

Performance Evaluation of Exotic and Indigenous Neutral Cellulolytic Enzymes as Denim Stone Wash Substitute

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(Received 20th August, 2005, revised 8th November, 2005)

Summary: Stone washing is an important process in the denim finishing or garment wet processing of denim industry. The stone wash effects are produced by the use of cellulases enzyme or pumice stones, individually or partially. The present paper investigates the influence of cellulase enzymes wash treatments under various levels of time and liquor concentration on physical properties (weight loss and strength) of denim fabric. Overall moderate dose and time is found most suitable for the end use requirement. As regards to the enzyme type, Ultima LV 100 (Denykem) retained maximum strength, Cellulases U (Department of Biochemistry, University of Agriculture, Faisalabad) and Cellulases N (Department of Industrial Bio-Technology, NIBGE, Faisalabad) showed satisfactory performance for warpwise and fillwise tear strength respectively as well as regarding weight loss.

Introduction

Denim is an all time favorite fabric and the denim jeans are the world's most popular, versatile garments, particularly the denim jeans with old look or washed-out jeans with traditional dark blue appearance are very famous. The popular washed down or worn look in denim jeans is usually obtained by the use of pumice stones that are soaked in sodium hypochlorite or potassium permanganate (oxidizing agents). When jeans are tumbled with these bleach soaked stones, the dye is destroyed whereas the stones are rubbed against the fabric. Since the decomposition of dye occurs in a random manner, a distinctive "look" is obtained. Needless to say, the chemicals used in the process are by no means environment friendly. In the 1980's it was discovered that the same "look" could be obtained by the use of cellulase enzyme.

The production of "aged" denim garments with cellulases is the most successful enzyme process that has emerged in the textile industry in the last decade. With the advent of finishing treatments of stone washing and enzymatic stone washing it has become more acceptable over the last few years [1]. The faded look traditionally produced by rubbing the dyed garments against pieces of a soft stone like pumice is now being augmented by action of cellulase enzymes. In fact the enzymatic hydrolysis has found wide spread textile use in three areas; desizing, stone washing, and bio-polishing. [2-4].

The treatment of cellulase and protease enzymes changes the physical properties of the fabric (for example fabric strength and weight) [5]. Influence of enzymatic treatment on different characteristics of cotton by selecting multiple variables has also been reported [6,7]. While Hartzell and Hsieh [8] studied the ability of pectinase, cellulase, protease and lipase enzymes to improve the surface wetting properties of raw and pretreated cotton fabrics, and evaluated that Pectinase and Cellulase significantly improve water wetting and retention properties. Cellulases treatments on scoured fabric further enhance water wetability and whiteness, but with significant reduction in fabric weight, thickness and strength.

The present study explores the performance of some imported and locally produced cellulase enzymes to achieve aging set up cotton denim jeans, under variant doses of enzymes and treatment times.

Results and Discussion

Weight of Fabric after Treatment (Weight loss)

The data regarding the effect of four types of neutral cellulolytic cellulases enzymes (E), three different dose levels (D), and three treatment times (T) of weight loss, subjected to analysis of variances, reveals highly significant effects of all sources of variances (Table-1). The comparison of individual means (Table-2) indicates that the effect of different

Table -1: Analysis of variance for physical properties of denim fabric

Sources of variance	Degrees of Freedom	Sum of Squares			F. Value		
		Tear (w)	Tear (f)	Weight	Tear (w)	Tear (f)	Weight
Enzyme (E)	3	2577035.9	24332845.	7.057	123.147**	1788.79**	5195.463**
Dose (D)	2	12962174.	9739123.3	11.96	929.125**	1073.93**	13209.55**
Time (T)	2	10661495.	9332935.8	9.685	764.213**	1029.14**	10695.76**
E x D	6	3355641.1	3476514.4	0.153	80.17**	127.785**	55.223**
E x T	6	3223990.2	3129095.2	0.21	77.032**	115.015**	77.313**
D x T	4	692925.55	566878.33	0.22	24.834**	31.255**	121.493**
E x D x T	12	1188408.8	1518777.2	0.309	14.197**	27.913**	57.433**
Error	148	1032370	671080	0.067			

