotometer (Unico Instruments Co., Ltd., China). The relative enzyme activities of the cellulase before and after incubation were subsequently calculated. One unit of cellulase activity was defined by the amount of enzyme that produced 1.0  $\mu$ mol of reduced sugar per minute.

Considering that the absorbance at 280 nm reflected the concentrations of soluble proteins, a standard curve was made plotting the absorbances at 280 nm against the concentrations of cellulase proteins. After incubating the cellulase with additives of salt or soda for a certain time, the mixed solution was centrifuged at 5000 r.p.m for 10 min, and the concentration of supernatant cellulase proteins was determined according to the standard curve.

## Reactive dyeing and enzymatic polishing

Two-bath combined treatments for the dyeing and bio-polishing of cotton fabrics were selectively carried out. Cotton fabrics were firstly dyed with 0.5 g/l of bifunctional reactive dye at 60 °C and pH 7.0 for 40 min at a liquor ratio of 25:1. Afterwards 10 g/l of sodium sulphate was used to promote the dye exhaustion, and then 5 g/l of sodium carbonate was added to the dyebath to increase its pH and initiate dye fixation. After 40 min of dye fixation the fabrics were soaped with 2 g/l of non-ionic detergent at 95 °C for 10 min to remove unfixed and hydrolysed dyes from the fabrics, which were finally washed with distilled water thoroughly and air-dried. Enzymatic polishing of the cotton fabric with 1 or 5 g/l of cellulase was subsequently carried out at pH 7.0 and 60 °C for 40 min at the same liquor ratio of 25:1.

For the one-bath method of polishing and dyeing cotton fabric, cellulase was added to the dyeing solution before initiating reactive dyeing. Dye fixation and the soaping procedure were subsequently carried out, respectively, under the same conditions as applied in the two-bath method.

## Colour strength and dyeing fastness measurements

The colour strength of the dyed fabric was determined using a Color-Eye 7000A spectrophotometer (GretagMacbeth, USA) according to the Kubelka-

Munk method, and the result was calculated using *Equation 1*.

$$K/S = \frac{(1 - R_{\infty})^2}{2R_{\infty}}$$
 (1)

where, K and S represent the coefficients of absorption and scattering, respectively, and  $R_{\infty}$  denotes the reflectivity of the fabric at the wavelength of maximum dye absorption [14].

The rubbing fastness of the dyed cotton fabric was tested according to AATCC Test Method 8-2007 using a YG(B)571-II rubbing fastness tester (Wenzhou Darong Company, China). Colour fastness to washing was determined according to AATCC Test Method 61-2009 (2A) with a SW-12A launderometer (Wenzhou Darong Company, China).

## Investigation of interactivity between cellulase and dyes

To investigate the interactivity between cellulase proteins and reactive dyes, 1 - 10 g/l of cellulase was added to a solution mixture containing 0.25 g/l of reactive dye, 10 g/l of sodium sulfate and 0 - 5 g/l of sodium carbonate. After incubating at 60 °C for 40 min, 2 M of trichloroacetic acid (TCA) was added to the solution and the TCA-soluble products were separated by centrifugation at 5000 r.p.m at room temperature for 10 min. The absorbances of the supernatants transferred were measured and the percentages of dye fixation on cellulase proteins calculated according to *Equation 2*.

$$F(\%) = (1 - \frac{A_1}{A_0}) \times 100\%$$
 (2)

where,  $A_0$  and  $A_1$  represent the absorbances of the original solution and the supernatant at the maximum absorption wavelength, respectively. The visual appearances of the protein deposits were also observed.

## Weight loss of cotton fabric after cellulase treatment

The weight loss was calculated as the difference in the weight of fabric samples before and after the cellulase treatments according to *Equation 3*.

Weight loss (%) = 
$$(1 - \frac{m_1}{m_0}) \times 100\%$$
 (3)

where,  $m_0$  and  $m_1$  represent the absolute dry weights before and after cellulase treatment, respectively.

## Fabric properties of drapability, smoothness appearance and tearing strength

The tearing strengths of cotton fabric before and after enzymatic polishing were evaluated according to ISO 13937.1:2000 using a YG(B)033A drop hammer textile tearing tester (Wenzhou Darong Company, China). The samples were conditioned at 25 °C and RH 60% for 24 h prior to testing.

The fabric drapability of the cotton fabric was determined according to ISO 9073.9:2008 using a XDP-1 fabric drape tester (Shanghai New Fibre Instrument Co., Ltd., China) and the drape image test method. The static drape coefficients of the fabric with and without polishing were recorded.

