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Effects of Lipid Removing by Lipase on Physical Properties of Cotton Fabrics

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Abstract

Advances in biotechnology have enabled the development of novel applications for enzymes in many fields. The enzyme treatments of textile cotton are becoming increasingly important over the last few years. In this study, samples of cotton fabrics treated with lipase (porcine pancreas) in different times (1, 42, 141, 240, 281 min) and concentrations (0.17, 1, 3, 5, 5.83 g/l) and some of their physical properties including weight reduction percentage (WRP), rate of water absorbency (RWA), moisture regain (MR) and reactive dye absorption (RDA) were measured. The Enzyme activity was determined based on titrimetric method and the amounts of produced fatty acid resulting from enzymatic hydrolysis were considered as the rate of the progress of the treatment. Thirteen experiments were carried out based on Central Composite Design (CCD). The results showed that increment in enzyme concentration and treatment time can improve the dye and water absorption significantly. Most cases showed close relationship between two studied variable parameters and response models were mostly quadratic.

Keywords: Cotton, Lipase, Physical Properties, Central Composite Design, Fatty acid

Introduction

Biotechnology has a great effect on the textiles industry through the development of more efficient and environmentally manufacturing processes, as well as the design of improved textile materials. Through biotechnology, enzymes are used to treat and modify fibers during textile manufacturing, processing, and in caring for the finished product (Cavaco Paulo.A, 2003)(Cavaco Paulo.A, 1998).

Enzymes have a high degree of specificity and can therefore be exploited to degrade particular groups of toxic chemical into harmless or less toxic productsBioremediation of pesticide contaminated water using anorganophosphate degrading enzyme immobilized on nonwovenpolyester textiles.

Some applications include:

De-sizing of cotton - IntroductionSizing is a complementary process that is performed on warpyarns that have insufficient tenacity/filaments with zero twist.Starch has primarily been used to size cotton fabrics because itis relatively inexpensive, readily available and plentiful in nature.Due to its improved tenacity, elasticity and abrasion properties,sized yarn has a higher weaving efficiency during the subsequentweaving stage. Sizing materials should not adversely affect thesubsequent processes, such as dyeing and finishing. Thus, starchmust be removed in the desizing stage after weaving. The desizingof cotton fabric can be accomplished by rot steeping, acid steep-ing or treatment with enzymes and oxidizing agents. Enzymes,particularly _-amylases, which catalyze the hydrolysis of starchmacromolecules, are preferably used in textile desizing due to theirspeed, selectivity, specificity and environmental gains (Cavaco Paulo.A, 2003).

Retting of flax- Traditional retting methods consume large quantities of water and energy. Bacteria, which may be bred or genetically engineered to contain necessary enzymes, could be used to make this a more energy efficient process (Shah.J.N, 2004**)**.

Breakdown of hydrogen peroxide- Catalase enzymes specifically break down hydrogen peroxide and may be used to remove this reactive chemical before further dyeing (Cavaco Paulo.A, 2003) (Costa.S et al, 2001).

Biostoning and Biopolishing-cellulase enzymes may be used to stonewash and polish fabrics effectively without damaging the fibers (Cavaco Paulo.A, 2003)(Cavaco Paulo.A, 1998)(Koo.H et al, 1994)(Cavaco Paulo.A, 1998).

Detergents – Enzymes allow detergents to effectively clean clothes and remove stains. They can remove certain stains, such as those made by grass and sweat, more effectively than enzyme-free detergents (Ee J. Misset O. and Baas E, 1997)**.**

A lipase is a water-soluble enzyme that catalyzes the hydrolysis of ester bonds in water-insoluble, lipid substrates. Lipases are ubiquitous throughout living organisms, and genes encoding lipases are even present in certain viruses (Anstrup, P, k, 1974).

The lipid content of cotton fibers is a major obstacle in water and dye absorption and producing hydrophill cotton textiles, in this study it is intended to investigate the effect of hydrolysis of ester bonds of lipid content of cotton fibers by lipase from procine pancreas in a neutral pH on removal of cotton lipids, and to study the effects of this treatment on some physical properties of cotton fabric including: Weight loss percentage, rate of water absorbency, moisture regain, produced fatty acid by hydrolysis reaction, and reactive dye absorption (Cavaco Paulo.A, 2003).

Materials and Methods

Cotton fabric (woven twill, 166 g/m^2), lipase enzyme (Sigma L3126, from porcine pancreas, EC 3.1.1.3).Hydrolysis of sample was carried out by AHIBA Laboratory dyeing machine. In this research, we use spectrophotometer UV- VIS to measure the amount of dye in bath dyeing.

