Emre Karapinar,
Merih Ones Sariisik*

University of Manchester,
Institute of Science and Technology
Department of Textiles and Paper
PO Box 88, Manchester M60 1QD, United Kingdom
E-mail: E.Karapinar@postgrad.umist.ac.uk

***Dokuz Eylul University**
Department of Textile Engineering
35100 Bornova, Izmir, Turkey
E-mail: merih.sariisik@deu.edu.tr

Scouring of Cotton with Cellulases, Pectinases and Proteases

Abstract
*Cotton may contain between 4 and 12% by weight of impurities in the form of waxes, proteins, pectins, ash, and miscellaneous substances such as pigments, hemicelluloses and reducing sugars. These impurities are removed from the fabric by scouring, since their hydrophobic nature negatively affects the enhancement of the fabric's wettability and absorbency. In this work, pectinase, protease and cellulase were used in various combinations for different treatment times, either in the baths containing one enzyme or different enzyme combinations, in order to evaluate the effects of these enzymes on 100% cotton fabric's wettability and absorbency. At the end of the enzymatic and alkaline scourings, the wettability and absorbency properties of the garments were evaluated in terms of wettability, CIE*L, WI values and pectin analysis by Ruthenium Red dyeing. Furthermore, the effects of bioscouring on bleaching and dyeing were also investigated. At the end of the evaluation tests, it was found that in order to achieve adequate wettability and absorbency, cellulase + pectinase and cellulase + pectinase + protease gave better results than other enzymatic combinations.*

Key words: *cellulase, protease, pectinase, cotton scouring, wettability.*

Introduction

The cotton fibre is a single biological cell with a multilayer structure. These layers are structurally and chemically different, and contain approximately 10% by weight of non-cellulosic substances such as lipids, waxes, pectic substances, organic acids, proteins/nitrogenous substances, non-cellulosic polysaccharides, and other unidentified compounds included within the outer layer of the fibre [7,9-12]. These non-cellulosic materials create a physical hydrophobic barrier which protects the fibre from the environment throughout development; they provide lubrication during textile processing, and affect the enhancement of the fabric's wettability and absorbency [3,5].

The common industrial removal of these impurities is conventionally carried out by treating the fabric with sodium hydroxide. Although alkaline scouring is effective and the cost of sodium hydroxide is low, the process is costly because it consumes large quantities of energy, water, and auxiliary agents. The potential for the environmental contamination and depletion of natural resources is also serious. The strict pH and temperature requirements for alkaline scouring are damaging to many fibres [1,4-5].

A wide range of studies have been undertaken to understand the effects of enzymes in cotton bioscouring. These previous works include either only the use of one kind of enzyme, or a combination of proteases, pectinases, lipases and cellulases. The effect of different proteases on bioscouring was investigated by using fabrics boiled in water at 100°C by Hsieh and Cram [6]. This study was expanded by the direct scouring of cotton fabrics with proteases. It was concluded that the wettability results obtained after both studies were quite similar; furthermore, the researchers concluded that similar wettability properties could be achieved by using a lower amount of energy when compared to the use of pre-treated fabrics at 100°C water [12]. Hartzell and Durrant studied the effect of agitation in pectinase scouring, and concluded that agitation during scouring improves the fabric absorbency [5]. The effects of pectinase and cellulase treatment on cuticle properties were investigated by Li & Hardin [10], and the correlation between absorbency and the cuticle image of bioscoured fabric was explained. These researchers also studied the effects of surfactants, agitation, and enzyme selection, and concluded that the effects of surfactants and agitation depend on the enzyme structure and the characteristics of cotton fibre [11].

However, there has been no statistical approach to the fabric properties obtained after bioscouring. Keeping these concerns in mind, the goals of this paper are to compare the effect of different enzymatic combinations on different pre-treated fabrics, to find out the possible effect of reaction time on bioscouring, to control the effect of different bioscourings on the bleaching & dyeing properties of the bioscoured cotton fabrics, and to determine the optimum treatment conditions for bioscouring, with the aid of results obtained from SPSS statistical analysis.

