Analysis of Enzyme Washing of Denim using Experimental Design

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ABSTRACT

Denim fabric with its wide acceptance has been subjected to various kinds of finish effects by processors, the worn out look being the most prominent. The process of imparting this fading effect has also evolved with time, particularly with use of enzymes as an option. In this context, herein three denim fabrics are treated with a modified neutral cellulase to study the wash down effect. Two of the fabrics are indigo vat dyed, one in light blue shade and the other dark blue shade, while the third one is of sulphur black. The enzyme wash was done by varying the concentration of enzyme, time of treatment and mechanical agitation. To reduce the number of trials, Box Behnken design of experiment was used and the process in the design region was optimized by ridge analysis. Other than studying the effect of process parameters, back staining during the enzyme action was also evaluated. The extent of colour fading was checked by finding the colour strength in terms of K/S (Absorption/ Scattering) values, while surface fibre removal due to cellulase action was determined by weight loss method. The trends and optimized conditions were observed to be dye and shade specific with reasonable coherence. Although back staining was not found much in the vat dyed fabrics but it was of prominence in sulphur black, possibly due to redeposition of the dye.

Keywords: Back staining, cellulase, colour strength, denim, enzyme, indigo, sulphur black

INTRODUCTION

Denim has become a popular apparel material across the world, because of a distinct advantage of durability and comfort. The international appeal for this cotton twill fabric is among all age groups. With many variants of the fabric coming in, it has become almost synonymous for high fashion garments. Indigo is widely used in denim dyeing, as it gives the characteristic blue colour to denim. Natural indigo has now been completely replaced by synthetic indigo. Conventionally, the warp threads of the fabric are dyed with the vat colour, while weft yarns remain plain white. Moreover, the warp is ring dyed, with a partial penetration of the dye in fibre. Consequently, the dye fades and abrades easily and this is what is required for getting the worn out look. The changing fashion trends have however led to use reactives, direct dyes and even pigments for dyeing denim [1]. Again, sulphur dyes are of low cost and can be applied on denim, giving more natural look than direct and reactive colours. They have a soft, dull appearance, allowing versatile wash down effects in laundering [2].

As regards washing of denim, use of pumice stones to accelerate the colour fading effect was followed for quite a period. But it was found to have destructive effect on equipment. In addition, these stones get entrapped in pockets of denim garments and need to be removed manually, thus increasing labour and production cost. Also, the stone particles and grit cause serious effluent problem. Owing to these reasons, cellulase enzymes were introduced as a benign alternative. The primary use of enzymes is to promote hydrolysis of specific substrates, thus converting water-insoluble material to products that dissolve in water and can be washed away [3-5]. Cellulase treatment on cotton denim fabric reduces fuzz and pills, increases softness and smoothness other than imparting the wash down effect. These cellulases, can again be acid stable or neutral stable, the former being more aggressive. Neutral cellulase has the advantage of less weight loss and higher strength retention. In fact, in the present work, a modified neutral cellulase has been used, on two differently dyed indigo denim and one sulphur black dyed denim. The laboratory scale treatments were done varying the concentration of the enzyme

and time of treatment. Standard steel balls were also used in the bath to make the treatment rigorous. The process parameters were changed based on design of experiment.

MATERIALS AND METHODS

Materials

Grey denim fabrics of three different colours were taken for the study. The fabrics of 2/1 twill with 7s warp count and 10s weft count had following specifications.

Light Blue indigo dyed: epi 49; ppi 49; gsm 200

Dark Blue Indigo dyed: epi 49; ppi 51; gsm 210

Sulphur Black dyed: epi 50; ppi 51; gsm 220

These classes of dyes are mostly used in industrial production of denim and hence were chosen for investigation. The enzyme used for the denim washing is a modified neutral cellulase, called Cellusoft CR, from Novozymes. For desizing of the sized warp yarn Invazyme ADC of Huntsman (now Archroma) was used while acetic acid used was of L R grade. A bleached plain weave cotton fabric with 40s count yarn was used for back staining.

