Science-Based Options for Application of Cellulase Biotreatment and Reactive Dyeing to Cotton Fabrics

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Abstract: Current work addresses three approaches. The first approach is based on two- step process where a systematic investigation was undertaken on factors affecting biotreatment of cotton fabrics using cellulase enzyme and, the onset of this on the dyeing properties of the fabrics when the latter where dyed using mono- and bifunctional reactive dyes. In the second approach, one- bath process for biotreatment and dyeing was established through controlling sequence of addition of the ingredients of both enzymatic biotreatment and reactive dyeing. The third approach refers to a post treatment process where dyeing was carried out first then thus obtained dyeings were treated with cellulase enzyme. Enzymatic effect was expressed as variation in the enzyme activity, loss in fabric weight, wrinkle recovery angle, tensile strength, elongation at break and colour strength in addition to overall fastness properties for selected samples. Results of these properties obtained with the three processes reveal that the two-step process is by far the best then comes the one step-process. The pos-treatment process occupies the last position in this order. Differences among the three processes were explained in terms of the environment created during applications of each of these processes and, to what extent does this environment acts in favour of the interaction of the enzyme and/or the dye with the cotton fabrics.

[A. Hebeish, M.M. Kamel, H. M. Helmy and N. S. El Hawary. **Science-Based Options for Application of Cellulase Biotreatment and Reactive Dyeing to Cotton Fabrics.** *Life Sci J* 2013;10(4): 3281-3289]. (ISSN:1097-8135). http://www.lifesciencesite.com. 436

Keywords: Science-Based Option; Cellulase Biotreatment; Reactive Dyeing; Cotton Fabrics

1- Introduction

The end of the last century witnessed the introduction of new processing techniques based on harnessing of biotechnology in different disciplines of textile industry. Techniques based on biotechnology using enzymes are advantageous for the following reasons: mild treatment conditions, replacing harsh chemicals, specificity of action, safe and easy to control, environmentally friendly, biodegradable, and are also economical [(1-5)]. There are continuous efforts to replace harsh chemicals with enzymes in textile processing. Cellulase is a commonly used enzyme for processes such as bio-finishing, bio-polishing and softening of cotton fabrics.

We undertake current work with a view to shed insight on science- based options that are feasible for application of cellulase enzymatic treatment and reactive dyeing to cotton fabrics within the realm of two step process for biotreatment and dyeing. Emphasis was placed on the impact of this biotreatment on the colour strength and other technical properties when thus treated fabrics were subjected to reactive dyeing. Biotreatment is carried out under different conditions including enzyme concentration, material to liquor ratio, pH, temperature and duration. The enzymatic effect was expressed as variation in the enzyme activity, loss in fabric weight, strength properties and colour strength in addition to overall fastness properties for selected samples. Another option was to use one bath process for performing the biotreatment and reactive dyeing. The third option involves a post treatment process where dyeing was carried out first followed by the biotreatment in a separate step.

2- Experimental

2.1. Materials

- Plain weave (1/1) scoured and bleached woven cotton fabric (130 g/m^3) was supplied by El-Amerya Company for Fine Spinning and Weaving, Alexandria, Egypt.
- Cellusoft Conc. L (with an activity 750 EGU/g) supplied by Novo-Nordisk- Denmark; imported by Port Said Company, Port Said, Egypt.
- Acetic acid, sodium carbonate and non-ionic detergent (Hostapal® CV, Clariant), All used chemicals were of reagent grade.
- Two different commercial reactive dyes were used:
- Levafix B Red E-2RN, (a mono functional reactive dye).
- Procion Yellow H-E3B, CI Reactive yellow120, (bi-functional reactive dye).

2.2. Methods

2.2.1. Dyeing

The cotton fabric samples were dyed -using Thermostatic Atlas Rotadyer- with Levafix B Red E-2RN, (a mono functional reactive dye) and Procion yellow H-E3B, CI Reactive yellow120, (bi-functional reactive dye), (2% each, owf), keeping a material to liquor ratio (M:LR) 1:20. Fabric samples were introduced into cups of Thermostatic Atlas Rotadyer followed by addition of the dye solution and agitation for 25min. at 40°C. Sodium chloride (50g/l) was then added and agitation continued for 40min. At this end sodium carbonate (20g/l) was added and the temperature raised to 60°C and dyeing continued for 60min. Washing of the dyed samples was effected, using cold water, followed by washing in a solution containing non-ionic detergent at 45°C for 30min. and finally, rinsed with water and dried.

