Effect of Swelling and Reactive Dyeing on the Accessibility of Cotton to Cellulase Enzymes

Roshan Paul,* Mangesh D. Teli

Department of Fibres and Textile Processing Technology, Institute of Chemical Technology, Matunga, Mumbai 400019, India

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ABSTRACT: The main objective of this study was to determine the effect of intracrystalline and intercrystalline swelling agents and reactive dyes on the accessibility of cotton cellulose to commercial cellulase enzymes. Both types of swelling agents improved the accessibility, and intracrystalline swelling agents showed better results, as the accessibility to difficult-to-reach crystalline regions was increased. As expected, the reactive dyes inhibited the accessibility of the enzymes to some extent. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 1946–1950, 2011

Key words: dyes/pigments; enzymes; fibers; swelling

INTRODUCTION

With cellulosic fiber substances, most reactions and chemical modifications usually take place at the hydroxyl groups of the anhydroglucose units, as in the case of dyeing with reactive dyes. To enhance the efficiency of a process, it is necessary to increase the accessibility of the cellulosic hydroxyl groups to the reagent, both in the readily accessible region and in the difficult-to-reach portions of the fiber. The mature cotton fiber has a spiral fibrillar structure, which can be observed underneath the primary wall. So swelling has to be considered as a combination of interfibrillar and intrafibrillar processes. The state of aggregation of the fibrillar units and the void spaces and their distribution are important factors in the chemical reactivity and morphology of cotton, and the way in which they are altered during swelling can have a profound effect on the extent of subsequent reactions.

Sodium hydroxide is a well-known intracrystalline swelling agent that is capable of imparting the recrystallization of cotton cellulose. The most important effect of mercerization on the fine structure of cellulose fibers consists of changing the crystal lattice from cellulose I to cellulose II.[1,2] Ethylenediamine is also a powerful decrystallizing agent, which causes intramolecular swelling of cellulose and forms complex with cellulose by linking two anhydroglucose units of two cellulose chains, thereby retaining the intact cellulose I structure. The partial conversion of the cellulose I lattice structure into a cellulose II lattice as a result of the swelling action of ethylenediamine has also been reported.[3,4]

Morpholine is an intercrystalline swelling agent, which is capable of swelling the cellulose without any appreciable change in the crystalline regions of the fiber. A high degree of swelling is observed at the microfibrillar level, and the microfibrillar width and thickness change without any appreciable change in the infrared crystallinity index.[5] The extent of swelling of cellulose governs its properties, such as the moisture sorption, reactivity to various reagents, and diffusion of dyes and chemicals inside the fiber structure.

Biopolishing is a finishing process in which the cellulase enzyme acts on the cellulosic fabric surface to improve its surface characteristics by conferring properties such as brighter luminosity of colors, softer feel, and more resistance to pilling.[6,7] One of the most important industrially used strains for cellulase enzymes is *Trichoderma reesei*. The cellulolytic system of *T reesei* is composed of two cellobiohydrolases (CBH I and CBH II), at least four endoglucanases (EG I, EG II, EG III, and EG V), and at least one β-glucosidase.[8–10] Cellulases act synergistically in the hydrolysis of crystalline cellulose. Endoglucanases randomly attack the amorphous regions in cellulosic substrates; this results in a rapid decrease in the cellulose chain length, whereas cellobiohydrolases can also act on crystalline regions of cellulose, releasing cellobiose from the end of cellulose chains.[11,12] The commercial cellulase enzymes used today belong to one or two groups, namely, acid and neutral cellulases. The use of acid cellulases is

**Present address:* LEITAT Technological Center, Calle de la Innovació, 2 - 08225 Terrassa (Barcelona), Spain.
Correspondence to: R. Paul (paulrosh@yahoo.com).

recommended for fast treatment. In the meantime, neutral cellulases are generally recommended because of their resistance to the backstaining of garments.[13] Previous studies have indicated that mercerization is effective in increasing the accessibility of fibers to the cellulase enzyme,[14,15] but there is very limited information available[4] on the effect of other intracrystalline swelling agents or an intercrystalline swelling agent on the enzymatic treatment.