Methods

In all the denim qualities, fabric was first desized with 5g/l of the amylase enzyme at 60-65^{0}C for 60 min. The pH was maintained between 5 and 6 using acetic acid while MLR was kept 1:30. After desizing, the enzyme was deactivated by raising the temperature of water bath to 80^{0}C for 10 min. Then fabric was rinsed with warm water followed by cold water. It was finally dried in an oven and checked for size removal with Iodine drop test. After passing the test, fabric was taken for further treatments.

Enzymatic fading

The fabric samples were treated with the cellulase enzyme under recommended temperature of 40-55^{0}C and pH 5.5 to 7. The enzyme in ready to use form, was applied in different concentrations with varying parameters like time and mechanical agitation. The denim fabrics as mentioned were of different colours and enzymatic fading action was supposed to differ from fabric to fabric. The enzyme was applied at concentrations of 0.5, 1.25 and 2% and treatment time was 30, 45 and 60 min. Desized samples were given the treatment in Infra red dyeing machine at 45 rpm with required number of steel balls. An MLR of 1:30 was maintained while pH of the bath was adjusted with acetic acid. After the specified time of treatment, hot and cold wash were given followed by soaping with anionic detergent, Lissapol D 1g/l at 80^{0}C for 15 min. Then hot and cold wash were given and finally samples were dried.

Experimental design

To study the individual and interactive effects of the concentration of enzymes, treatment time and mechanical agitation and optimize the results, Box-Behnken design was used. These three variables were chosen as they were expected to potentially affect the fading process. The limits of each variable were chosen based on the recommended conditions and preliminary investigations. Colour strength, weight loss percentage and back staining values were taken as response factors. The three levels of the variables and the set of experimental combinations are shown in following tables (Tables 1, 2). The contour plots obtained from the values in the observation tables were used for interpretation. Further, ridge analysis was used for optimization of values in the experimental design region.

Table 1: Experimental conditions for the enzyme fading

Variables	**Coded levels**		
	-1	0	+1
Concentration (%)	0.5	1.25	2
Time (min)	30	45	60
Mechanical agitation (Number of steel balls)	0	5	10

Table 2: Experimental design based on Coded levels

Sample	**Enzyme concentration**	**Time**	**Mechanical Agitation**
E1	-1	-1	0
E2	1	-1	0
E3	-1	1	0
E4	1	1	0
E5	-1	0	-1
E6	1	0	-1
E7	-1	0	1
E8	1	0	1
E9	0	-1	-1
E10	0	1	-1
E11	0	-1	1
E12	0	1	1
E13	0	0	0
E14	0	0	0
E15	0	0	0

Test methods

The extent of fading was determined by measuring the colour strength of the treated samples in Premier Colorscan SS 5100A spectrophotometer. The measurement was done using the formula

Colour strength, $K/S = (1-R)^2/2R$

Where R = Reflectance

The effect of enzyme on cellulose was tested by finding the %age weight loss using the following simple calculation.

$$\text{Weight loss \%} = \frac{(\text{Weight before washing} - \text{Weight after washing}) \times 100}{\text{Weight before washing}}$$

Since enzyme action on denim often causes undesirable back staining, the intensity of the staining was also measured in terms of K/S.

RESULTS AND DISCUSSION

Effect of Modified cellulase on light blue indigo dyed denim fabric

The impact of process parameters namely, enzyme concentration, time and mechanical agitation was studied on the K/S value i.e. colour strength of both the vat dyed denim qualities as well as Sulphur black dyed denim fabric using Box-Behnken design. The values so obtained for the light blue vat dyed fabric are,

Multiple R	:	0.968
Squared Multiple R	:	0.937
Adjusted Squared Multiple R	:	0.823
Standard Error of Estimate	:	0.270

The response model evaluated in this study is significant, with coefficient of determination R^2 of 0.937 at a confidence level of 95%. The response surface equation obtained for the K/S value is as follows.

$$K/S = 13.74 - 0.281X - 0.427Y - 0.346 + 0.632X^2 - 0.150Y^2 - 0.363Z^2 - 0.018XY + 0.168YZ + 0.048XZ$$

where, X = Concentration, Y= Treatment time and Z= Mechanical agitation

The model is very significant, as is evident from its F-value ($F_{model} = 8.236$) and very low probability value ($p = 0.016$) obtained from analysis of variance given below.