2.2.2. Enzymatic treatment

Enzymatic treatment using cellulase was carried out as per three methods. The first method involved treatment of the cotton fabric with the enzyme prior to dyeing; hence it is called the pre treatment method. In the second method the enzyme is incorporated in the dyeing bath to effect concurrent dyeing and enzymatic treatment in one-step. The third method entailed dyeing first then subjecting the dyeings to the enzymatic treatment; this method is referred to as post treatment method. More details about the three methods are given under.

2.2.2.1. Pre-treatment method

Samples of cotton fabric were treated at the outset with aqueous solutions containing different concentrations of cellulase enzyme (1-5%, owf) using material to liquor ratio (1:10-1:50) at different pH's (3-8) using acetate buffer or sodium carbonate at different temperatures (20-80°C) for varying lengths of time (10-70min). After desirable time the temperature was raised to 100°C for 10 min to stop the enzyme action. Thus treated samples were then dyed with reactive dyes as described above.

2.2.2.2. One- bath process

In this method enzymatic treatment was preformed first followed by dyeing using the same bath. That is, the enzyme was allowed to interact with cotton cellulose and thus treated fabric was dyed using reactive dyes through introduction of the dye and its auxiliaries to the same bath. The fabric was then washed and dried as described above.

2.2.2.3. Post-treatment method

In this method cotton fabric samples were dyed first with the reactive dye, and then the dyed fabrics obtained were treated with enzyme. Following this, samples were subjected to washing. Dyeing, enzymatic treatment, washing and drying were carried out as described above.

2.3. Testing

2.3.1. Fabric weight loss due to cellulase treatment was recorded as dried samples weight loss. Samples were repeatedly dried at 105°C till constant weight. The sample was weighed after cooling in dissector. The following equation was used to calculate the weight loss (Wt. L %):

$$\textbf{Wt. L \%} = (W_1 - W_2/W_1) \times 100$$

Where: W_1 & W_2 are the weights of the fabric before and after treatment.

Wight loss was used for determination of bioactivity of cellulase.

2.3.2. Tensile strength and elongation at break

Tensile strength and elongation at break were monitored by the strip method according to ASTM method [11].

2.3.3. The color strength of dyed samples was evaluated by light reflectance technique using the Perkin-Elmer, UV/V Spectrophotometer (Model, Lambda 3B) [7] using the following Kubelka- Munk equation:

$$K / S = \frac{(1 - R)}{2R} - \frac{(1 - R_o)}{2R_o}$$

Where:

R = Decimal fraction of the reflectance of the dyed fabric.

R0= Decimal fraction of the reflectance of the undyed fabric.

K = Absorption coefficient.

S = Scattering coefficient.

2.3.4. Wrinkle recovery angle (WRA) was measured as per AATCC test method 66-1990[10]

2.3.5. The dyed samples were tested for fastness to washing, acid and alkaline perspiration, dry and wet rubbing and light fastness according to AATCC standard methods. The effect on the color of the test specimen was expressed and defined by reference to grey scale for staining and color change [6-10].

2.3.6. Scanning Electron Microscopy (SEM)

Surface characterization of selected fabric samples before and after being treated as per the aftertreatment process for enzymatic/ dyeing treatment were studied using a scanning electron probe microanalyzer (JXA-840A), Japan. The specimens in the form of films were mounted on the specimen stabs and coated with thin film of gold by the sputtering method. The micrographs were taken at magnification of 1000 using (KV) accelerating voltage.

3- Results and Discussion

3.1. Dependence of the enzymatic effect on conditions of biotreatment and the onset of this on the performance of reactive dyeings obtained thereof

As already indicated, cellulase enzyme was used in current work to expedite enzymatic effect. The term enzymatic effect is used here to account for the changes in the molecular structure, surface morphology in particular, of cotton fabric brought about by the cellulase enzyme as monitored by activity of the enzyme, loss in fabric weight, tensile strength and elongation at break, as well as the color strength of the enzymatically treated fabric when dyed with reactive dyes. Dependence of the enzymatic effect on essential factors affecting the enzymatic treatment was studied. Also studied was the onset of these factors on the color yield when the enzymatically treated fabric samples were dyed independently with two reactive dyes. The latter encompassed Levafix B Red E-2RN and Procion yellow H-E3B representing mono and bifunctional reactive dyes respectively.