Materials and Method

The three different pre-treated fabrics (weight 156 g/m^2) used in this research were 100% unscoured plain woven cotton fabrics, whose warp and weft yarn densities were both 24 per cm. The different pre-treatments applied were as follows: fabric A, which was only boiled with water at 100°C; fabric B, which was only de-sized with α amylase; and fabric C, which was both boiled with water at 100°C and de-sized with α amylase. The enzymes used in this study are described in Table 1.

According to Table 2, each different pre-treated fabric was treated with 6 different enzyme combinations for 3 different reaction times (30, 60, and 90 minutes), and these experiments were repeated 3 times. In total, 3×6×3×3=162 bioscouring experiments (pre-treatment variant; enzymatic combination variant; treatment time variant; repeat for each treatments) were carried out. In addition

Table 1. *Enzymes and their properties.*

Enzyme	Source or EC number	Temperature, °C	pH
α Amylase	Bacterial	65 - 70	6.2 - 6.8
Serin-type protease	*Bacillus*	60 - 70	8.0
Pectinase	EC 4.2.2.2	50 - 65	8.0 - 9.5
Cellulase	EC 3.2.1.4	45 - 50	4.5 - 5.5

Table 2. Enzymatic treatment conditions (a - treatments carried out in first bath; b - treatments carried out in second bath).

Parameter	Treatment conditions					
	Comb. 1	Comb. 2	Comb. 3	Comb. 4	Comb. 5	Comb. 6
Protease, g/l	2.0	-	2.0	-	2.0[b]	2.0[b]
Pectinase, g/l	-	0.2	0.2	0.2[b]	-	0.2[b]
Cellulase, g/l	-	-	-	4.0[a]	4.0[a]	4.0[a]
Wetting agent, g/l	0.5	6.0	6.0	1.0[a] – 6.0[b]	1.0[a] - 0.5[b]	1.0[a] – 6.0[b]
Sequestering agent, g/l	-	2.0	2.0	2.0[b]	-	2.0[b]
Reaction temperature, °C	70	55	60	50[a] - 55[b]	50[a] - 70[b]	50[a] - 60[b]
Reaction pH	8.0	8.0	8.0	5.5[a] - 8.0[b]	5.5[a] - 8.0[b]	5.5[a] - 8.0[b]
Liquor ratio	20:1	20:1	20:1	20:1	20:1	20:1

Table 3. Variation analysis of wettability values of bioscoured fabrics.

Variation source	Type (III) sum of squares	df (degree of freedom)	Mean square	F value	Significance level
Combination	17518.2	5	3503.7	880.4	0.00
Fabric	61.6	2	30.8	7.8	0.00
Time	31.8	2	15.9	4.0	0.02
Comb*Fabric	2099.3	10	209.9	52.8	0.00
Comb*Time	19.8	10	2.0	0.5	0.89
Fabric*Time	17.4	4	4.3	1.1	0.37
Comb*Fabric*Time	60.7	20	3.0	0.8	0.75
Error	429.8	108	4.0	-	-
Total	20238.7	161	-	-	-

to this, fabrics A, B, and C were also subjected to alkaline scouring, whose treatment conditions were 2%wt. NaOH, 2% soda, and 2% wetting agent at 90°C for 30 and 60 minutes with a liquor ratio of 20:1. Treatments including cellulase were carried out in two different baths. Both enzymatic and alkaline scourings were carried out in a Linitest, Atlas Machine with 42 rpm, whereas a CM-3600d Minolta Spectrophotometer was used in spectrophotometric measurements. The wetting agent used in this research was non-ionic Perlavin NIC, and the sequestering agent was Perlavin AHS, which were both supplied by Dr Petry, Istanbul.

After enzymatic and alkaline scourings, evaluation tests such as wettability (which was carried out according to DIN 53924), whiteness index (Stephensen), CIE*L, and pectin analysis with Ruthenium Red were carried out. The whiteness index was measured according to equation 1:

$$W = 2B - A \qquad (1)$$

where:

W - the whiteness index value,

B, A - remission values at 460 nm and 620 nm, respectively.