Source	**df**	**Mean Squares**	**F-ratio**	**p-value**
Regression	9	0.599	8.236	0.016
Linear	3	1.018	14.002	0.007
Quadratic	3	0.737	10.141	0.014
Interaction	3	0.041	0.566	0.661
Residual Error	5	0.073		
Total Error	14			

The trends in colour fading under actual experimental conditions are shown by the K/S value in Table 3, while the contour plots for the interactive effects are in Figures 1. a, b &c. With respect to the effect of concentration of enzyme, the K/S value shows an increase in fading of the denim fabric from 0.5 to around 1.5%. The trend is not continued at still higher concentration, which could be due to some redeposition of removed dye particles. The colour strength is also found to decrease with increase in mechanical agitation. This may be supported by the fact that on increasing the number of steel balls in the treatment, the abrasion on fabric surface increases thus

creating more accessible sites for enzyme attack. Similarly, the K/S value is found to decrease with increasing treatment time (Figure 1c). This could be due to the fact that longer treatment time, will prolong enzymatic degradation of cellulose and the time for further abrasion. The weight loss values as observed in Table 3, appear to be well within acceptable range, which indicates no significant strength loss of the substrate. Neutral cellulase being moderate in its action, is likely to give the desired fading effect with out causing noticeable degradation of fabric.

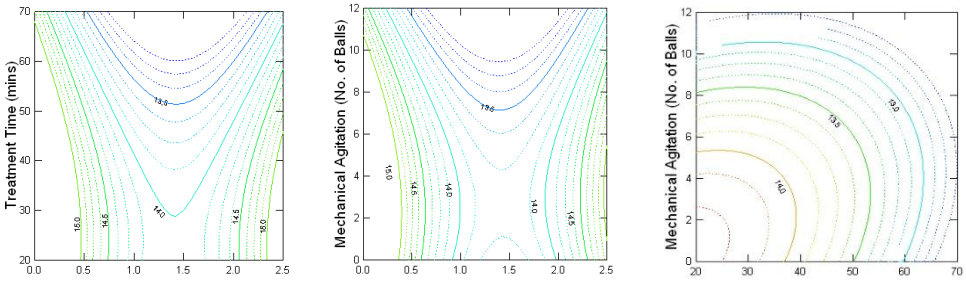
Optimum conditions for the K/S value

Using ridge analysis the optimum conditions to obtain fading effect (minimum K/S value) are 1.25 – 1.33 % enzyme concentration for 45-50 mins with 5-10 steel balls.

Table 3: Effect of process variables on washing of Denim qualities

Sample No.	Light Blue denim			Dark Blue denim			Sulphur dyed denim		
	K/S	Weight loss (%)	Back staining	K/S	Weight loss (%)	Back staining	K/S	Weight loss (%)	Back staining
Control	15.14*	-----	0.03	22.32*	----	0.03	26.88*	---	0.03
E1	15.08	1.59	0.64	21.94	1.03	0.67	23.33	0.62	1.23
E2	14.59	1.62	0.66	21.82	1.88	0.68	22.49	0.92	2.21
E3	13.89	2.55	0.70	20.23	1.76	0.73	21.74	0.79	1.94
E4	13.33	3.50	0.69	19.44	2.4	0.82	20.58	1.83	3.44
E5	14.70	1.84	0.62	20.88	0.4	0.67	22.02	0.59	1.10
E6	14.00	1.03	0.65	21.11	0.65	0.71	22.52	0.63	1.18
E7	13.92	3.05	0.69	18.27	3.01	0.87	21.88	2.25	1.32
E8	13.42	3.5	0.73	17.93	3.98	0.91	20.74	2.52	2.84
E9	13.99	0.64	0.62	21.22	1.68	0.62	23.04	0.70	1.81
E10	13.17	2.32	0.64	19.23	2.43	0.73	21.32	0.78	1.99
E11	12.95	1.81	0.65	20.07	2.97	0.65	22.38	1.26	2.09
E12	12.80	3.03	0.71	17.82	4.25	0.81	20.11	2.54	2.17
E13	13.98	2.1	0.61	19.11	2.23	0.63	22.11	0.80	1.08
E14	13.63	1.71	0.63	20.46	1.82	0.61	22.12	1.51	1.11
E15	13.61	1.89	0.66	19.52	1.3	0.65	21.72	1.11	0.98

