Biopolishing of Cotton Fabrics with Total Cellulases of *Trichoderma reesei* and Optimization Using Taguchi Methods

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Received 14 July 2008; accepted 18 November 2008
DOI 10.1002/app.29826

ABSTRACT: Biopolishing of cotton fabrics enhances appearance and handle of the fabrics without compromising on essential properties. Process of biopolishing is influenced by concentration of cellulases, temperature, pH, and duration of treatment, besides the activity levels of enzymes, method of mechanical agitations and construction features of fabrics. Optimization of process parameters, including mechanical agitations and fabric construction features, has been carried out using Taguchi methods followed by analysis of variance and confirmation tests. All the design parameters, used in the study, have predominant influence on weight loss, fabric strength after biopolishing while thickness, bursting strength, abrasion losses, and flexural rigidity of the fabrics were significantly influenced by the concentration of cellulases together with duration of treatment. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 3402–3409, 2009

Key words: amorphous; biopolishing; degradation; factor effects; signal-to-noise ratio; strength

INTRODUCTION

Biopolishing of cotton fabrics, using acid cellulases, is aimed to remove cellulosic impurities, individual and loose fiber ends that protrude from fabric surfaces thereby smoothening the fabrics with enhanced appearance and handle, with or without the aid of mechanical agitations but without degrading properties of the fabrics significantly. Fungal sources like *Aspergillus, Mucor, Trichoderma, Myrothecium, Humicola* have been used in the processing of cotton materials. The effect of enzyme treatment on the surface mechanical properties of cotton fabrics has been investigated using simple sliding friction apparatus recently.[1–3] Besides the nature of the substrates, efficiency of hydrolysis is also influenced by process conditions and mechanical agitations used in the reaction systems.[4–14] Various levels of agitations are used, in practice, using pad-batch, winch machines, jet systems, and the level of agitations used in the process has profound effects on weight loss values of the fabrics[4–10,15–19] depending upon the structure of yarns, fabrics.[18,20–25]

Taguchi's robust design method replicates the experiments with an outer array that deliberately includes the sources of variation (noise factors) that a product or process would come across in service, while such uncontrollable variables are kept under observation during experimentation in many other experimental designs. Inclusion of those factors in the experimentation makes the design a robust and prone to less variation in the output levels.[26–28] Although efficiency of biopolishing has been stated to be influenced by the process conditions like temperature, time, pH, and concentration of enzymes, combinations of these parameters, often, decide the efficiency of biopolishing. Analysis of variance (ANOVA) followed by confirmation tests using optimum values obtained in the Taguchi methods substantiate the results obtained in the experiments. Inclusion of factors related to fabric constructions and different agitation levels as noise factors, analysis of variance followed by the confirmation tests could provide robust design parameters that give stable output performances, under various process conditions.

EXPERIMENTAL

Biopolishing

Total cellulase extracts from *Trichoderma reesei* with an optimum pH of 5.5 at 50°C was generously given by the Department of Microbiology, PSG College of Arts and Science, Coimbatore, and biopolishing was carried out in a shaker bath at a speed of 120

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Contract grant sponsor: Department of Biotechnology, Ministry of Science and Technology, Government of India.

strokes/min with the range of design parameter values obtained after characterization.

Estimation of cellulase activity

0.5-mL cellulase enzyme, diluted in citrate buffer of pH 5.5, and 0.5 mL of 1% carboxy methyl cellulose were mixed well and incubated at 45°C, 50°C, 55°C, and 60°C for 15 min. To this, 0.5 mL of dinitrosalicylic acid (DNSA) was added and boiled for 15 min before adding 1 mL of sodium potassium tartarate and cooling the contents. Color change at the wavelength of 540 nm was measured, subtracted from that of enzyme blank, and translated into glucose production using a standard curve.[29]

Experimental design and selection of noise factors

Design factors

Biopolishing, with the listed design parameters at different levels (Table I), was carried out using L9 orthogonal array randomly, to avoid systematic errors. Four design parameters at three different levels were selected for optimization purpose in this study.