At the end of the evaluation tests, a statistical analysis of the results was carried out by a SPSS statistics program with a 95% confidence interval. Moreover, the effects of enzymatic scouring on bleaching and dyeing were also determined. All experiments were carried out at the Department of Textile Engineering of Dokuz Eylul University.

Results and Discussion

Wettability and absorbency properties

It is statistically understood from the variation analysis that the combination type, fabric type and reaction time had an effect on the wettability properties of the bioscoured samples (Table 3), since each variation source relating to these quantities had a significance level value below 0.05. When combinations were compared among each other, protease was the combination which caused a significant difference in wettability properties. In fact, the wettability values of the protease scoured of all fabric types (A, B, C) were the lowest. Similarly, when fabric types and treatment times were compared among each other, it was seen after bioscouring that the wettability properties of fabric A were statistically different from fabric B and C, whereas fabric B and fabric C did not show any difference. In the case of treatment times, it was found that the wettability values achieved after 60 min and 90 min reaction times had no statistical difference, whereas on the other hand the wettability values achieved after 30 min showed differences according to the enzymatic combination applied.

It was concluded from the wettability test and SPSS analysis that after the wettability and absorbency tests, although the wettability values of most of the samples had increased when compared with the control fabric (fabric B before bioscouring), the treatment of fabric B with cellulase + pectinase for 60 min was the most effective enzymatic treatment with respect to Figure 1. The wettability results were also supported by AATCC 39-1980, and all the combinations except protease had a wettability time of less than 1 second.

Whiteness index and CIE*L values

The whiteness index values (Stephensen) of fabrics were measured with respect to the view that although the removal of natural pigments in cotton fibre is achieved during the bleaching step, some of them can also be removed in scouring, since these pigments are adsorbed to the pectic and proteinic substances in cotton fibre. Thus, some increases in WI values were noted upon the removal of these pectic and/or proteinic substances from cotton fibre. Statistical analysis of the WI values of the bioscoured fabrics proved that combination type, fabric type and treatment time had an effect on the WI properties of the bioscoured samples (Ta-

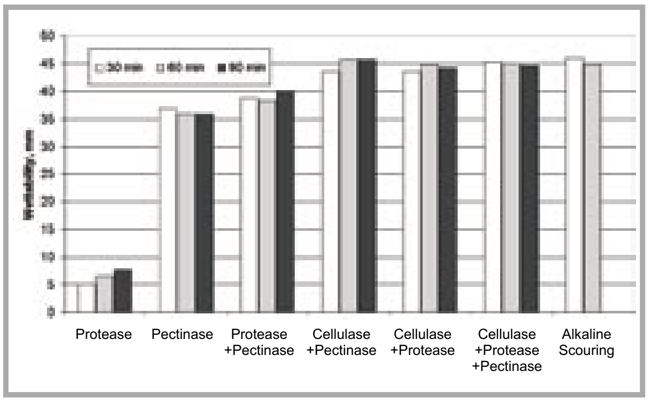

Figure 1. Wettability measurements in warp direction of fabric B (with respect to DIN 53924).

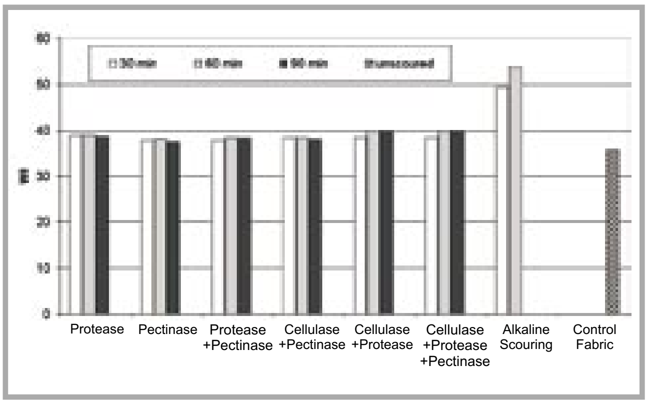

Figure 2. *WI values of fabric B after different enzymatic and alkaline scourings.*