*Control – Without any enzyme treatment



Concentration (%) **Concentration (%)** **Concentration %)**

Figure 1: Colour fading trends for Light Blue Indigo denim- a) K/S Vs Concentration & Treatment Time,

K/S Vs Concentration & Mechanical agitation, c) K/S vs Treatment Time & Mechanical agitation

Effect on Dark blue indigo dyed denim fabric

The correlation aspects for treatment of above fabric with the cellulose enzyme are as follows.

Multiple R	:	0.951
Squared Multiple R	:	0.905
Adjusted Squared Multiple R	:	0.735
Standard Error of Estimate	:	0.685

The response model evaluated in this study is significant, with coefficient of determination R^2 of 0.905 at a confidence level of 95%. The response surface equation obtained for the K/S value is here below.

$$K/S = 19.697 – 0.127X – 1.041Y – 1.044Z + 0.562X^2 + 0.599Y^2 – 0.711Z^2 – 0.167XY – 0.065YZ – 0.143XZ$$

Where, X = Concentration, Y= Treatment time and Z= Mechanical agitation

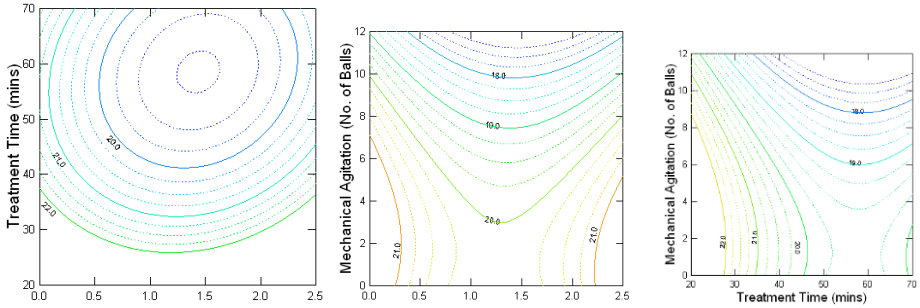
The model is quite significant, as observed from its F-value (F_{model} = 5.305) and very low probability value (p = 0.040) obtained from analysis of variance. The different values are-

Source	**df**	**Mean Squares**	**F-ratio**	**p-value**
Regression	9	2.488	5.305	0.040
Linear	3	5.840	12.451	0.009
Quadratic	3	1.555	3.316	0.115
Interaction	3	0.070	0.150	0.926
Residual Error	5	0.469		

The concentration and treatment time are clearly optimized in Figure 2a, with decrease in K/S on increasing concentration of the enzyme. The actual experimental values as seen in Table 3, indicate a medium concentration of the enzyme, gives a reasonably good fading of colour from the dark blue denim dyed fabric. The change in weight by individual treatment, is indicative of the enzyme action to varying degrees and so is the decrease in K/S values. The contour plots (Figures 2 b & c) indicate the positive influence of mechanical agitation on colour removal from the warp dyed substrate. The co-operative action of enzyme and mechanical action facilitates removal of indigo dye trapped in cellulose fibre [6]. Increase in treatment time further helps in the process, as in case of previous denim quality.