Noise factors

Mechanical agitations and construction of fabrics were considered as the two different noise factors because they influence biopolishing process individually to significant extent. Agitation levels were increased (N2) by adding 20 numbers of stainless steel balls, each weighing 1 g, besides the agitations levels available in the shaker bath (N1). Areal density of fabrics at high (N3), low levels (N4) were selected as two levels of noise factors and according to the response variable of interest; signal-to-noise ratios were selected from the Table II.[28]

Fabric parameters

Ends per centimeter and picks per centimeter were calculated as per ASTM D 3775-96. Linear densities of the yarns in the fabric were calculated as per the ASTM D 1059-2001. Areal density of the fabric was calculated as per the ASTM D 3776-96, using a standard fabric cutter. Thickness of the fabric was calculated as per ASTM D 1777-07 at ten different places for each specimen and average was taken for further analysis and Table III shows specifications of fabrics used in the experiments.

TABLE I
Coded Variables and Their Levels

Design factors	Notation	Levels 1	2	3
Time (min)	A	35	40	45
Temperature (°C)	B	50	55	60
pH	C	4.0	4.5	5.0
Enzyme concentration (gpl)	D	5.5	6.0	6.5

TABLE II
Signal-to-Noise Ratio and Its Relevance

S. No.	*S/N* ratio	Nature of response variable
1	$S/N\ (\theta) = 10\log_{10}(\tau^2/s^2)$	Target is the best
2	$S/N\ (\theta) = -10\log_{10}(y_i^2/n)$	Smaller-the-better
3	$S/N\ (\theta) = 10\log_{10}[(1/y_i^2)/n]$	Larger-the-better
4	$S/N\ (\theta) = 10\log_{10}(p/1-p)$	Binary scale (GO/NO GO)

Weight loss

Weight loss after biopolishing was calculated as a ratio of difference in weights, before and after treatment, to the original weight. Before weighing, every sample was allowed to reach equilibrium under standard conditions with a relative humidity of 65 ± 2%, at a temperature of 25 ± 2°C in an environmental chamber.

Abrasion resistance

Abrasion resistance of the fabric was calculated as per ASTM D 4966, using Martindale abrasion tester. Abrasion loss percent was calculated from the ratio of difference in weights, before and after the test, to the original weight of the specimen. Five tests were carried out for each specimen and average was taken for analysis purpose.

Tensile strength

Tensile tests were carried out in Tensomax 7000 tensile testing instrument, using strip test method with a sample size of 8 × 2 in. 2½ inch width was cut from both control and biopolished fabric samples,

TABLE III
Specifications of Raw Fabrics

Fabric parameter	N3	N4
Weave	Plain	Plain
Warp count (Tex)	30	15
Weft count (Tex)	30	15
Ends per cm	26	28
Picks per cm	26	23
Areal density (g/m^2)	171	83.5
Thickness (mm)	0.43	0.33

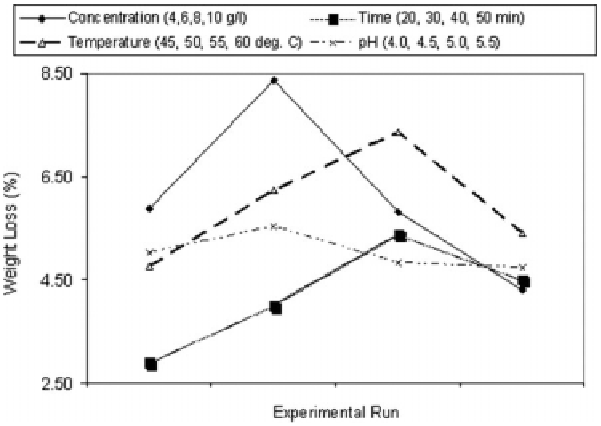

Figure 1 Activities of cellulases on bleached cotton fabrics.

and threads from both edges were removed until the width reduced to 2 in. The specimen was mounted centrally and load for breaking at the center was noted and average value of five tests was considered for strength loss calculations.

Bursting strength

Bursting strength test was carried out according to the procedure[30] available (test method has been withdrawn from ASTM test methods since 1996), using hydraulic-diaphragm bursting strength tester that uses glycerine as the liquid medium and screw driven piston to increase the hydraulic pressure. Five tests were carried out for each specimen and average was reported.