Enzyme Activity: To determine the activity of enzyme the Sigma quality control test (titrimetric method) was applied and olive oil was chosen as the substrate (enzymatic assay of lipase (EC 3.1.1.3) Sigma prod.No.L-3126, 1997)
The following reagents were prepared:
A. 200 mMTrisHCl Buffer, pH 7.7 at 37 ˚C (with 1 M HCl.)
B. Olive Oil Substrate Solution
C. 95% Ethanol
D. 0.9% (w/v) Thymolphthalein Indicator Solution (TPH Indic)
E. 50 mM Sodium Hydroxide Solution-Standardized (NaOH)
F. Lipase Enzyme Solution(containing 500 - 1,000 units/ml of Lipase in cold deionized water).
The blank (3.5 ml deionized water, 1 ml reagent A, and 3 ml reagent B), and test (same as blank but 2.5 ml deionized water and 1 ml reagent F) solutions were prepared and incubated at 37 ˚C for 30 minutes, then each of them was added to a 50 ml Erlenmeyer flask(containing 1 ml reagent F for blank solution).Reagent C (3 ml) and 4 drops of reagent D was added to both the test and blank solutions, and each solution titrated with reagent E to a light blue color. Activity of enzyme was calculated by equations 1-3.

$$\text{Units/ml enzyme} = [(\text{NaOH})\ (\text{Molarity of NaOH})\ (1000)\ (2)\ (\text{df})] / \text{l} \quad (1)$$
$$\text{Units/mg solid} = (\text{units/ml enzyme}) / (\text{mg solid/ml enzyme}) \quad (2)$$
$$\text{Units/mg protein} = (\text{units/ml enzyme}) / (\text{mg protein/ml enzyme}) \quad (3)$$

(NaOH) = Volume (ml) of reagent E used for test minus volume (ml) of the reagent E used for Blank.
1000 = Conversion factor from milliequivalent to micro equivalent
2 = Time conversion factor from 30 minutes to 1 hour (Unit Definition)
df = Dilution factor
l = Volume (ml) of enzyme used

One unit will hydrolyze 1.0 micro equivalent of fatty acid from a triglyceride in one hour at pH 7.7 at 37˚C. (This is equivalent to approximately 10 micro liters of CO_2 in 30 minutes.)

Experimental Design: Central Composite Design "CCD", representing the most frequently used response surface methodology, was introduced for eighteen experiments to determine the effects of enzymatic treatment on weight reduction, moisture content, rate of water absorption, color absorbency, and the amount of produced fatty acid. There were three groups of design points: a two-level fractional design, axial (star) points, and center points (Montgomery, D. C, 1998)
For the enzymatic treatment, there are two variable parameters involved in the design, they are, time and concentration of the enzyme. The design scheme is shown in Table I, and the order of eighteen experiments (randomized by the program) is shown in Table II.
In all the experiments, Cotton fabric samples sank into a buffer solution including Trizma-base and HCl (pH=7.7), then the required amount of enzyme was added according to table II to start the treatment. Enzymatic hydrolysis was carried out at 40 °C in the specified times (table II). Solution temperature increased to 90 °C, and it was kept constant for 10 minutes to deactivate the enzyme. Finally samples were rinsed and dried at room temperature.

Table I: Design points of the factors for enzymatic treatment.

Factor	Parameter	Unit	Low level	High level	Start point	Center point
A	Time	Min	42	240	1 ; 281	141
B	concentration	g/l	1	5	0.17 ; 5.83	3

Table II: The order of eighteen experiments.

		Concentration (g/l)				
		0.17	1	3	5	5.83
Time (min)	1	-	-	12	-	-
	42	-	3	-	4	-
	141	2	-	1,5,6,10,13	-	11
	240	-	7	-	8	-
	281	-	-	9	-	-

To investigate the effect of buffered solution on the studied properties, some samples were treated in the absence of enzyme in different times: 0.00, 0.99, 42.00, 141.00, 240.00, and 281.00 (min).

Weight loss

Percentages of weight loss of samples were obtained according to the equation (4) after two days of conditioning at room temperature.

$$\% \text{ Weight Loss} = (WD_1 - WD_2) / WD_1 * 100 \qquad (4)$$

WD_1= Weight of sample before enzymatic treatment.
WD_2= Weight of sample after enzymatic treatment.

Moisture regain of samples were calculated by equation (5) after 24 hours in standard condition.

$$\% \text{ Regain} = m_1 - m_2 / m1 * 100 \qquad (5)$$

To determine water absorbency of samples the time of absorption for a single drop of water was measured.