Reactive dyes, in contrary to vat dyes, may create barriers for the action of cellulase enzymes, as there is covalent bond formation. Although the effect of reactive dyes on cellulase efficiency has been reported previously,[16] no attempts have been made to correlate the effect of enzymes on preswollen and reactive dyed cotton.

EXPERIMENTAL

Materials

Scoured, bleached, plain-weave 180 g/m^2 cotton fabric, supplied by Century Textiles and Industries, Ltd. (Mumbai, India) was used for this study. Hot Brand (HB) dye, Procion Brilliant Blue H-GR (CI Reactive Blue 5), Cold Brand (CB) dye, Procion Brilliant Red M-8B (CI Reactive Red 11), High Exhaustion (HE) dye, and Procion Yellow H-E6G (CI Reactive Yellow 135) were supplied by Atic Industries, Ltd. (Valsad, Gujarat, India). Laboratory-reagent-grade sodium hydroxide, ethylenediamine, morpholine, acetic acid, sodium acetate, sodium carbonate, and sodium sulfate were purchased from S. D Fine Chem, Ltd. (Boisar, India). The acid cellulase Bactosol JA and the neutral cellulase Bactosol JN were supplied by Clariant Chemicals (India), Ltd. (Mumbai, India). Nonionic detergent was supplied by Uniqema India, Ltd. (Thane, India).

Swelling treatments

We mercerized the cotton fabric (S samples) without tension by soaking it in a 7.5*N* aqueous sodium hydroxide solution for 1 h at 27°C, with the material-to-liquor (M:L) ratio kept at 1 : 20, and subsequently washing the samples in acidified water followed by distilled water until the alkali was removed completely. Another set of samples were treated with a 75% w/w aqueous solution of ethylenediamine (E samples) at 27°C for 1 h, with constant stirring; we kept the M:L ratio at 1 : 20. The samples were subsequently washed thoroughly with distilled water and dried in air.[17,18] Cotton samples were also treated with a 40% w/w aqueous solution of morpholine (M samples) at 20°C for 1 h with the M:L ratio kept at 1 : 20.[5] These samples were subsequently washed thoroughly with distilled water and dried in air. In all cases, comparison was made with the control (C) sample.

Dyeing of the samples

The dyeing of the cotton fabric samples with reactive dyes was carried out in an open-bath beaker dyeing machine manufactured by EEC (Mumbai, India). In the case of the HB dyes, the dye bath was prepared for 2% shade (on weight of fabric) with the M:L ratio kept at 1 : 20. The samples entered at 60°C, and dyeing was carried out for 15 min. Later, 20 g/L sodium sulfate was added in two lots with a time interval of 10 min. The temperature was raised to 80°C, and dyeing carried out for 20 min. This was followed by the addition of 20 g/L sodium carbonate in two lots, and dyeing was carried out for 30 min. Samples were washed with cold water, soaped with 2 g/L nonionic detergent at boiling for 20 min, washed, and air-dried. The dyeing was carried out with CB dyes at 28°C with a similar procedure. Additionally, cotton was dyed with HE dyes with the same procedure used for dyeing with HB dyes.

Enzyme application

The enzyme treatment was carried out in a high-temperature, high-pressure dyeing machine manufactured by EEC that ensured continuous rotation. An enzymatic bath was prepared with 2% w/v acid cellulase enzyme. The pH of the bath was maintained between 4.5–5 with a sodium acetate/acetic acid buffer solution. The fabric sample was introduced into the enzymatic bath at room temperature, with the M:L ratio kept at 1 : 40. The temperature of the bath was raised to 40–45°C and kept there for 1 h. The enzyme-treated sample was then rinsed with hot water followed by cold water, soaped with 2 g/L nonionic detergent at 60°C for 20 min, washed, and air-dried. In the case of neutral enzyme, the pH was kept at 7, and the treatment temperature was 50–60°C.

Weight loss

We assessed the extent of enzymatic activity by carefully measuring the weight loss of the conditioned samples after the enzymatic treatment in a Mettler Toledo MonoBloc balance (Mumbai, India).