Flexural rigidity

Flexural rigidity of the fabric was calculated as per the ASTM D1388-96, using fabric stiffness tester, using the principle of cantilever bending. Flexural rigidity was calculated using

$$\text{Flexural Rigidity } (G) = W \times C^3 \text{ (mg/cm)}$$

where W is the fabric mass per unit area in mg/cm^2, and C is the bending length in cm. Average of five test results, measured in warp direction, was reported.

RESULTS AND DISCUSSIONS

Characterization of enzyme activity

Efficiency of enzymes varies with reference to nature and form of substrates, when compared with the xactivity levels obtained at the time of secretion from various sources during the growth period due to differences in metabolizable nature of substrates. Total crude cellulases used in the experiment was characterized using a small piece of bleached cotton fabric to identify the range of time, temperature, pH, and concentration for further optimization, keeping weight loss as the sole response variable. All the response curves showed initially an increasing tendency followed by a decreasing trend, after reaching a peak weight loss value, adhering to the characteristic curve of the enzyme activity (Fig. 1). Activity of cellulases by DNSA method showed a maximum of reducing sugar 200 μg/mL of cellulase at a temperature of 55°C, comparable to the values reported in the literatures.[31–36]

Weight loss

Reactions of cellulases on cotton fabrics, conversion of long chain cellulose polymers into soluble

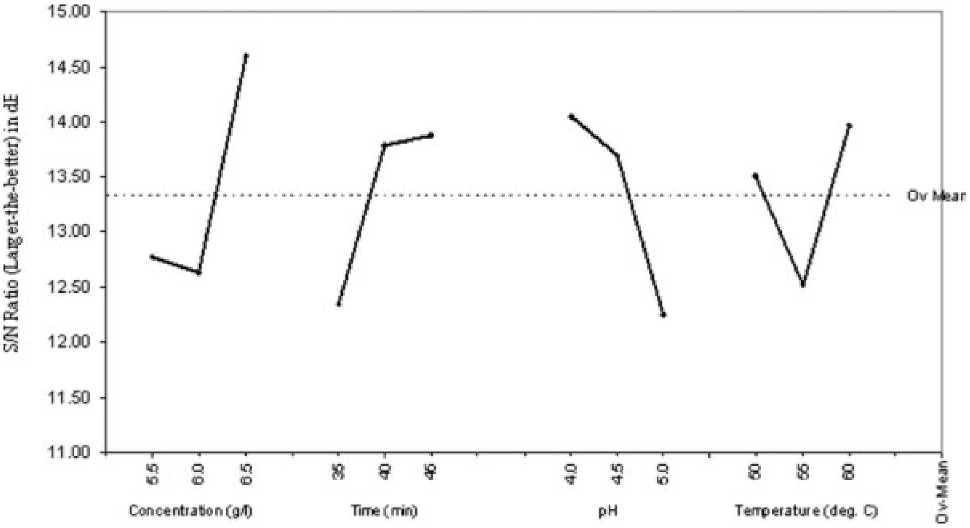

Figure 2 Effect of design parameters on of weight loss.

TABLE IV
Effect of Design and Noise Parameters on Response Variables

					Signal–to–noise ratio					
	Design parameters				Larger-the-better	Smaller-the-better	Larger-the-better	Larger-the-better	Smaller-the-better	Smaller-the-better
No.	Conc.	Time	pH	Temp.	Weight loss	Thickness	Bursting strength	Tensile strength	Abrasion resistance	Flexural rigidity
1	1	1	1	1	12.68	8.61	39.65	14.48	−12.66	−4.86
2	1	2	2	2	12.77	8.39	40.23	22.18	−13.35	−4.03
3	1	3	3	3	12.86	8.52	39.80	10.17	−9.54	−3.81
4	2	1	2	3	12.65	8.67	39.81	27.99	−10.17	−3.75
5	2	2	3	1	12.18	8.08	40.46	21.14	−13.59	−4.02
6	2	3	1	2	13.08	8.51	39.91	8.23	−11.46	−3.57
7	3	1	3	2	11.71	8.81	38.80	18.43	−14.85	−3.88
8	3	2	1	3	16.39	8.51	39.42	−0.09	−11.81	−3.40
9	3	3	2	1	15.68	9.27	38.94	20.83	−14.71	−3.20

oligomers, reducing sugars, weakening, and removal of small protruding fibers from the fabric or yarn surfaces result in weight loss. Design factors used in the experiments appeared to exercise strong influence over weight loss values of fabrics in all the treatments (Fig. 2). Highest weight loss value in the given set of experiments was found to be at 6.53% and the lowest weight loss value at 3.25%, whereas the overall average was about 4.83%, values that were different from those obtained during characterization treatment.