Measurement of Water Absorption

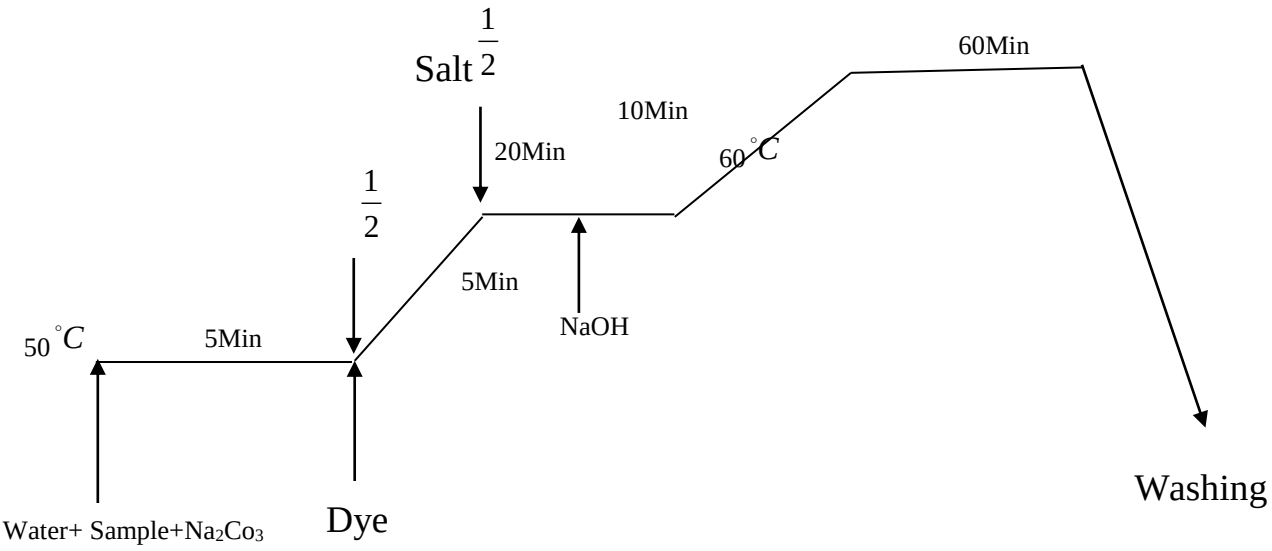
Duration of water absorption is equal with the time that one drop is absorbed by fabric. After enzymatic treatment, the time needed for water absorption by the treated fabric was measured and compared to the time needed for samples treated by buffer solution.

Dye Absorption

Samples was dyed according to figure one. Dyeing bath consisted of 1% (o.w.f.) CibacronTurquosie 3G-E , 30 g/l NaCl, and 20 g/l sodium carbonate, and L/R selected 30:1. To determine the amount of dye exhaustion, the equation 6 was used.

$$\% \text{ Exhaustion} = m - n / m * 100 \qquad (6)$$

m: Total dye consumed concentration.



Measurement of Produced Fatty Acid

The amount of fatty acid determined by equation 7 estimate the degree of hydrolysis by lipase enzyme.

$$\text{Fatty acid (mole)} = 5^{*}\ 10^{-5}{}^{*}\ V_{NaOH} \quad (7)$$

Results and Discussion

The effects of buffered solution on investigated properties are given in Table IV. Increasing the time of treatment shows a significant increase in (according to Duncan's Test) weight loss, relative humidity, dye absorption, and water absorbency. Differentiation the effect of buffer solution from enzymatic treatment, the measurements obtains in similar times were subtracted from the results obtained in enzymatic treatment.

Table IV. Effects of buffered solution on cotton fabric in different times.

		Quantity			
		Weight Loss (%)	Moisture Regain (%)	Water Absorption (S)	Dye Absorption (%)
Time (min)	0.00	0.000	5.545	900	81.23
	0.99	0.009	5.520	901	81.40
	42.00	0.012	5.597	830	82.09
	141.00	0.060	5.632	800	82.53
	240.00	0.113	5.676	650	82.90
	281.00	0.150	5.707	632	83.03

According to response to surface analysis there exist quadratic response models for all the investigated parameters (table V). The diagnostic analysis of the studentized residuals proved that all the chosen models were satisfied for the data (Figure 2).The significant effects of the variables on investigated properties are depicted in figures 3.

Table V. Response model equations.