Color values

We analyzed all of the samples by measuring the K/S values under illuminant D_{65} with a 10° observer on a Spectraflash SF 300, a computer color matching system of Datacolor International (Lawrenceville, NJ). The λ_{max} values of the HB, CB, and HE dyes were 610, 550, and 400 nm, respectively.

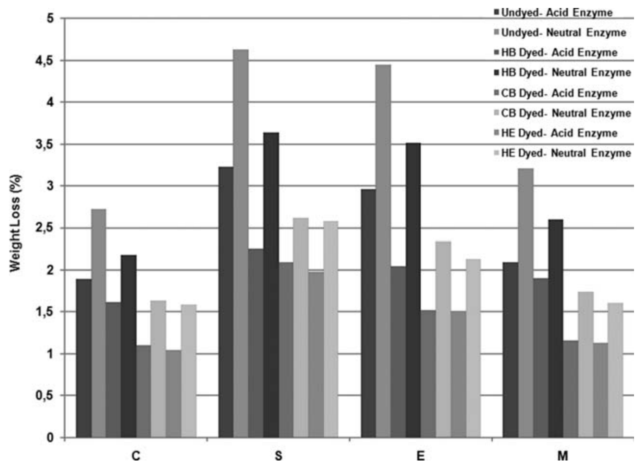

Figure 1 Effect of acid and neutral enzymes on various pretreated and reactive dyed cotton substrates.

RESULTS AND DISCUSSION

The bleached fabric was subjected to slack mercerization (S samples), treatment with ethylenediamine (E samples), and treatment with morpholine (M samples). All of the swelling treatments were carried out in the slack state to facilitate the maximum swelling action. These cotton fabric samples with three different pretreatments along with sample C were then subjected to treatment with acid and neutral cellulase enzymes. These samples were then washed and dried carefully, and the weight loss percentage of the conditioned samples was determined.

It is clear from Figure 1 that among all the pretreatments, S samples exhibited maximum weight loss, regardless of the type of enzyme used. This was followed by E samples and M samples. As the processes—mercerization, ethylenediamine treatment, and morpholine treatment—are known for bringing about swelling in the fiber structure,[2,3,5] the attack of enzymes and accessibility of the fiber for enzymes were greater, and hence, they showed higher weight loss in comparison to sample C. Out of the two enzymes used, the neutral enzyme was found to be more effective and degraded the cellulose to a larger extent. The cellulase enzymes are known for attacking the 1,4-β-glycoside linkages and thus breaking down the cellulose molecular chain,[12] which in turn, during abrasion, is removed out of the fabric, causing a loss in weight.

The S samples, because of their extraordinary level of intracrystalline swelling, enhanced the response of the enzyme to a higher extent.[14,19,20] In the case of ethylenediamine also, intracrystalline swelling has been reported to occur;[18] this results in a higher response of enzymes. However, the morpholine treatment causes only intercrystalline swelling,[5] and accessibility to the enzyme inside the crystalline region was denied, which restricted the weight loss. Another probability is that the swelling made the cellulose more accessible to the enzyme, and it could act more effectively on the larger surface area of the crystallites in the swollen sample.

The pretreated samples were further subjected to dyeing with various reactive dyes, namely, HB, CB, and HE types. The dyed samples were then subjected to enzyme treatment, and the weight loss was again determined (Fig. 1). In this case, the presence of reactive dyes in the fabric seemed to have hindered the enzymatic activity to some extent, as reported earlier in the case of cotton without any swelling treatment,[16] and thus, the weight loss values were commensurately lower in the dyed samples compared to the undyed ones. The extent of reduction in the weight loss value was maximum in the case of the HE dyes, followed by the CB and HB dyes. This could have been due to the higher crosslinking ability of the HE dyes, which contained homobireactive systems and resulted in a higher extent of blockage of the accessibility due to the reaction with the hydroxyl groups of cellulose.

The efficiency of the neutral enzyme was more than that of acid enzyme, as observed in earlier cases. However, the order of the extent of weight loss still remained the same with respect to the pretreatments. In other words, dyeing with various brands of reactive dyes did not change the order in which the weight loss varied due to the enzymatic attack. The presence of reactive dye, however, definitely decreased the extent of attack on the cellulose, and hence, the magnitude of weight loss did decrease.