3.1.1. Time of biotreatment

Table 1 shows the effect of time of biotreatment on the enzymatic effect, expressed as activity of the cellulase enzyme, loss in fabric weight, strength properties and color strength (K/S) of the enzymatically treated and dyed fabrics. As is evident the activity of the cellulase enzyme enhances marginally by prolonging the time of the biotreatment from 10 to 30 min. Thereafter the enzyme activity decreases slowly within the range studied.

It is logical that the first 30 min. of the biotreatment act in favour of: (1) transfer of the enzyme molecules from the aqueous phase to fibre phase, (2) adsorption of the enzyme molecule on the surface of cotton fabric, (3) bio catalysis of the surface hydrolytic reactions by the cellulase enzyme and (4) release and transfer of this enzymatic reaction products to the aqueous phase. These four steps may also be favoured after 30 min biotreatment but to a much lower extent. This is evidenced by maximum cellulase activity of (0.378 EGU/g) at 30 min. vis-à-vis a lowest activity of (0.230 EGU/g) at 70 min.

The decrement in enzyme activity after 30 min biotreatment may be interpreted in terms of partial inhibition of the cellulase enzyme under the abundance of the enzymatic reaction products in the aqueous phase as well as those redeposited on the fibre-fabric substrate. It is likely that such products resist complexation with the enzyme by virtue of their lower interaction with the cellulase enzyme. The bio hydrolyzed cellulose products - unlike the original cotton cellulose - lack the ability of attachment (binding) to the active sites of the enzyme thereby reducing the bio catalytic activity of the latter. As a result the cellulase enzyme activity decreases.

For more clarification, it is understandable that the binding and the catalytic groups of the cellulase enzyme combine with the cotton substrate to form an intermediate-enzyme complex [1]. Within this complex a series of atomic and electronic rearrangement take place followed by releasing of the enzyme along with several products of cotton decomposition. Leaving these products for longer periods of time (more than 30 min) seems to exert adverse effect on the activity of cellulase enzyme most probably for reasons cited above. Indeed, results of loss in fabric weight, strength properties and the color strength of the enzymatically treated fabrics are in conformation with this as shown under.

Table 1 signifies that the percent loss in fabric weight increases appreciably by increasing the time of the bio treatment from 10 to 30 min. whereby this loss attains a maximum value of 1.29% at 30 min. This is against a value of 0.76% at an enzymatic treatment time of 70min. The significant decrement in loss in fabric weight by prolonging the time of the enzymatic treatment beyond 30min. is rather substantiating the redeposition of the decomposition products of cotton cellulose of the cotton fabric. Once this is the case, the expected continuous loss in fabric weight would be compensated for by such redeposition. As already pointed out, the enzymatic decomposition products of cotton formed under the conditions used seem to have remarkable resistance to further attack by the cellulase enzyme.

Table 1 shows the effect of biotreatment time on the strength properties. Obviously, the tensile strength and elongation at break decrease by increasing the time of the biotreatment from 10 to 30 min. A loss of 9.5% in tensile strength is observed after 30min.bio treatment. No significant losses in tensile strength by prolonging the time of the biotreatment are observed thereafter. Similar trend is obtained with elongation at break which displays loss of about 28.8% after 30min.biotreatment. However, the effect of changes in the molecular structure of cotton on losses in strength properties of the cotton fabric cannot be ruled out. That is strength losses could be associated with bio-molecular scission of the cellulose chains of cotton under the hydrolytic attack of the cellulase enzyme on non - crystalline domains along with variation in the degree of orientation of the microfibrils of cotton fibre under the biotreatment conditions used.

Table 1 depicts the color strength (K/S) of cotton fabrics as a function of the bio treatment time when the fabrics were first subjected to enzymatic treatment for different times followed by reactive dyeing using bi-functional and mono functional reactive dyestuffs. As expected the former dye brings about much higher K/S values than does the latter dye by virtue of the double reactivity of the former. Alkaline hydrolysis of one reactive group in the bio-functional dye converts it to mono functional reactive dye in contrast with the reactive dye containing one reactive group which is converted to the inactivated form and behaves like a direct dye. This is observed with both untreated and enzymatically treated fabrics. Nevertheless the color strength (K/S) values are significantly greater in case of the treated fabrics than the untreated fabrics, irrespective of the dye used.

It is also observed that the time of the biotreatment within a range of 10-30min. acts in favor of the color strength. Longer times adversely affect the color strength. This is rather in accordance with the results of enzyme activity, strength properties and loss in fabric weight discussed above. Within the time range studied, profound changes in the molecular structure of the cotton cellulose of the fabric brought about by the cellulase enzyme under the conditions used seem to occur at 30 min- bio treatment.