The smoothness appearance of the cotton fabric was evaluated by the visual assessment method using a D65 standard illuminant and observation angle of 10°. The results were divided into five levels, where *Level 1* meant no difference from the untreated one, and *Level 5* - no noticeable fuzz-fibres on the fabric surface, and others were accordingly defined as *Levels 2*, 3 or 4 according to the residual fuzz-fibres on the fabric surface.

All results in this work were expressed as the means of three replicates, the relative standard deviations (SD) for the measured data were also calculated.

#### Result and discussion

## Effects of the dyeing agents of salt and alkali on enzyme activity of cellulase

Dyeing agent - sodium sulphate could promote the adsorption of reactive dyes onto the fibre surface by reducing the coulomb repulsion between anionic dyes and cellulose fibres [15]. Meanwhile the colour fixing agent of sodium carbonate might enhance the efficacy of dye fixation, endowing fabric with high colour depth and dyeing fastness as well. For the combined treatment of polishing and dyeing in one bath, the addition of salt and alkali might affect the enzyme activity of cellulase. In this study, an amount of sodium sulphate and sodium carbonate was added to the cellulase solutions, respectively, and enzyme activity before and after incubating investigated, shown in Figures 1 and 2.

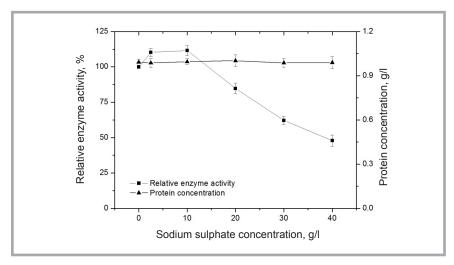
It can be seen in Figure 1 that the sodium sulphate had a noticeable impact on cellulase activity. When the salt concentration was 10 g/l the cellulase activity increased to approximately 110%, implying a slight activation of the enzyme, which was consistent with previous reports [16]. Contrarily, high concentrations of sodium sulphate exhibited noticeable inhibiting effects on the cellulase. The residual enzyme activity gradually decreased and reached 50% at a salt concentration of 40 g/l, which was much lower than that of the salt-free assays (approximately 82%). As the concentration of supernatant proteins after centrifugation saw no obvious change, as shown in Figure 1, the reduction in enzyme activity could be mostly attributed to conformational changes in the cellulase structure or inhibited binding of cellulase to the cellulosic substrates [17, 18] instead of protein aggregation or salting out by the high concentration of salt.

Remarkably sodium carbonate inhibited the neutral cellulase, as shown in Figure 2, with even a low concentration of 1 g/l of sodium carbonate leading to a 30% decrease in enzyme activity. The dramatic decrease might be similarly interpreted as being caused by incubating with sodium sulphate. Furthermore cellulose proteins could be partially hydrolysed under alkaline conditions, which also enlarged the degree of enzyme inhibition. Thus for the acceptable one-bath process of bio-polishing and dyeing, cellulase treatment should be commenced with reactive dyeing in neutral solution, which might avoid potential enzyme inhibition and unsatisfactory dye hydrolysis as well. After polishing and dyeing for a certain time, an mount of sodium carbonate could be added to the dyebath to increase the pH and subsequently initiate dye-fibre reactions.

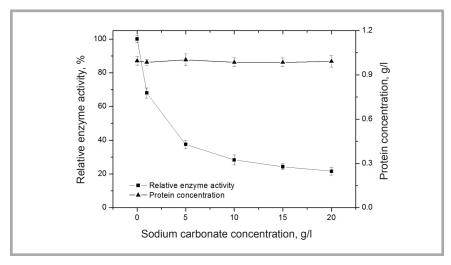
## Colour strength and dyeing fastness of cotton fabrics after polishing and dyeing processes

To investigate and compare the effects of enzymatic polishing on the dyeability of cotton fabrics, one-bath and two-bath methods of polishing and dyeing were carried out, respectively. The colour strengths and colour fastnesses of different fabric samples were determined and depicted in *Figure 3* (see page 112).

The K/S value of the cotton fabric without polishing was higher than the others,



**Figure 1.** Effects of sodium sulphate on enzyme activity and concentration of cellulase proteins. The error bars show the SD of three replicates.