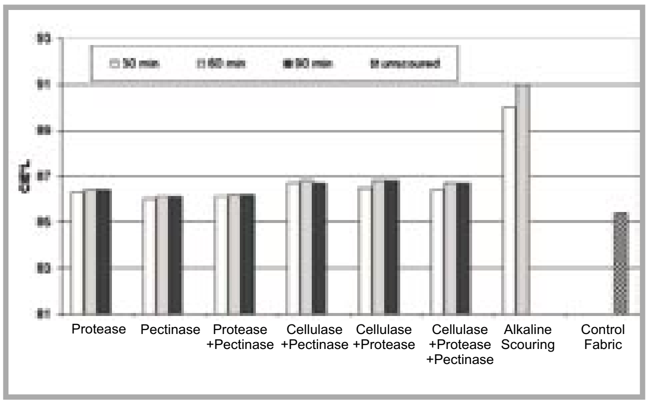

Figure 3. *CIE*L values of fabric B after enzymatic and alkaline scourings.*

ble 4). Combinations including protease resulted in different WI values. In addition, the WI values of fabric B and fabric C after bioscouring were statistically the same, whereas the WI values of bioscoured fabric A were statistically different and lower than those of fabric B and C. It was concluded that 60-min enzymatic scourings with cellulase+protease and only with protease caused the highest statistical increase in WI values among other enzymatic combinations, whereas alkaline scouring showed a significant rise when compared with the control fabric (fabric B before bioscouring) (Figure 2). Similar effects were obtained when the results were evaluated in terms of CIE*L (Figure 3).

Evaluation of pectin removal by Ruthenium Red dyeing

K/S is calculated as in equation 2, known as the Kubelka Munk formula, where R is the reflectance of a sample measured.

$$K/S = (1-R)^2/2R \qquad (2)$$

Since Ruthenium Red dyes only pectic and proteinic substances in cotton fibre, the lower the K/S value is, the less of the pectic and proteinic substance is present in cotton fibre. On this basis, the K/S values of enzyme- and alkaline-scoured fabrics after Ruthenium Red dyeing was measured at 540 nm, which was the wavelength at which maximum absorption was seen. Thanks to variation analysis, it was found that combination type, fabric type and treatment time had an effect on the K/S values of the bioscoured samples (Table 5). It was also concluded at the end of the Ruthenium Red dyeing that when the combinations were multiply compared among each other, the protease-scoured fabrics gave lower K/S values which were statistically different from the others; in addition, combinations including cellulase gave better results. In the case of fabric type, the K/S values of bioscoured fabric B and C were statistically the same. The K/S values obtained from 30- and 60-min bioscoured fabrics were the same; therefore there was a statistical difference between the ones obtained from 30- and 90-min bioscoured fabrics.

At the end of the measurements, the treatment of fabric B with cellulase + protease + pectinase for 90 minutes was seen to be the most effective combination of all the enzymatic combinations, whereas alkaline scouring was seen as just a more effective treatment than cellulase + protease + pectinase combination, but the difference between them was very small (Figure 4).

Effects of enzymatic and alkaline scouring on the bleaching and dyeing properties of scoured fabrics

At the end of the spectrophotometric measurements, it was noted that the type of enzymatic combinations did not affect the WI values of the scoured fabrics after bleaching. As can be seen in Figure 5, a significant difference was obtained between the WI values of enzyme-and alkaline-scoured fabric samples. However, the WI values of the bioscoured samples were already similar (Figure 2). After bleaching with peroxide, an increase in the WI values of each enzymatic combination was obtained. These increases were expected, due to the effect of hydrogen peroxide. The WI values of most combinations except protease treatment reached levels around 70; however, the

Table 4. *Variation analysis of WI values of bioscoured fabrics.*

Variation source	Type (III) sum of squares	df (degree of freedom)	Mean square	F value	Significance level
Combination	56.8	5	11.4	31.0	0.00
Fabric	85.2	2	42.6	116.4	0.00
Time	8.2	2	4.1	11.2	0.00
Comb*Fabric	2.7	10	0.3	0.7	0.70
Comb*Time	11.3	10	1.1	3.0	0.02
Fabric*Time	2.4	4	0.6	1.6	0.17
Comb*Fabric*Time	1.8	20	88.4	0.2	1.00
Error	39.5	108	0.4	-	-
Total	207.9	161	-	-	-