** Highly Significant at 5% level of probability

Table-2: Individual Comparison of Treatment Means for Physical Properties of Denim Fabric

Enzyme	Tear strength		Weight of fabric (oz/yd ²)	Dose	Tear Strength		Weight of fabric (oz/yd ²)	Time	Tear Strength		Weight of fabric (oz/yd ²)
	(warp)	(fill)			(warp)	(fill)			(warp)	(fill)	
E ₁	6071 C	5009 B	13.46 D	D ₁	6374 A	5170 A	14.00 A	T ₁	6375 A	5133 A	13.95 A
E ₂	8242 A	5286 A	13.48 C	D ₂	6163 B	4820 B	13.63 B	T ₂	6111 B	4885 B	13.67 B
E ₃	6131 B	4280 D	13.85 B	D ₃	5729 C	4606 C	13.37 C	T ₃	5780 C	4677 C	13.38 C
E ₄	5911 D	4886 C	13.88 A								

Note: Means within each column not followed by the same letter are significantly different at 5% level of probability as judged by DMR test

cellulases enzymes E₁ (DeniMax BT), E₂ (Ultima LV100), E₃ (Cellulase U) and E₄ (Cellulase N) upon fabric weight is highly significant and their mean values are recorded as 13.46, 13.48, 13.85 and 13.88 ounces per square yard respectively. The results show that E₄ retained maximum weight of fabric after treatment (only 3.885 % weight loss), while E₁ revealed minimum fabric weight (maximum weight loss).

The decrease in the weight of fabric after enzyme wash treatment may be due to the cellulolytic action of cellulase enzyme on 1-4 β glucosidic bond between cellulose chains. The hydrolysis of cellulose weakened the yarn and especially protruding fibre and by the action of agitation and fabric-to-fabric abrasion with each other and with the tumbler drum, the fabric weight reduced [9].

The individual comparison of treatment mean values under variant time applied, elaborated in the Table-2, describes that T₁ (20mins) recorded the maximum fabric weight after treatment with the mean value 13.95 ounces per square yard, followed by T₂ (40mins) and T₃ (60mins) with their mean values 13.67 and 13.38 ounces per square yard respectively. At minimum time (T₁) maximum weight of fabric is recorded with only 3.39 % weight loss, while T₃ revealed minimum weight of fabric with 7.34 % weight loss as compared with 14.44 ounces per square yard weight of control specimen.

The trend is evident that as the treatment time increased the weight loss of the denim fabric increased. When a cellulolytic enzyme was given more time for action, its glucosidic bond breaking efficiency also increased, hence more reduction in weight observed.

The statistical comparison of individual mean regarding the effect of enzyme dose on weight of fabric (Table-2) revealed that D₁ (200 g) recorded maximum weight of fabric with the mean value 14.00 ounces per square yard (3 % weight loss) followed by D₂ (400 g) and D₃ (600 g) with their mean values 13.63 and 13.37 ounces per square yard (5.61 % and 7.41 % weight loss) respectively. These values depict a trend that as the dose quantity increased the weight of fabric decreased. When dose and time increase, the chances of cellulolytic reaction also increase which degrade the 1-4 β glucosidic bond and weaken the cellulose, hence resulted more weight loss during tumbling. Many researchers [6, 9-10] agree that cellulolytic enzyme degrade and hydrolysis the 1-4 β glucosidic bond of cellulose chains of cotton.

Tear Strength (warp direction)

The analysis of data (Table-1) of tear strength (warp direction) of the specimen as observed under the effect of four types of neutral cellulolytic cellulases enzymes (E), three different dose levels (D), and three treatment times (T) reveals highly signifi-

cant effects for all sources of variances viz, enzyme, dose, time, second order interaction as well as third order interactions. The overall co-efficient of variation of experimental data on tear strength in warp direction was as low as 1.37 %, which means that the process was well controlled.