Table 1: Effect of time of biotreatment using cellulase enzyme on major technical properties of the enzymatically treated and dyed cotton fabrics.

Time of biotreatment (min.)	Activity of cellulase enzyme (EGU/g)	Loss in fabric weight (%)	Tensile strength (Kg/F)	Elongation at Break (%)	K/S of reactive dyeings	
					Mono functional dye	Bi Functional dye
Untreated fabric	----------	------	63.00	24	4.00	6.06
10	0.320	0.82	58.35	23	4.22	9.36
20	0.353	0.89	57.35	17	5.27	9.43
30	**0.378**	**1.29**	**56.67**	**17**	**6.66**	**11.00**
40	0.280	0.99	56.87	17	6.43	10.88
50	0.256	0.93	56.88	17	5.20	10.68
60	0.237	0.78	56.89	16	5.17	9.00
70	0.230	0.76	56.90	15	5.00	8.88

Bio treatment conditions: [Cellulase enzyme], 1%; M: LR, 1:20; pH, 5; Temp, 50°C.
Dyeing conditions: [Reactive dye], 1%; M: LR, 1:20; [NaCl], 50g/l; [Na_2CO_3], 20g/l.

3.1.2. Temperature of biotreatment

Table 2 shows the effect of temperature of the bio treatment of cotton fabrics using cellulase enzyme on major technical properties of the cotton fabrics. Properties examined embrace activity of the enzyme, loss in fabric weight, mechanical strength and color strength of the enzymatically treated fabrics when the latter were dyed independently with the two reactive dyes under investigation.

Results of table 2 disclose that the activity of the cellulase enzyme increases significantly and reaches a maximum upon raising the biotreatment temperature from 20°C to 40°C. Raising the temperature further to 60°C is accompanied by decrement in the enzyme activity. More marginal decrement is observed upon elevating the temperature to 80°C. It follows from this that the cellulase enzyme activity is in full swing at 40°C when the biotreatment was carried out under the conditions employed in current investigation. This state of affairs is reflected clearly on all the technical properties examined. Maximum losses in both fabric weight and tensile strength occur at 40°C. Meanwhile the most appreciable value of color strength and elongation at break are obtained when the biotreatment was carried out at 40°C and the enzymatically treated fabrics were dyed with the reactive dyes. Differences in color strength among the two dyes used, were observed before and could be explained on similar lines.

Based on the above, it is clear that 40°C constitutes the most appropriate temperature for the biotreatment using cellulase enzyme under the conditions employed in the present work. The conditions used as a whole seem to function in favour of the cellulase enzyme with its three dimensional complex structure. For instance active sites of this enzyme including hollow spaces or columns containing binding and catalyzing groups would be promoted in such a way that their ability to bind the cellulase and cotton fabric in lock and key fashion can be easily fulfilled. Similarly, all ranges of microenvironments for all catalysis are facilitated. In short, a biotreatment temperature of 40°C along with other parameters specified in the footnote of table 2 allow the cellulase enzyme to fully operate as per the mode of enzymatic action reported in a recent review article [12].

Table 2: Effect of temperature of bio-treatment using cellulase enzyme on major technical properties of the enzymatically treated and dyed cotton fabrics.

Temp. of biotreatmnt (°C)	Activity of cellulase enzyme (EGU/g)	Loss in fabric Wight (%)	Tensile strength (Kg/F)	Elongation at Break (%)	K/S of reactive dyeing	
					Mono functional dye	Bi functional dye
Untreated fabric	----------	------	63.00	24	4.00	6.06
20	0.75	0.373	56.79	28	4.22	10.43
40	**1.40**	**0.485**	**55.00**	**28**	**6.80**	**11.55**
60	1.10	0.447	56.83	28	6.12	11.30
80	1.00	0.394	56.85	28	5.81	10.81

Bio treatment conditions: [Cellulase enzyme], 1%; M:LR, 1:20; pH, 5; Time 30min.
Dyeing conditions: [Reactive dye], 1%; M: LR, 1:20; [NaCl], 50 g/l, [Na_2CO_3], 20 g/l

3.1.3. pH of biotreatment

Table 3 shows the dependence of the enzymatic effect of cellulase on the pH of biotreatment. The latter was carried out at 40°C using cellulase at a concentration of 1% and a material to liquor ratio1:20. Enzymatic effect is expressed as enzyme activity, loss in fabric weight, strength properties and color strength of the enzymatically treated and dyed cotton fabrics. The results reveal that the enzyme activity increases by increasing the pH from pH 3 to pH5 then decreases sharply upon further increases to pH6 and pH 7. That is, the cellulase enzyme exercises its maximum activity at pH5 under the conditions used; below or above pH5 the activity of the cellulase enzyme decreases. Indeed, this state of affairs is directly reflected on the technical properties of the enzymatically treated cotton fabrics. The latter exhibit their maximum loss in loss in fabric weight and tensile strength at pH 5. Similarly substantial improvement in both elongation at break and color strength of the enzymatically treated dyed cotton fabrics could be achieved at pH 5.