Signal to noise ratio, larger-the-better, showed the highest value for the sample treated with the highest concentration and temperature levels (Table IV), confirming roles of these factors as reported in the literature.[37] However, all the design parameters appeared to influence the weight loss values in biopolishing, shown by analysis of variance in terms of factor effects and *F* values (Table V). Although concentration of enzymes plays a dominant role followed by pH and time, the temperature of the treatment appears to play comparatively lesser role as far as weight loss is concerned.

Thickness

Actual thickness of fabrics reduces with biopolishing, while the apparent thickness appears to increase with very high mechanical actions that lead to fibrillation on the surfaces of fibers.[38] Removal of fibers from surface of fabrics, hydrolysis of cellulose and consolidation of fabric structure leads to decrease in thickness of fabrics after biopolishing and in this case too, signal-to-noise ratio of "smaller-the-better" was adopted for analysis. Thickness of the fabrics, measured after the biopolishing apparently differed within a small range in terms of signal-to-noise ratios (Fig. 3, Table IV). Concentration of cellulase and duration of treatment seemed to have equally higher influences than pH and temperatures used in the experimental design (Table V).

Bursting strength

Bursting strength of fabrics represents the ability to withstand simultaneous, multidirectional stresses introduced by a hydraulic system. Height of the specimen during extension in the bursting strength

TABLE V
Factor Effects and *F*-values of Response Variables

		Weight loss		Thickness		Bursting strength		Tensile strength		Abrasion resistance		Flexural rigidity	
Design parameter	Degree of freedom	Factor effect (%)	*F* Value	Factor effect (%)	*F* value	Factor effect (%)	*F* value	Factor effect (%)	*F* value	Factor effect (%)	*F* value	Factor effect (%)	*F* value
Concentration	2	35	3587	40	19	73	438	19	8179	28	8	44	1044
Time	2	22	2202	40	19	27	161	36	15,345	6	2	40	942
pH	2	27	2730	18	9	0	–	31	13,463	4	–	7	175
Temp.	2	16	1634	2	–	0	–	14	6015	62	16	9	219

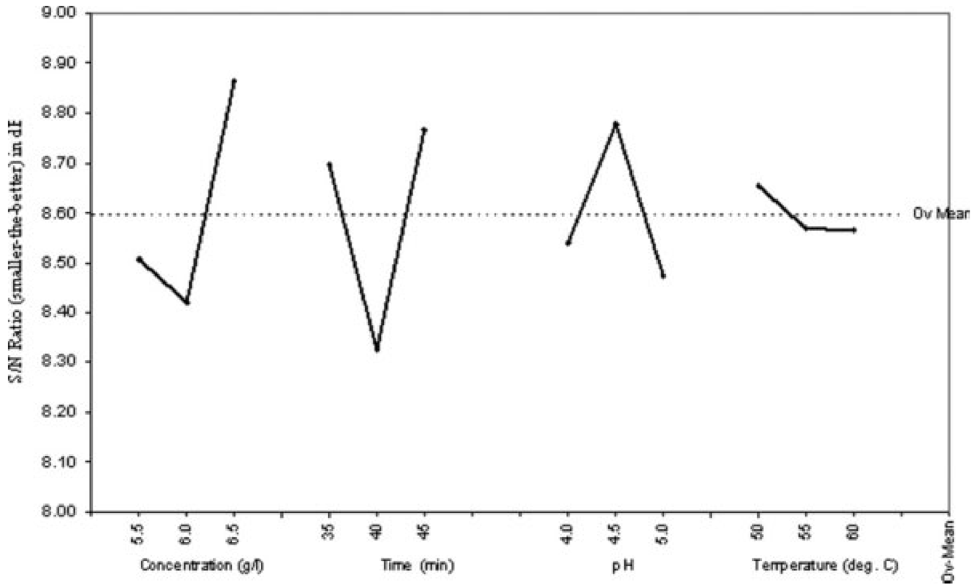

Figure 3 Effect of biopolishing on fabric thickness.