Optimum solution

As per ridge analysis the optimum conditions to obtain fading effect (minimum K/S value) in the dark blue indigo dyed denim are 1.25 – 1.31 % enzyme concentration for 45-50 mins with 5-10 steel balls.



Concentration (%) **Concentration (%)** **Concentration (%)**

Figure 2: Colour fading trends for Dark Blue Indigo denim- a) K/S Vs Concentration & Treatment Time, b) K/S Vs Concentration & Mechanical agitation, c) K/S vs Treatment Time & Mechanical agitation

Effect on Sulphur Black dyed denim fabric

The correlation aspects for treatment of black denim fabric with the neutral cellulase are as follows.

Multiple R	:	0.983
Squared Multiple R	:	0.967
Adjusted Squared Multiple R	:	0.908
Standard Error of Estimate	:	0.270

The response model evaluated in this study is significant, with coefficient of determination R^2 of 0.967 at a confidence level of 95%. The response surface equation obtained for the K/S value is -

$$K/S = 21.983 – 0.33X – 0.936Y – 0.474Z + 0.065\ X^2 – 0.013Y^2 – 0.258Z^2 – 0.08XY – 0.138YZ – 0.41XZ$$

Where, X = Concentration, Y= Treatment time and Z= Mechanical agitation

The high significance of the model is indicated by the F-value (F_{model} = 16.298) and negligible probability value (p = 0.003) obtained from analysis of variance shown below.

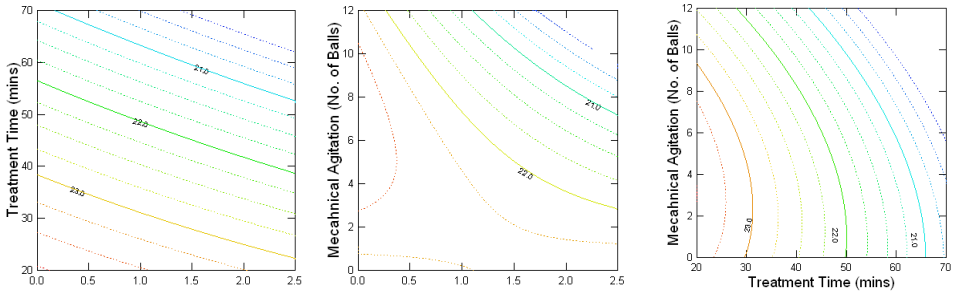
Source	**df**	**Mean Squares**	**F-ratio**	**p-value**
Regression	9	1.192	16.298	0.003

Linear	3	3.226	44.126	0.001
Quadratic	3	0.091	1.240	0.388
Interaction	3	0.258	3.527	0.104
Residual Error	5	0.073		

As seen from Figures 3b and 3c, the fading of the denim fabric increases with increase in the level of mechanical agitation. Sulphur dyes are present in high concentration and abrasion of fabric with steel balls favours the removal of dyes from fabric surface. Other than sulphur dye bonds getting broken from substrate, removal of the fibres from surface due to enzyme action, also causes removal of colour. This effect is more pronounced at higher enzyme concentration and for longer duration of washing.

Optimum solution

By ridge analysis the optimum conditions to obtain fading effect (minimum K/S value) on the black denim are 1.25 – 1.53 % enzyme concentration for 45-55 mins with 5-9 steel balls.



Concentration (%) **Concentration (%)**

Figure 3: Colour fading trends for Sulphur Black denim- a) K/S Vs Concentration & Treatment Time, b) K/S Vs Concentration & Mechanical agitation, c) K/S vs Treatment Time & Mechanical agitation

Effect on back staining of Sulphur Black dyed denim fabric

No significant back staining was observed in case of vat dyed denim fabrics. Neither in light blue nor in dark blue fabrics perceptible staining of white yarns took place. However, significant back staining was observed on sulphur black dyed denim fabric. Experimental design was applied to study this aspect of the black denim. The statistical values obtained are given below.