Obviously, then, the activity of the cellulase enzyme under investigation determines the properties of the enzymatically treated cotton fabric and, this activity attains its maximal at pH5. At this particular pH and within the range and conditions studied, the changes in the active sites of the enzyme seem to be affected so that its binding to the surface of cotton fabric is accentuated. In combination with this is the effect of the charges away from the active sites, which are needed to create the active sites and keep the native structure of the enzyme.

Table 3: Effect of pH of bio-treatment using cellulase enzyme on major technical properties of the enzymatically treated cotton fabrics.

pH of bio treatment	Activity of cellulase enzyme (EGU/g)	Loss in fabric Wight (%)	Tensile strength (Kg/F)	Elongation at Break (%)	K/S of reactive dyeing	
					Mono functional dye	Bi functional dye
Untreated fabric	----------	------	63.00	24	4.00	6.06
3	0.80	0.87	58.10	26	5.50	10.91
4	0.99	0.91	58.11	26	6.00	11.00
5	**1.20**	**1.10**	**56.26**	**28**	**6.83**	**11.59**
6	0.71	0.78	57.70	29	6.80	11.50
7	0.51	0.63	56.72	28	5.40	10.80

Bio treatment conditions: [Cellulase enzyme], 1%; M: LR, 1:20; Temperature 40°C; Time 30 min.
Dyeing conditions: [Reactive dye], 1%; M: LR 1:20; [NaCl], 50g/l; [Na_2CO_3], 20g/l

3.1.4. Liquor ratio of biotreatment

Table 4 depicts variations of the enzymatic effect of cellulase enzyme with material to liquor ratio. Biotreatment of cotton fabrics was carried out at 40°C and pH 5 for 30 min. using 1% cellulase enzyme. It is seen that the activity of the cellulase enzyme is improved gradually by increasing the material to liquor ratio (M: RL) from 1:10 to 1:50. Further increase of M: RL to 1:60 decreases the activity of the enzyme. This is rather reflected on the enzymatically treated cotton fabrics. These fabrics exhibit the highest losses in both fabric weight and tensile strength upon using M: RL 1:50 whereby maximum activity of the enzyme is achieved. Enhancement in both elongation at break and color strength of the reactive dyeings, is also achieved at M: LR 1:50. However, dyeings obtained with the bifunctional dye display much higher color strength than the monofunctional dye as indicated above.

It is further noticed that the biotreatment using cellulase enzyme causes significant improvement in the color strength. This is the case regardless of the M: LR used (Table 4). Reasons for the improved susceptibility of cotton fabric by biotreatment are stated in the foregoing paragraphs.

The increased enzymatic effect by increasing the liquor ratio up to 50 is a manifestation of greater amount of the cellulase enzyme on the surface of the cotton fabric which, in turn, augments the availability of the active sites of the enzyme to attack the cotton cellulose of the fabrics. Higher liquor ratio (M:LR 60) seems to exert excess active sites of the enzyme due to greater availability of the latter on the fabric surface, a point which suggests that these extra enzyme molecule may not find vacant cellulose molecules to attack.

3.1.5. Cellulase enzyme concentration

The effect of cellulase enzyme concentration on its enzymatic effect, expressed as, activity of the enzyme, and the onset of this on loss in fabric weight and strength properties (tensile strength and elongation at break) as well on the color strength (K/S) of this enzymatically treated cotton fabrics dyed by the said reactive dyes. Cellulase enzyme concentration ranged from 1 to 6% and the biotreatment was carried out at 40°C for 30 min, and pH 5 using a material to liquor ratio1:50. Reactive dyeing of the obtained biotreated fabrics was performed using two reactive dyestuffs, namely, Levafix B Red E-2RN (a monofunctional reactive dye) and Procion yellowH-E3B, CI Reactive yellow120; (bi-functional reactive dye). Dyeings were thoroughly rinsed with running tap water then washed extensively as described in the experimental section.