The results from Figures 2–4 indicate that as a result of pretreatments, there was a change in the extent of dyeability with various reactive dyes. Regardless of the class of reactive dye used, all of the dyes gave the highest dye uptake for S samples followed by E and M samples. All of the three

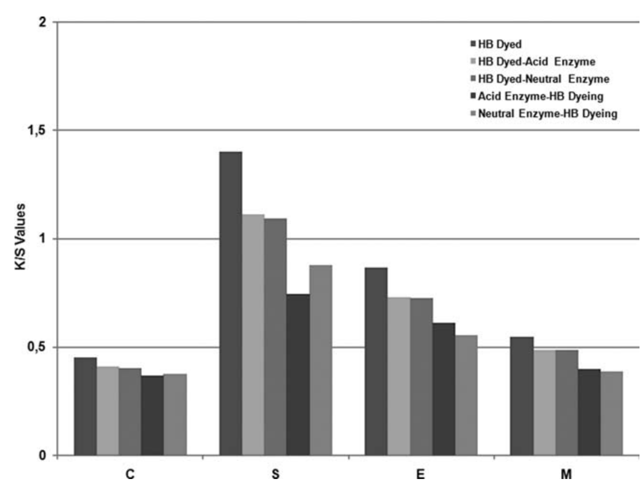

Figure 2 Effect of the enzyme treatments after and before HB dyeing on various pretreated cotton substrates.

pretreatments developed slack swelling of cotton fabric, in which case the collapsed structure of the cotton fibers in the fabric, acquiring cylindrical and swollen structures, tremendously increased the accessibility to various dye molecules.

The intercrystalline swelling agents were in a position to break the hydrogen bonds between the cellulose molecules, as observed in the case of the M samples. However, if the swelling agent was more powerful, it did not confine its activity in bringing about intercrystalline swelling but further penetrated into individual crystals of cellulose and brought about intracrystalline swelling. Sodium hydroxide is said to possess such capability to the highest extent, and ethylenediamine has it to a relatively lesser extent. For this reason, the extent of accessibility of hydroxyl groups in the cellulose structure toward various dyes was effectively maximum in case of sodium hydroxide, followed by ethylenediamine and morpholine.

The HB reactive dye showed that the maximum improvement in the depth of shade could be attributed to its least reactivity, and in such cases, the change in the internal structure of the fiber due to the pretreatment of fabric showed the maximum influence. As the dye reactivity increased, slowly such improvement decreased relatively because it was not only swelling but also the higher reactivity of the dye, which brought about better dyeability.

After we dyed the pretreated cotton fabric samples, such materials were further subjected to enzyme treatment, and their *K*/*S* values were determined and compared with those before enzyme treatment. The results of HB, CB, and HE dyes in terms of *K*/*S* values are given in Figures 2–4, respectively. It was obvious that as the enzyme treatment was given after dyeing, a reduction in the *K*/*S* value was evident, as during the enzyme action, surface leaching of the fiber took place; this resulted in a decrease in the *K*/*S* value. It was clear from the data that the original *K*/*S* values of the S and E samples were relatively higher, and the magnitude of the *K*/*S* values after the enzyme treatment was lower than before the enzyme treatment. The extent of reduction was highest with mercerization followed by the ethylenediamine treatment, which, in turn, was followed by the morpholine treatment. In other words, just as mercerization gave the highest extent of dye uptake and the E samples gave a value slightly lower than that of mercerization during dyeing, similarly, the reduction in the *K*/*S* value was also highest for the S samples followed by the E samples.