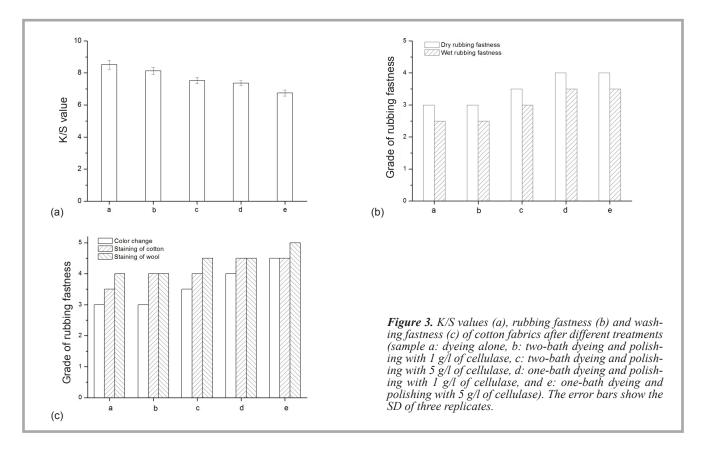
**Figure 2** Effects of sodium carbonate on enzyme activity and concentration of cellulase proteins. The error bars show the SD of three replicates.

reaching 8.51 after the reactive dyeing. Enzymatic treatment with cellulase decreased the colour strengths of the dyed fabrics to different extents. For fabric sample b, as shown in Figure 3.a, separately incubating with 1 g/l of cellulase after dyeing led to a slight decrease in K/S values, although the dry and wet rubbing fastnesses obtained were similar to those of sample a, which was mainly attributed to cellulase degrading fuzzfibres on the fabric surface and reducing the fabric surface roughness and colour strength accordingly. For fabric samples d and e in the one-bath method of dyeing and polishing, the colour strengths decreased more when compared to the results for sample b and c, together with better colour fastnesses to rubbing and washing (*Figures 3.b* and 3.c). Since the dye concentrations and dyeing time for the fabric samples were almost same, the remarkably low K/S values of fabric

samples d and e should not be interpreted only as the degradation of fuzz-fibres on the fabric surface, some other aspects might be responsible for the decrease in color strength.

## Interactivity between cellulase protein and reactive dye

When polishing and dyeing in one bath, reactive dyes could adsorb onto cellulose fibres and cellulase proteins as well via hydrogen bonds and van der Waals forces. When sodium sulphate was added to the incubating solutions, reactive groups of the dyes might simultaneously react with the hydroxyl groups of cotton fibres and amine groups of cellulase proteins. To verify the assumption above, an investigation of the interactivity between cellulase and reactive dye was carried out as described in section 2.5, the results of which are shown in *Table 1* (see page 112).



The data in *Table 1* disclosed that reactive dyes could react with cotton fibres and cellulase proteins simultaneously under the alkaline condition. Without the addition of sodium carbonate, the deposits of enzyme proteins after centrifugation showed a native white appearance, and the absorbances of the residual dye-

ing solutions did not obviously change. When 5 g/l of sodium carbonate was added, the absorbances of the residual solutions decreased remarkably after protein aggregation by TCA, and the deposits of cellulase proteins appeared to be coloured. The covalent reactions between dye molecules and cellulase proteins

**Table 1.** Degree of dye fixation on cellulase and visual appearance of enzyme protein deposited.

Additives in solutions	Fixation percentage (F), %	Visual colour of the deposit	
1 g/l cellulase	0	White	
1 g/l cellulase, 5 g/l Na <sub>2</sub> CO <sub>3</sub>	24.0 ± 1.2	Red	
5 g/l cellulase	0.7 ± 0.4	White	
5 g/l cellulase, 5 g/l Na <sub>2</sub> CO <sub>3</sub>	70.3 ± 1.7	Red	
10 g/l cellulase	2.5 ± 0.6	White	
10 g/l cellulase, 5 g/l Na <sub>2</sub> CO <sub>3</sub>	75.1 ± 1.3	Red	

**Table 2.** Properties of tearing strength, fabric drapability and smoothness of appearance for cotton fabrics after polishing-dyeing treatments.