Results of table 5 reveal that cellulase enzyme activity increases gradually from 0.87 to 0.94 by increasing the enzyme concentration from 1% to 5%. Increasing the enzyme concentration further to 6% leaves the enzyme activity unaltered. As a consequence, the enzymatically treated cotton fabrics display loss in fabric weight values which increase gradually by increasing the enzyme concentration up to 5% then levels off. The adverse effect of the increased activity of the enzyme by increasing its concentration on the tensile strength is also apparent. The tensile strength decreases from 59 Kg at enzyme concentration of 1% to 55.43 Kg at enzyme concentration of 6%. On the other hand, the elongation at break exhibits a value of 26% at 1% enzyme concentration, this is against a value of 29% at enzyme concentrations of 5%and 6%.

The above findings indicate that presence of increased amounts of the cellulase enzyme in the biotreatment medium causes progressive interactions in a key and lock fashion between the enzyme and the cotton cellulose of the fabric. And, in so doing, cotton cellulose chains undergo molecular scission at 1,4-β-glucosidic linkages thereby decreasing the degree of polymerization of cotton. This together with the loosening of the fabric structure by virtue of its loss in fabric weight would account for the decrease in tensile strength and the enhancement in elongation at break.

Table 5 discloses that the enzymatically treated cotton fabrics acquire much higher color strength than the untreated fabric. The colour strength is higher the higher the concentrations of the enzyme in the biotreatment till 5%concentration. Above this enzyme concentration the color strength decreases. The highest color strength (K/S=7.25) is attained when the fabric was pretreated with the enzyme at a concentration of 5% then dyed with the monofunctional reactive dye and a lower value (K/S=6.98) at similar pretreatment but using enzyme concentration of 6%. Similar results are obtained with the bifunctional reactive dye, reflecting the positive effect of the enzyme in enhancing the susceptibility of the cotton fabrics towards reactive dyeing as previously explained in the foregoing sections. The superiority of the bifunctional dye vis-à-vis the monofunctional dye was also discussed previously and can be tackled on similar lines.

Table 4: Effect of Liquor ratio of biotreatment using cellulase enzyme on major technical properties of cotton fabrics.

M:LR	Activity of cellulase enzyme (EGU/g)	Loss in fabrics weight (%)	Tensile strength (Kg/F)	Elongation at Break (%)	K/S	
					Mono functional dye	Bi functional dye
Untreated fabric	----------	------	63.00	24	4.00	6.06
1:10	0.322	0.21	56.79	26	5.60	10.50
1:20	0.340	0.55	56.79	26	6.50	10.63
1:30	0.348	0.56	56.79	28	6.53	10.76
1:40	0.400	0.69	56.78	29	6.70	10.98
1:50	**0.431**	**0.97**	**56.75**	**28**	**6.99**	**11.29**
1:60	0.352	0.43	56.81	28	6.99	11.29

Bio treatment conditions: [Cellulase enzyme], 1%; pH, 5; Temp., 40°C; Time 30min.
Dyeing conditions: [Reactive dye], 1%; M:LR 1:20; [NaCl],50g/l; [Na_2CO_3], 20g/l

Table 5: Effect of cellulase enzyme concentration on major technical properties of cotton fabrics.

Cellulase concentration	Activity of cellulase enzyme (EGU/g)	Loss in fabrics weight (%)	Tensile strength (Kg/F)	Elongation at Break (%)	K/S of reactive dyeings	
					Mono functional dye	Bi functional dye
Untreated fabric	----------	------	63.00	24	4.00	6.06
1%	0.87	0.20	59.00	26	6.55	10.65
2%	0.88	0.27	57.67	26	6.80	10.68
3%	0.88	0.27	57.33	28	7.00	10.98
4%	0.90	0.34	56.89	28	7.20	10.99
5%	**0.94**	**0.39**	**55.62**	**29**	**7.25**	**11.01**
6%	0.94	0.39	55.43	29	6.98	10.98

Bio treatment conditions: [cellulase enzyme], 1%-6%; M:LR, 1:50; pH, 5; Temp., 40°C; Time, 30min].
Dyeing conditions: [Reactive dye], 1%; M:LR, 1:50; [NaCl], 50g/l; [Na_2CO_3] 20g/l.

3.2. One- bath process for enzymatic Biotreatment and Dyeing (one-step process)

In the foregoing sections, factors affecting the biotreatment of cotton fabrics using cellulase enzyme then dyeing the enzymatically treated fabrics with reactive dyes were studied. The enzymatic biotreatment and dyeing were carried out in two separate steps and hence the method may be referred to as a two-step process.