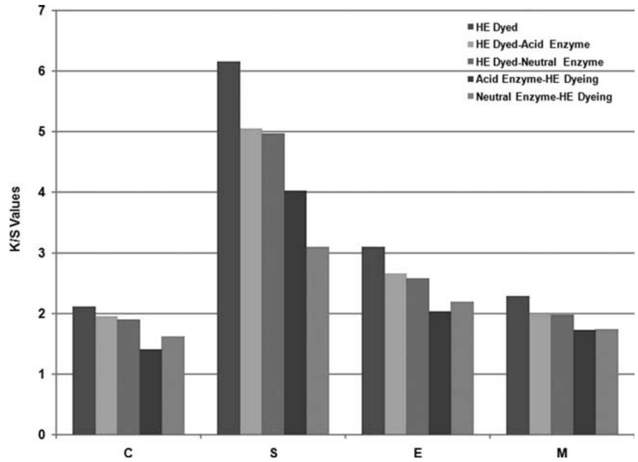

Figure 4 Effect of the enzyme treatments after and before HE dyeing on various pretreated cotton substrates.

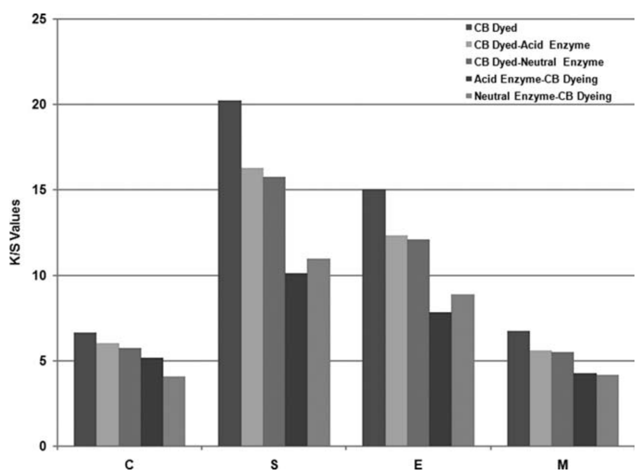

Figure 3 Effect of the enzyme treatments after and before CB dyeing on various pretreated cotton substrates.

It was thus the extent of swelling and accessibility, which during dyeing showed highest improvement in *K*/*S* values, resulted in highest reduction in *K*/*S* values after the enzyme treatment. Thus, the swelling agents used for pretreatment not only enhanced the dyeability regardless of the type of reactive dye used, but they also enhanced the extent of enzymatic attack. Once again, the *K*/*S* reduction with respect to the HB reactive dye was much greater compared to the other highly reactive types of dyes. However, the reduction in *K*/*S* values was distinctly higher in the case of neutral enzymes than that observed in the case of acid enzyme, as reported earlier.[4]

In the next set of experiments, the bleached cotton fabric, after having been pretreated with different swelling agents, was first enzyme-treated and then subjected to reactive dyeing. The results of the HB, CB, and HE dyes in terms of *K*/*S* values are also given in Figures 2–4, respectively. In this case, the white fabric dyed after pretreatment was compared with the fabric that was pretreated, enzyme-treated, and then dyed. Thus, the reduction in the *K*/*S* value was mainly due to the enzyme treatment that was given to the fabric sample before it was dyed. Once again, the trend in the reduction in dyeability followed the general trend, with the maximum reduction in the S samples followed by the E samples and, then, the M samples.

The initially pretreated samples with further treatment with enzymes leached out the amorphous region, brought down the accessibility of the fiber, and increased the surface area of the fabric. The cumulative effect of all these actions led to the reduction in the dyeability after the enzyme treatment. The enzyme was most effective in the S and least in the M samples. As observed before, all three reactive dyes showed a similar trend.

CONCLUSIONS

The accessibility of cotton cellulose to reactive dyes and cellulase enzymes increased significantly with pretreatments with intracrystalline and intercrystalline swelling agents. After pretreatment and dyeing, when enzymes were used for the final washing of the dyed samples, the K/S values decreased as a function of the extent of swelling. The pre-existing reactive dyes inhibited the action of the enzymes to some extent.

When the pretreatment was followed by enzyme treatment and then dyeing was carried out, because of the swelling caused by pretreatment, the enzyme action was found to be higher, and this resulted in decreased dye uptake. This decrease in the dyeability was attributed to the lower number of hydroxyl groups that were available for the further fixation of reactive dyes and also the increased surface area of the enzyme-treated fabrics. The swelling treatments, in general, led to a more efficient enzymatic processing of cotton.

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