tester, radius of sample decides the extension levels of the fabrics that can occur in the test, which in turns decides the bursting strength of fabric sample. In biopolishing of cotton fabrics, preferential removal of disordered regions in the fibers could, possibly, be responsible for alterations in the bursting strength. Retention of bursting strength to higher extent is, often, expected after biopolishing and larger-the-better type of signal-to-noise ratio was used to analyze the effect of cellulase biopolishing on bursting strength. In this case too, role of concentration of enzymes and duration of biopolishing were found to be very much significant when compared with pH and temperature (Fig. 4). Effect of various design and noise factors on the response variable, that is, bursting strength, is shown in Tables IV and V shows the effect of various factors, included in the study, on bursting strength of fabrics and the performance of individual control factors. Concentration of enzyme plays a dominant role followed by duration of treatment where pH and temperature have very low influences.

Flexural rigidity

Changes in morphological structure of cotton fibers, weight and thickness of fabrics after biopolishing could definitely have an impact on the flexural

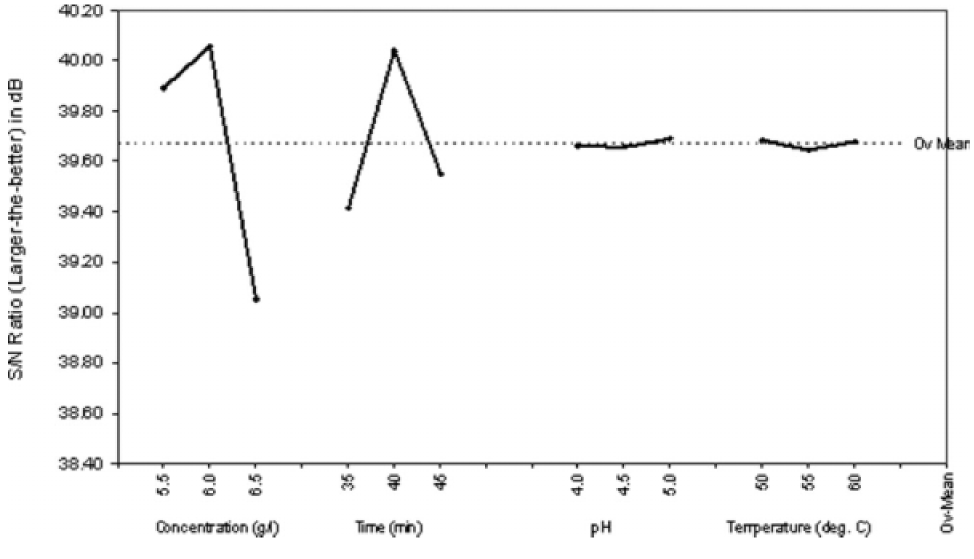

Figure 4 Effect of control factors on bursting strength of fabrics.