Table 5. *Variation analysis of Ruthenium Red dyeings of bioscoured fabrics.*

Variation source	Type (III) sum of squares	df (degree of freedom)	Mean square	F value	Significance level
Combination	1049.9	5	210.0	940.5	0.00
Fabric	2.7	2	1.3	5.9	0.01
Time	2.6	2	1.3	5.7	0.01
Comb*Fabric	0.9	10	0.1	0.4	0.93
Comb*Time	5.5	10	0.6	2.5	0.02
Fabric*Time	0.9	4	0.2	1.0	0.44
Comb*Fabric*Time	3.4	20	0.2	0.8	0.75
Error	12.1	54	0.2	-	-
Total	1077.7	107	-	-	-

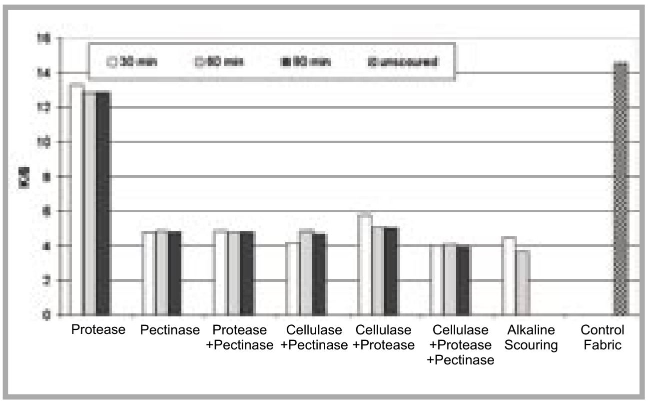

Figure 4. *K/S values of fabric B after Ruthenium Red dyeing.*

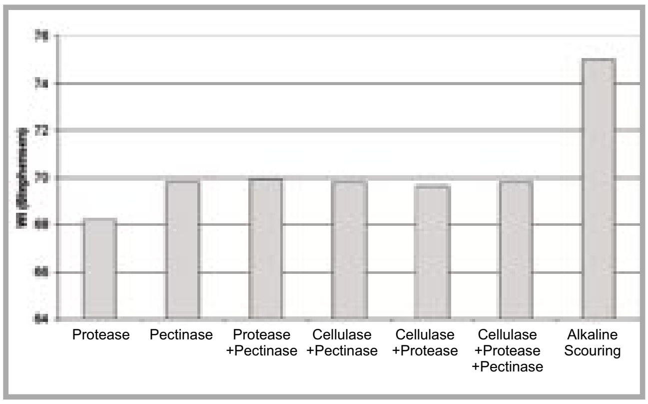

Figure 5. *WI values of fabric B after bleaching.*

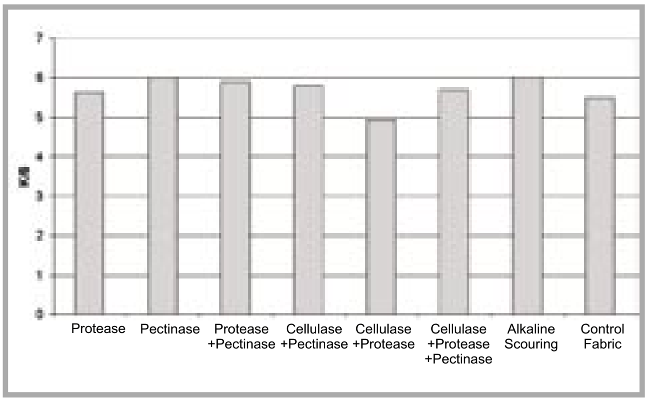

Figure 6. *K/S values of scoured fabrics B after reactive dyeing of 60 min.*

WI values achieved for the protease-treated fabric was just above 68. It can be assumed that the effects of different enzymatic scourings on bleaching are negligible.