In this section experiments were designed to establish a single-step process for enzymatic biotreatment and reactive dyeing; monofunctional and bifunctional reactive dyes were used. Two series of experiments were undertaken to establish such a process.

The first experiment involved treatment of the cotton fabric in an aqueous bath containing the cellulase enzyme 2% at 40°C for 50 minutes with agitation. After neutralization of the treatment bath and raising the temperature to 60 ^{o}C, the dye (2% OWF) was added followed by half NaCl dosing (25 g/l) for 15 minutes with continuous agitation. The other half of NaCl (25 g/l) dosing was exercised along with Na_2CO_3 first dose (10 g/l). The dyeing was continued for 15 minutes at 60 ^{o}C followed by addition of the second half of Na_2CO_3 (10 g/l). The so treated fabrics were then washed and dried.

Cotton fabrics processed according to the one bath process were monitored for the color strength, tensile strength and wrinkle recovery angle (WRA). The results obtained are set out in table6. Keeping aside the differences in values of color strength between the mono- and bifunctional dyes as they have been previously explained, there are other differences in tensile strength among samples processed as per the two experiments described above.

For a given dye, the color strength of fabric processed by the first experiment is higher than that of the second experiment, indicating that sequence of addition of the dye, salts (NaCl and Na_2CO_3) and enzyme plays a role in determining the color strength. The same holds true for the tensile strength, but with an opposite effect; the tensile strength obtained with fabric processed using the first experiment is lower than that of the second experiment. This could be interpreted in terms of difference in time elapsed at the temperature used during incorporation of each ingredient in both experiments.

Table 6: Color strength and other technical properties of cotton fabrics processed as per the one- bath process for enzymatic treatment and reactive dyeing.

Treatment	Mono Functional dye			Bi- Functional dye		
	WRA	K/S	Tensile strength Kg/ F	WRA	K/S	Tensile strength Kg/ F
Dyed fabric only	180	4	63	180	6.06	63
Fabric subjected to enzymatic and dyeing processing in one- bath (one- bath for enzymatic and dyeing fabric)	191	6	56	191	9	54

Conditions used: as detailed in the text.

3.3. Post-treatment method

In this method, reactive dyeing was first effected using mono- and bifunctional reactive dyes, then, the dyeings obtained were treated with cellulase enzyme in a subsequent step. These dyed-enzymatically treated fabrics were evaluated for color strength, tensile strength and WRA. The results obtained are given in table 7.

It is obvious that the values of retained tensile strength obtained with the two dyes used are equal. Similar situation is encountered with WRA. To our surprise the color strength, though differs with the mono- than the bifunctional dye for reasons cited before, increases by post treatment using cellulase enzyme. Biobolishing of the dyeings seems to accentuate the reflectance of dyeings by removing short cellulosic chains and hairness thereby enhancing the color strength.

Table 7: Color strength, tensile strength and WRA of dyed and enzymatically treated cotton fabric as per the post-treatment method

Treatment	Mono Functional dye			Bi- Functional dye		
	WRA	K/S	Tensile strength Kg/ F	WRA	K/S	Tensile strength Kg/ F
Dyed fabric only	180	4	63	180	6.06	63
Dyeing followed by treatment with cellulase	191	5.53	58	191	6.51	58

Dyeing conditions: [Reactive dye], 1%, M: LR 1:20, [NaCl]50g/l, [Na_2CO_3] 20g/l
Biotreatment conditions: [Cellulase enzyme], 1%-5%; M: LR, 1:50; pH, 5; Temp., 40°C, Time, 30min.

3.4. Comparison

The foregoing sections clarify that the three methods applied to expedite combined effect of reactive dyeing and enzymatic treatment using cellulase enzyme were successful. These methods include the pretreatment method where enzyme treatment was first carried out followed by dyeing; one bath for enzymatic treatment and reactive dyeing and; the post treatment method where dyeing was first effected followed by enzymatic treatment. A close examination of the results obtained pertaining to color strength, tensile strength and WRA would reveal that the pretreatment method is by far the best then comes the one bath method. The post treatment method occupies the last position in this order.