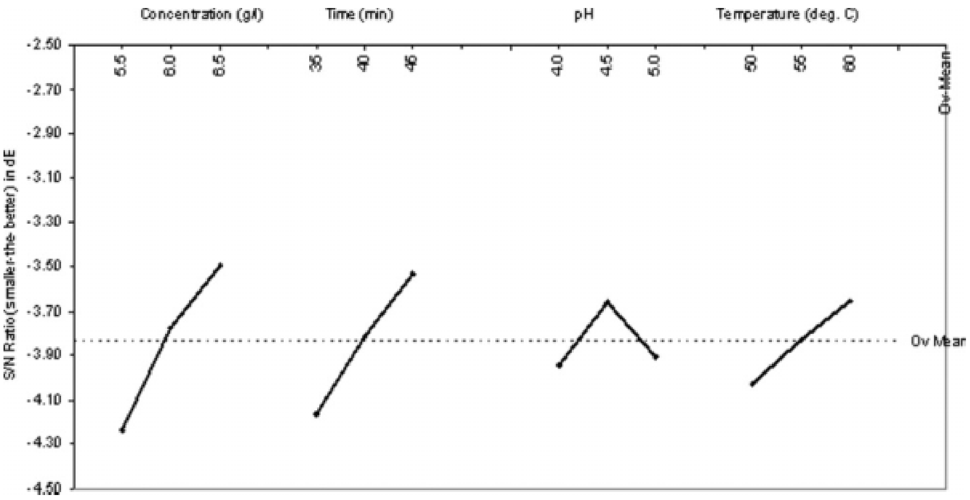

Figure 5 Effect of control and noise factors on flexural rigidity.

rigidity of fabrics. Any decrease in these parameters, obviously, might result in lower rigidity values of samples and smaller-the-better type of signal-to-noise ratio characterizes the flexural rigidity of fabrics to the best possible extent. Signal-to-noise ratios, in this case, appeared to vary in a smaller range, similar to thickness values, than other response variables (Table IV, Fig. 5). In the case of flexural rigidity, concentration and time appeared to play a major role compared to temperature and pH of process bath (Table V).

Abrasion resistance

Abrasion resistance increases due to smoothness of the fabric surface, after biopolishing, depending on the yarn structures.[30,40] Abrasion losses in the fabrics occur mainly due to series of repeated applications of stress and, similar to other properties, behavior of fibers to abrasion is expected to vary significantly after biopolishing, which in turns, would influence the abrasion resistance. Lower the abrasion loss, better is the condition of the biopolished fabrics, and hence, "smaller-the-better" type of signal-to-noise ratio was preferred in the case of abrasion resistance (Table IV). Abrasion resistances of fabrics appeared to be highly influenced by concentration and temperature of the biopolishing treatment (Fig. 6). Table V shows the effect of various design parameters with their contribution in the abrasion loss in terms of percent factor effects with *F* values.

Tensile strength

Gradual degradation of fibers along the spiral plane during cellulase hydrolysis is considered to be the reason for initial tensile strength loss and once the

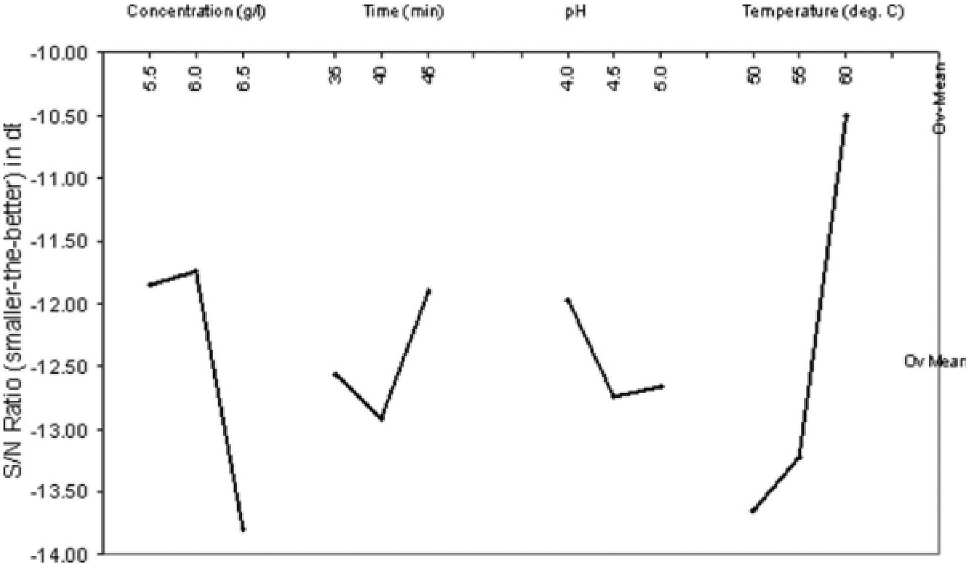

Figure 6 Effect of process parameters on abrasion loss.