The statistical comparison of individual means with regard to warp wise tear strength, presented in Table-2 depicts that E₂ recorded maximum tear strength in warp direction with the mean value of 6242 g followed by E₃, E₁ and E₄ with their mean values 6131, 6071 and 5911 g respectively as compared with control (untreated) specimen fabric's strength i.e. 6875 g. This may be due to the fact that cellulolytic activity of E₄ was maximum and it degraded the 1-4 β glucosidic bond more efficiently than the other three enzymes, hence recorded minimum tear strength (warp direction). The loss of warp wise tear strength was minimum in case of E₂ (9.21 %), while in case of E₄ the tear strength loss was maximum (14.02 %).

Anticipations attributing to treatment for time given in Table-2 reveals that T₁ (20 mins) recorded maximum warp wise tear strength with the mean value 6375 g followed by T₂ (40 mins) and T₃ (60 mins) with their mean values 6111 and 5780 g respectively. The trend of the results shows that as the treatment time increased the warp wise tear strength decreased. The prolong exposure of denim fabric to cellulolytic reaction raised degradation when time increase.

Results pertaining to dose quantity inferred that the dose D₁ (200 g) recorded maximum warp wise tear strength with the mean value 6374g (7.29 % strength loss) followed by D₂ (400 g) and D₃ (600 g) with their mean values 6163 and 5729g (10.36 % and 16.67 % strength loss) respectively. The trend is evident i.e. higher dose recorded maximum strength loss.

Tear Strength (fill-direction)

The analysis of variance regarding the tear strength in fill direction of denim fabric under the effect of four types of neutral cellulolytic enzymes (E) which are DeniMax BT, Ultima LV100, Cellulases U and Cellulases N under varying treatment times (T) and dosages (D) reveals highly significant effects of all sources of variances viz E, D, and T and second order interaction as well as third order

interaction as evident from Table-1. The over all co-efficient of variation of experimental data on tear strength in fill direction was as low as 1.38 %, which shows that the process was well controlled.

The comparison of individual means with regards to tear strength in fill direction given in Table-2, indicated that E₂ recorded maximum tear strength in fill direction with the mean value of 5286g(8.15 % tear strength loss). Followed by E₁, E₄ and E₃ with their tear strength in fill direction 5009, 4886 and 4280g (12.96 %, 15.10 % and 25.63 % strength loss) respectively, at same abrasion level. Different enzymes (cellulolytic nature) show different level of activity hence cause different level of tear strength and weight loss.

The individual mean with regard to the tear strength (fill direction) of denim fabric treated with different treatment times, T₁, T₂ and T₃ are recorded as 5133, 4885 and 4577 g (10.81%, 15.12 % and 20.47 % strength loss) respectively. These results indicated the trend that as the treatment increased fill wise tear strength decreased and vice versa. This may be due to the reason of prolonged exposure of denim fabric to cellulolytic reaction which raised degradation when time increase.

The Table-2 revealed the effect of dosage treatment on the fill wise tear strength. The D₁ (200 g) recorded the maximum fill wise tear strength with its mean value of 5170g (10.17 % tear strength loss), followed by D₂ (400 g) and D₃ (600 g) with their mean values of 4820 and 4606g (16.25 % and 19.97 % tear strength loss) respectively. These values show that dose treatment and fill wise tear strength has inverse relationship. As the dose increased the tear strength decreased and vice versa. Regardless of enzymes types, when denim fabric is treated with cellulase enzyme a degradation reaction occur and breakdown the strength and weight of fabric. The loss of strength is due to the fact that cellulolytic nature enzyme degrade and hydrolysis the 1-4 B glucosidic bond of cellulose chains of cotton [10-12].

Experimental

The research work was carried out at the Departments of Fibre Technology in collaboration with the Departments of Biochemistry, University of Agriculture, Faisalabad and Industrial Biotechnology Division, Nuclear Institute for Biotechnology & Genetic Engineering (NIBGE), Faisalabad. While all

the treatments and processing work was conducted at Crescent Greenwood Ltd. Pindi Bhattain, Dist Hafizabad, Pakistan.