In the case of the dyeing properties of fabrics after enzymatic and alkaline scourings, the K/S values of reactive dyed fabrics showed that removal of the pectic substances enhanced the dyeing properties of fabrics (Figure 6). The level of this enhancement differed depending on the amount of pectic substances removed. The hydrophobic characteristic of pectic substances prevented the uptake of the dye liquor in combinations where the amount of removed pectic substances was insufficient, as in the cellulase + + protease combination. The control fabric in Figure 6 refers to fabric B, which was de-sized only.

Conclusion

At the end of all the evaluation tests and their statistical analyses, it was noted that there were no statistical differences between the evaluation test results obtained from fabric B and fabric C. So, in order to prevent extra consumption of water and energy, de-sizing a fabric was alone enough for effective scouring. Furthermore, in the case of treatment times, 30 minutes of treatment time was in most cases insufficient for effective scouring when compared with reaction times of 60 and 90 minutes, whereas the evaluation test results obtained after 60 and 90 minutes were statistically the same. According to the evaluated results, combinations in which cellulase was present showed more improvements than the other enzymatic combinations. Most of the enzymatic scourings did not give better results than conventional alkaline scouring; however, the most similar results to alkaline scouring by means of wettability and pectin removal were achieved with cellulase + pectinase and cellulase + protease + pectinase.

References

1. G. Buschle-Diller, R. Radhakrishnaiah, H. Freeman, 'Environmentally Benign Preparatory Processes-Introducing a Closed-Loop System', National Textile Center Annual Report, 2001, C99-A07.
2. E. Csiszar, G. Szakacs, I. Rusznak, Combining Traditional Scouring with Cellulase Enzymatic Treatment. Textile Research Journal, Vol. 68, No. 3, 1998, 163-167.
3. J.N. Etters, Cotton Preparation with Alkaline Pectinase: An Environmental Advance. Textile Chemist and Colorist & American Dyestuff Reporter. Vol. 1, No. 3, 1999, 33-36.
4. M. Hartzell, Y.L. Hsieh, Enzymatic Scouring to Improve Cotton Fabric Wettability. Textile Research Journal, Vol. 68, No. 4, 1998, 223-241.
5. M. Hartzell, S.K. Durrant, The Efficiency of Pectinase Scouring with Agitation to Improve Cotton Fabric Wettability. Textile Chemist and Colorist & American Dyestuff Reporter, Vol. 32, No. 8, 2000, 86-90.
6. Y. Hsieh, L.A. Cram, Proteases as Scouring Agents for Cotton. Textile Research Journal, Vol. 69, No. 8, 1999, 590-597.
7. S.R. Karmakar, Application of Biotechnology in the Pre-treatment Processes of Textiles. Colourage Annual, 1999, 75-84.
8. C. Li, C.M. Ladisch, M.R. Ladisch, Pore Characterization of Cellulase Treated Cotton Fabric. Textile Research Journal, Vol. 71, No. 5, 2001, 407-414.
9. Y. Li, I.R. Hardin, Enzymatic Scouring of Cotton: Effects on Structure and Properties. Textile Chemist and Colorist & American Dyestuff Reporter, Vol. 29, No. 8, 1997, 71-76.
10. Y. Li, I.R. Hardin, Treating Cotton with Cellulases and Pectinases: Effects on Cuticle Properties. Textile Research Journal, Vol. 68, No. 9, 1998, 671-679.
11. Y. Li, I.R. Hardin, Enzymatic Scouring of Cotton- Surfactants, Agitation, and Selection of Enzymes. Textile Chemist and Colorist & American Dyestuff Reporter, Vol. 30, No. 9, 1998, 23-29.
12. C.H. Lin, Y.L. Hsieh, Direct Scouring of Greige Cotton Fabrics with Proteases. Textile Research Journal, Vol. 71, No. 5, 2001, 425-434.
13. A.K. Sarkar, J.N. Etters, Kinetics of Enzymatic Hydrolysis of Cellulose. AATCC Review, No. 3, 2001, 48-52.
14. M.K. Traore, G. Buschle-Diller, An Environmentally Friendly Scouring Processes. Textile Chemist and Colorist & American Dyestuff Reporter, Vol. 32, No. 12, 2000, 40-43.

Received 21.11.2003 Reviewed 08.04.2004