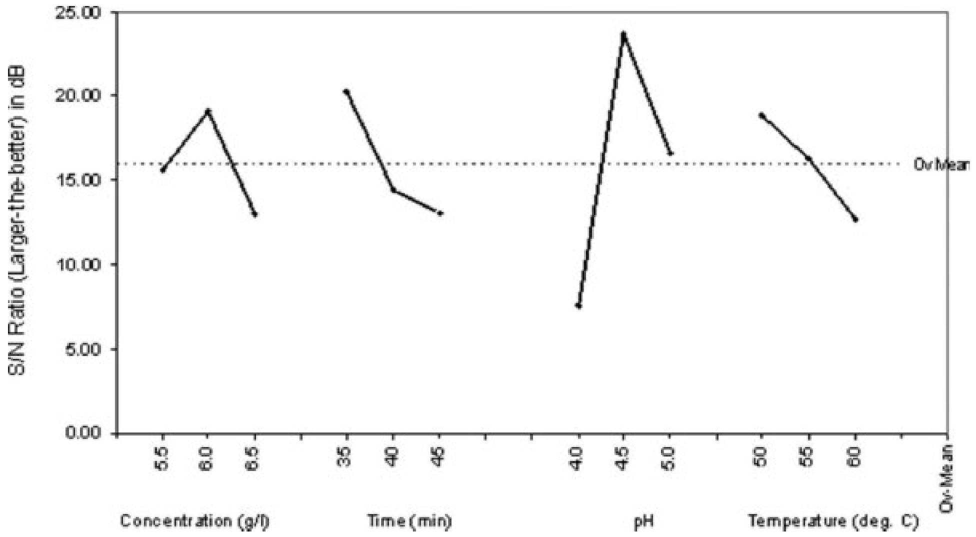

Figure 7 Effect of control factors on tensile strength loss.

fissures reach the lumen, further degradation in strength occurs rapidly.[7,13,34,38,39] Combination of high agitations synergistically tears away fiber surfaces, exposing fresh surfaces for further attack and could lead to further loss in breaking strengths. Similar to weight loss of specimens, tensile strength of the fabric samples after biopolishing, also appeared to be influenced by all the design factors included in the experimental set-up (Table V, Fig. 7). However, increasing the duration of biopolishing resulted in reduction of fabric strength in conformance to the literatures.

Confirmation test

Methodology advocated by Taguchi for optimization problems involve typically four stages namely, problem formulation, data collection/simulation, factor effects analysis, and confirmation tests.[28] Confirmation test in the Taguchi methods supplements, assures validity of the results obtained in the experimental design and orthogonal array selected in the study and various levels of the design parameters and their interactions. Confirmation tests carried out with every set of optimum parameters for all the response variable showed the closer results to that of original results and did not show any significant difference at 95% confidence levels. Table VI shows the values obtained in confirmation tests along with the original values for all response variables, considered in the study.

CONCLUSIONS

All the design factors selected in the experiments demonstrated pronounced effects on various parameters used in the assessment of biopolishing. Biopolishing of cotton fabrics using cellulases is often characterized by weight loss values of treated fabrics with enhanced physical properties. Weight loss and tensile strength of biopolished samples, comparable to that of reported in the literature, were obtained in all the experiments and were found to be influenced by concentration, temperature, time, and pH selected in the experiments, exhibited by scaling factor effects alone and none of them could be assigned for adjustment factors. Thickness, bursting strength, and flexural rigidity of the biopolished fabrics were dominantly influenced by concentration, time, whereas abrasion resistance appeared to be influenced by concentration and temperature of the treatment.

TABLE VI
Confirmation Tests with Scaling and Adjustment Factors

Parameter	Original result	Confirmation result	Significance at 95% confidence level (yes/no)	Scaling factors	Adjustment factors
Weight loss (%)	6.37	6.51	No	A3, B3, C1, D3	–
Thickness ($\times 10^{-3}$ m)	0.43	0.43	No	A2, C3, D2	B3
Flexural rigidity × 1000 (mg/cm)	3.04	3.75	No	A1, D1	B1, C1
Abrasion resistance (%)	98.03	97.89	No	B1, D3	A2, C3
Bursting strength (kPa)	759.00	776.25	No	A2, D2	B3, C3

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