Incubation conditions	Weight loss, %	Tearing strength, N	Static drape coefficient, %	Smoothness appearance level
Dyeing alone	0.52 ± 0.42	26.5 ± 0.9	63.2 ± 1.6	1
Polishing alone, with 1 g/l of cellulase	2.06 ± 0.27	24.3 ± 1.1	60.5 ± 0.8	4
Two-bath dyeing and polishing, with 1 g/l of cellulase	3.11 ± 0.36	20.6 ± 0.8	59.3 ± 1.2	3
Two-bath dyeing and polishing, with 5 g/l of cellulase	3.95 ± 0.29	18.8 ± 0.5	57.4 ± 0.9	4
One-bath dyeing and polishing, with 1 g/l of cellulase	2.36 ± 0.48	23.6 ± 0.9	59.2 ± 0.9	4
One-bath dyeing and polishing, with 5 g/l of cellulase	3.10 ± 0.49	22.0 ± 0.7	58.1 ± 1.1	4

might account for changes in the fixation percentage.

Increasing the concentrations of cellulase led to high percentages of dye fixation, and eventually 75% of the reactive dyes were bonded to the cellulase proteins deposited at a cellulase concentration of 10 g/l (Table 1). This further revealed that there were at least two competitive reactions occurring during the one-bath polishing and dyeing of cotton fabrics, i.e. the reactions of dye-fibre and dye-cellulase. Meanwhile if the reactive groups of dyes were bonded to the active sites of cellulase, it would undoubtedly affect the enzyme activity or stability of cellulase, which also partially explained why there was an inhibiting effect between cellulase and some reactive dyes.

# Fabric drapability, smoothness appearance and tearing strength of cotton fabrics in the dyeing-polishing processes

The objective of enzymatic polishing was to improve the fabric drapability and smooth appearance by degrading fuzz-fibres on the fabric surface. An acceptable process should endow fabric with a satisfactorily smooth appearance, considerable colour depth and lower strength loss. The properties of fabrics after different

treatments were evaluated and shown in *Table 2*.

For individual enzymatic polishing, the reduction in the static drape coefficient implied an improvement of fabric drapability. Meanwhile the smoothness of the fabric appearance was also at an encouraging level, together with a slight decrease in the tearing strength. In the case of dyeing and polishing in two baths with 1 g/l of cellulase, the fabric drape obtained was similar to that by the one-bath method, while the smoothness of the fabric appearance was at an inferior level, which could be attributed to the fact that the incubating conditions were not exactly the same. For the two-bath method, a long period of dyeing prior to polishing made the tightness of the cotton yarns decrease and more fuzz-fibres appeared on the fabric, which increased the accessible surface area of the interior of the yarn to cellulase and easily caused damage during the succeeding bio-polishing [19, 20]. Thus more fuzz-fibres were left on the fabric after dveing and polishing with 1 g/l of cellulase. When separately incubating with 5 g/l of cellulase after reactive dyeing, as shown in Table 2, the strength loss and weight loss of the fabric was relatively higher than for the others. For the one-bath polishing and dyeing, the degradation of fuzz-fibres on the fabric surface occurred simultaneously with reactive dyeing. Without a long period of previous reactive dyeing, the enzymatic hydrolysis of cellulosic cotton tended to proceed at the periphery of the cotton yarns, and the results of which were relatively better than those in the two-bath method. When polishing and dveing with 1g/l of cellulase in one bath, the fabric drapability and smoothness of the fabric appearance reached a similar level to that in treatment with cellulase alone. Considering that a low concentration of cellulase had fewer unsatisfactory impacts on the colour strength of cotton (Figure 3.a), 1 g/l of cellulase could be used in the one-bath combined treatment. The advantage of one-bath polishing and dyeing was that it not only endowed cotton fabric with a smooth appearance and lower fibre damage, but it also improved the efficacy of the dyeing-polishing by shortening the treatment time.

### Conclusions

Cellulase treatment was able to hydrolyse the cellulosic substrate of fuzz-fi-

bres, endowing fabric with a glabrous appearance. The efficacies of the combined bio-polishing and reactive dyeing of cotton fabrics were investigated. During the dye fixation phase, the reactive groups of dye molecules could react with cotton fibres and cellulase proteins simultaneously, resulting in decelerating influences on the dye-fibre reactions. For the onebath treatment of polishing and dyeing, the colour strength of the fabric was a bit lower than that of the fabric sample treated in two separate baths, mainly owing to partial covalent reactions between the reactive dye and cellulase proteins. However, one-bath treatment of polishing and dyeing endowed cotton fabric with acceptable fabric drapability and lower strength loss, and the smoothness of the fabric appearance and dyeing fastnesses obtained were also satisfactory. The effective method of one-bath bio-polishing and dyeing shortened the treatment time and reduced the overall consumption of water and energy as well.

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