Unequivocally, the above order is a manifestation of the environment created during applications of each of the three aforementioned methods and, to what extent does this environment acts in favour of the interactions of the enzyme and/or the dye with the cotton fabrics. Allowing the cellulase enzyme to act upon the cotton cellulose of the fabric would bring about clean, soft and smooth fabric surfaces with more accessible structure for reactive dyeing. Such situation may also occur during the one bath method but to lesser degree as a result of concurrent enzymatic attack and dyeing. On the other hand, in the post treatment method, dyeing takes place without interference of the cellulase enzyme. Improvement of the color strength and decrease in tensile strength are due to the attack of the enzyme on the cotton fabric. The attack seems to comprise purification of the fabric surface (biopolishing) thereby enhancing the color strength; meanwhile this attack causes molecular degradation of the cotton thus decreasing the tensile strength.

3.5. Fastness properties

Table 8 shows the fastness properties of fabric enzymatically treated using cellulase then dyed with the two reactive dyes in question vis-à-vis those of the only dyed fabric. As is evident cellulase treatment improves fastness to washing and to light while leaving fastness to crocking and perspiration unaltered.

Table 8: Fastness properties of enzymatically treated fabrics using cellulase enzyme

Fabric	Dyestuff	Crocking		Acidic Perspiration		Alkaline Perspiration		Washing fastness		Light fastness
		Dry	Wet	St.*	St.**	St.*	St.**	St.*	St.**	40hr
Only dyed	Mono dye	3-4	2	2-3	2	2-3	2	2	2	2
	Bi-functional dye	3-4	3	2-3	2-3	2-3	2	3	2-3	3-4
Treated with Cellulase then dyed	Mono dye	3-4	2	2-3	2	2	2-3	3	2-3	2-3
	Bi-functional dye	3-4	3	2-3	3	2-3	2-3	2-3	3	3-4

St.** = staining on wool St.* = staining on cotton

3.6. Scanning Electron Microscopy

Figure 1-A shows SEM image of the fibres of the cotton fabric that has been only dyed with Procion yellowH-E3B. This sample is referred to as the untreated cotton fabric. The SEM image shows surface irregularities of the normal cotton fibre.

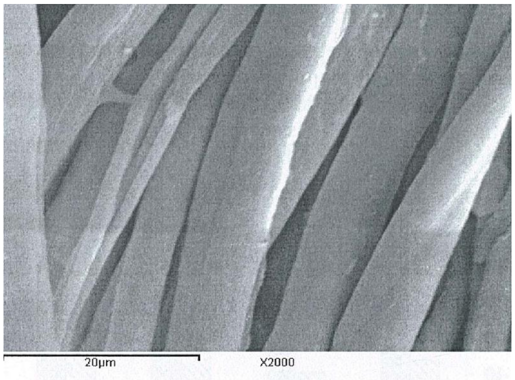

Fig. 1-A: SEM of fabric dyed only

Figure 1-B shows SEM image of the fibres of the cotton fabric that have been enzymatically treated with cellulase enzyme then dyed with Procion yellow H-E3B. As can be seen the enzymatic treatment smoothened the surface of the fibre with the certainty that the primary wall of the cotton fibre was the first target to the attack by the cellulase enzyme.

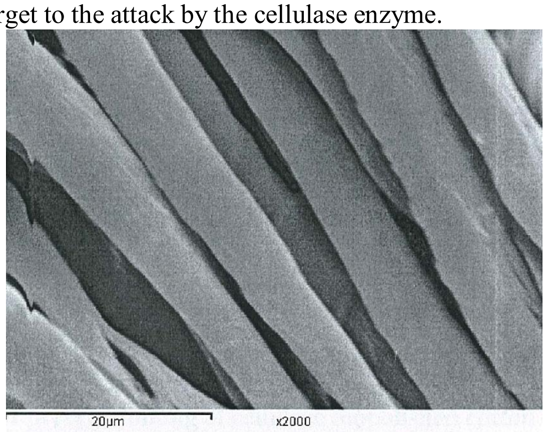

Fig. 1-B: SEM of cellulase treated cotton fabric, then dyed

Conclusion

Three science– based options were undertaken for application of cellulase enzymatic treatment and reactive dyeing to cotton fabrics. The first option was a manifestation of the successive treatments, namely, cellulase biotreatment followed by reactive dyeing. The second option involved performing both dyeing and biotreatment in one bath process through adjust of sequence of additions of ingredients pertaining to dyeing and biotreatment process. The third option, on the other hand, entailed treatment with the dye first then with the enzyme in a subsequent step. Mono and difunctional reactive dyes were used and the treated cotton fabrics were monitored for color strength, tensile strength, wrinkle recovery angle and overall fastness properties. The favorable effect of the enzymatic treatment on these technical properties was very significant when the first option was employed, then comes the second option while the third option occupied the last position.

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12/6/2013