Multiple R	:	0.949
Squared Multiple R	:	0.901
Adjusted Squared Multiple R	:	0.723
Standard Error of Estimate	:	0.375

The response model evaluated in this study is significant, with coefficient of determination R^2 of 0.949 at a confidence level of 95%. The response surface equation obtained for the K/S value is as follows -

$$K/S = 1.1 + 0.51X + 0.275Y + 0.292Z + 0.35X^2 + 0.755Y^2 + 0.16Z^2 + 0.13XY – 0.025YZ + 0.36XZ$$

Where, X = Concentration, Y= Treatment time and Z= Mechanical agitation

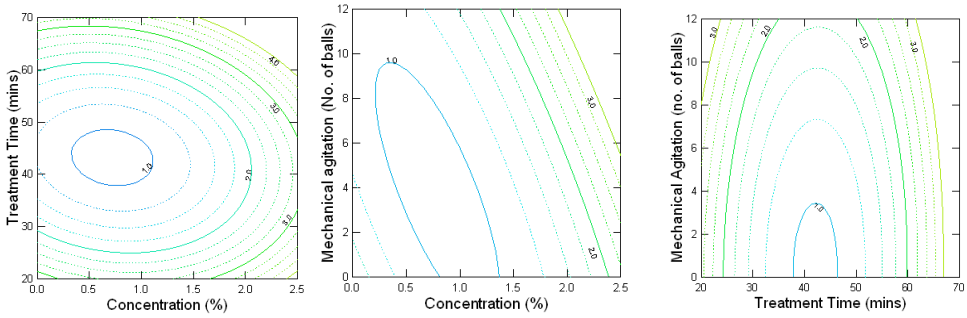
The model is significant enough, as is evident from its F-value (F_{model} = 5.067) and low probability value (p = 0.047), which is less than the threshold value of 0.05. The analysis of variance showing the values is -

Source	df	Mean Squares	F-ratio	p-value
Regression	9	0.712	5.067	0.047
Linear	3	1.123	7.999	0.024
Quadratic	3	0.815	5.805	0.044
Interaction	3	0.196	1.397	0.346
Residual Error	5	0.140		

The contour plots so generated are shown in Figures 4a to c. The staining is found to be more prominent particularly at higher concentration levels of the enzyme. This effect could be attributed to the possibility that cellulose is degraded hydrolytically by cellulose enzyme, and partially it becomes glucose [7]. The glucose thus formed, to some extent is capable of reducing the easily reducible sulphur dye both on fibre and in treatment liquor. This reduced form with limited affinity for cellulose fibre, soils the weft thread. This action is further assisted by mechanical agitation and the treatment time.

Optimum solution

Using ridge analysis the optimum conditions to obtain fading effect (minimum K/S value) with controlled level of back staining are 1.2 – 1.25 % enzyme concentration for 40-45 mins with 0-5 steel balls.



(a) (b) (c)

Figure 4: Back staining trends in Sulphur Black denim – Colour staining with respect to a) Concentration & Treatment Time, b) Concentration & Mechanical agitation, c) Treatment Time & Mechanical agitation

The optimized condition for all the three dyed qualities of denim are thus found to be nearly in similar range. The minimum enzyme concentration, time of treatment and mechanical agitation are found to be the same for all the colours, thus indicating the robustness of the process. However, for reduced back staining in sulphur dyed fabric, lower mechanical agitation appears to be a preferred option.

CONCLUSION

The studied cellulose enzyme normally used for biopolishing, is found to be quite effective for fading of denim fabrics under test. The fading effect is quantitatively established by the change in colour strength while the removal of surface fibre is evident from the loss in weight of the fabric. The enzyme being a neutral cellulase, the action was not severe. The vat dyed fabrics did not have significant back staining while the sulphur dyed denim had some back staining, due to redeposition of the colour. The experimental values have been adequately endorsed by the statistical calculations. The results can therefore be generalized for the desired worn out look.

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