The study was carried out in three phases viz., desizing, enzymatic stone washing and determination of physical characteristics of the denim fabric samples. The denim (untreated) fabric used in this proposed study having 76.5 kg tensile strength; 6880 grams tear strength (warp); 5780 grams tear strength (fillwise) and 14.4 oz/yd² weight was selected for the experiment. The denim fabric samples were desized (removal of sizing material) with Aquazym (desizer) at the liquor ratio 1:16 and marinating temperature of 140 °F [9, 13].

Enzymatic stone washing

Neutral cellulolytic enzymes were used for enzymatic stone washing of denim fabric. The sources and brand names of cellulases enzymes used in the experiment are as follows:

- E1 = Denimax BT (Novozymes)
- E2 = Ultima LV 100 (Denykem)
- E3 = Celulases U (Department of Biochemistry, University of Agriculture, Faisalabad)
- E4 = Cellulases N (Department of Industrial Bio-Technology, NIBGE, Faisalabad)

The range of time and concentration or dosage given to the cotton denim fabric in the experiment is listed below.

Recipe and Procedure

Enzyme Dosage (D)	=	D1:200; D2:400; and D3:600 g/lit.
Time (T)	=	T1:20; T2:40, and T3:60 minutes
Acetic acid	=	80 ml + 250 ml
Wetting agent (Foryl CP)	=	50 ml
Softener (Belfacin)	=	300 ml
Liquor ratio	=	1:11

Eight kilograms desized leg tubes were loaded in the UniMac tumbler machine at medium water level (26 gallons) and the temperature was adjusted at

140 °F (60 °C). After approaching 140 °F (60 °C), acetic acid (80ml) and cellulase enzyme were added at every possible combination of enzyme, dose and time (E x D x T). After required time, the bath was drained and the load was given a 2mins rinse at high water level at 120 °F (49 °C) and then extracted at 495 machine rpm.

After extraction the load was filled in the UniMac washer for softener application. The load was given 50 ml wetting agent for 5mins at medium water level and 120°F (49 °C) temperature then the bath was drained. The fabric was again given a 2 min rinse at high water level and 120 °F (49 °C) temperature and bath was drained again. Then, the softener (300 ml) was applied with 250 ml acetic acid at high water level and 120 °F (49 °C) temperature for 5mins. The bath was then drained and load was extracted at 495 machine rpm and then dried at 101 °C for 40mins [9,13-14].

Acetic acid was used to maintain the pH in between 5.5 ~ 6.5. Foryl CP was added to increase the wettability of fabric and Belfacin to give a soft texture and handle to fabric.

Determination of Physical Characteristics of Fabric

The weight of the fabrics was determined by LSEMA Test Method 10/ISO 3801. This procedure is to determine the weight per unit area of fabric in unwashed and or washed conditions. After calibrating the balance, the circular cut were weighed in grams to the nearest 0.01 g. The tearing strengths (warp-wise & fill-wise) were determined according to ASTM Standard method [15]. This method describes a procedure for the determination of the average force required to propagate a single rip tongue-type tear starting from a cut in a woven fabric by means of falling pendulum Elmendorf Apparatus.

Analysis of Data

The data thus obtained was analyzed statistically using Duncan's multiple Range Test [16], by using Mstat computer statistical program [17].

Conclusions

Different types of neutral cellulolytic enzymes, quantity of dose and treatment time, recorded significant effects upon physical properties (weight loss and tear strength) of cotton denim fabric. At minimum dose and time, the fabric retained

maximum strength and minimum weight loss, but the abrasion (aging effect) at this level was the least. On the other hand when time and dose of the treatments was raised, the abrasion increased but at the cost of strength and weight loss. Therefore overall moderate dose and time is most suitable for the end use requirement are economically less expensive. As regards to the enzyme type, Ultima LV 100 (Deny-kem) retained maximum strength, Cellulases U (Department of Biochemistry, University of Agriculture, Faisalabad) and Cellulases N (Department of Industrial Bio-Technology, NIBGE, Faisalabad) showed satisfactory performance. Therefore more efforts should be done to explore domestically prepared Cellulase in order to save the foreign exchange of the country

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