	Model accepted (in terms of coded factors)						Significant variables (prob.>F)
Response	**Constant**	**A**	**B**	**A2**	**B2**	**AB**	
Sqrt (weight loss)	$-2/31687\times10^{-4}$	$8/80615\times10^{-3}$	$-1/44804\times10^{-5}$	0/27279	0/016298	-0/34229	A,B,A^2
Ln(moisture regain)	$1/58511\times10^{-4}$	--------	--------	0/013768	$1/47499\times10^{-4}$	1/72747	A,B
L/Sqrt(water absorbtion)	$1/20960\times10^{-4}$	--------	--------	$1/15622\times10^{-3}$	$3/42096\times10^{-4}$	0/079394	A,B
Dye absorption	$6/06061\times10^{-6}$	--------	--------	$8/30985\times10^{-3}$	$1/45791\times10^{-4}$	0/79838	B
Ln(fatty acid)	$-7/69582\times10^{-4}$	-0/066671	$-4/89128\times10^{-5}$	0/92860	0/025157	1/94794	A,B,A^2

- Note: A represents the time of enzymatic treatment and B concentration of lipase enzyme.

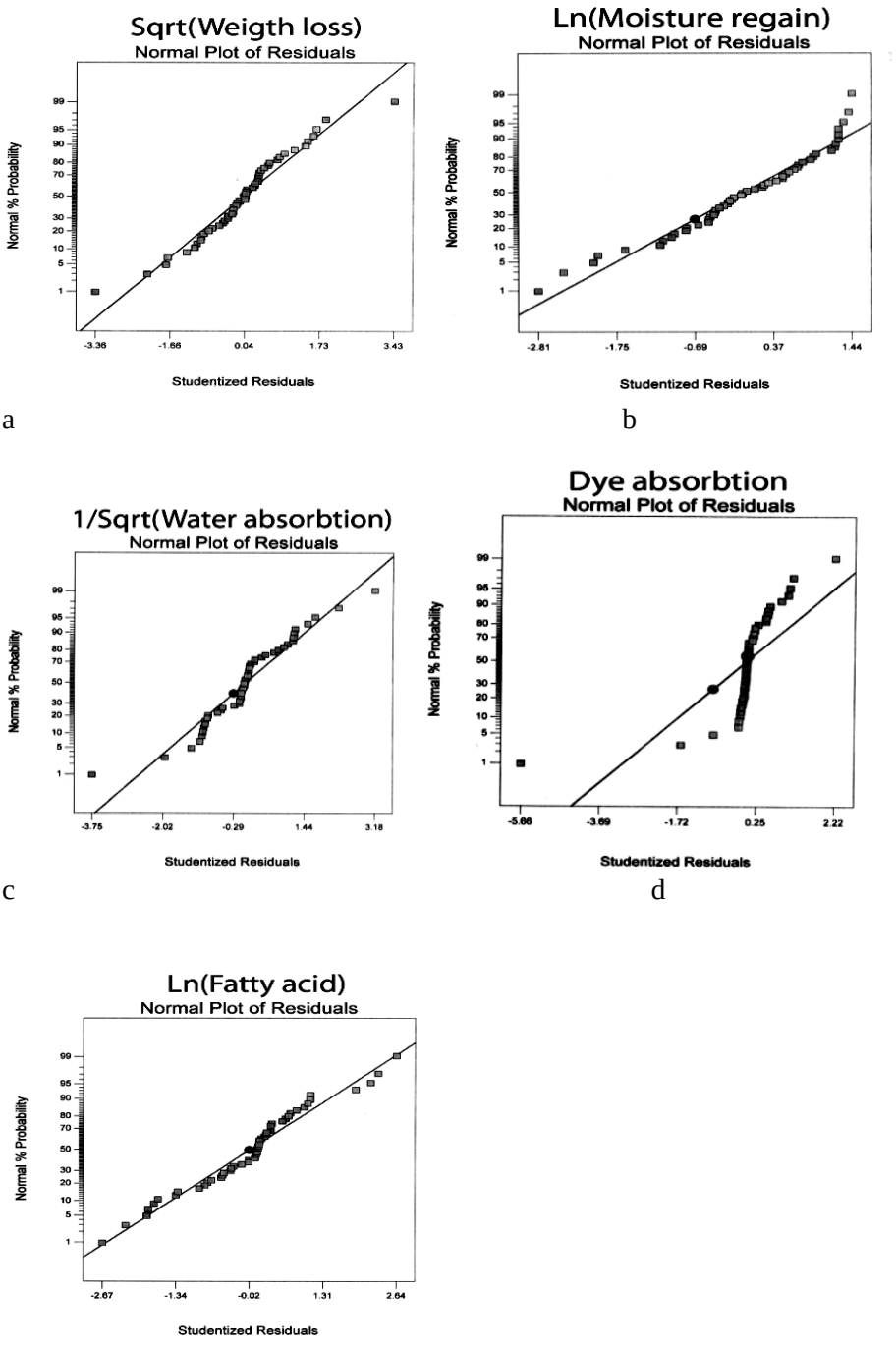


Figure 2. Diagnostic plots of the normal probability of studentized residuals for the response models of a) Weight loss, b) moisture regain, and c) water absorption, d) dye absorption, and d) fatty acid.

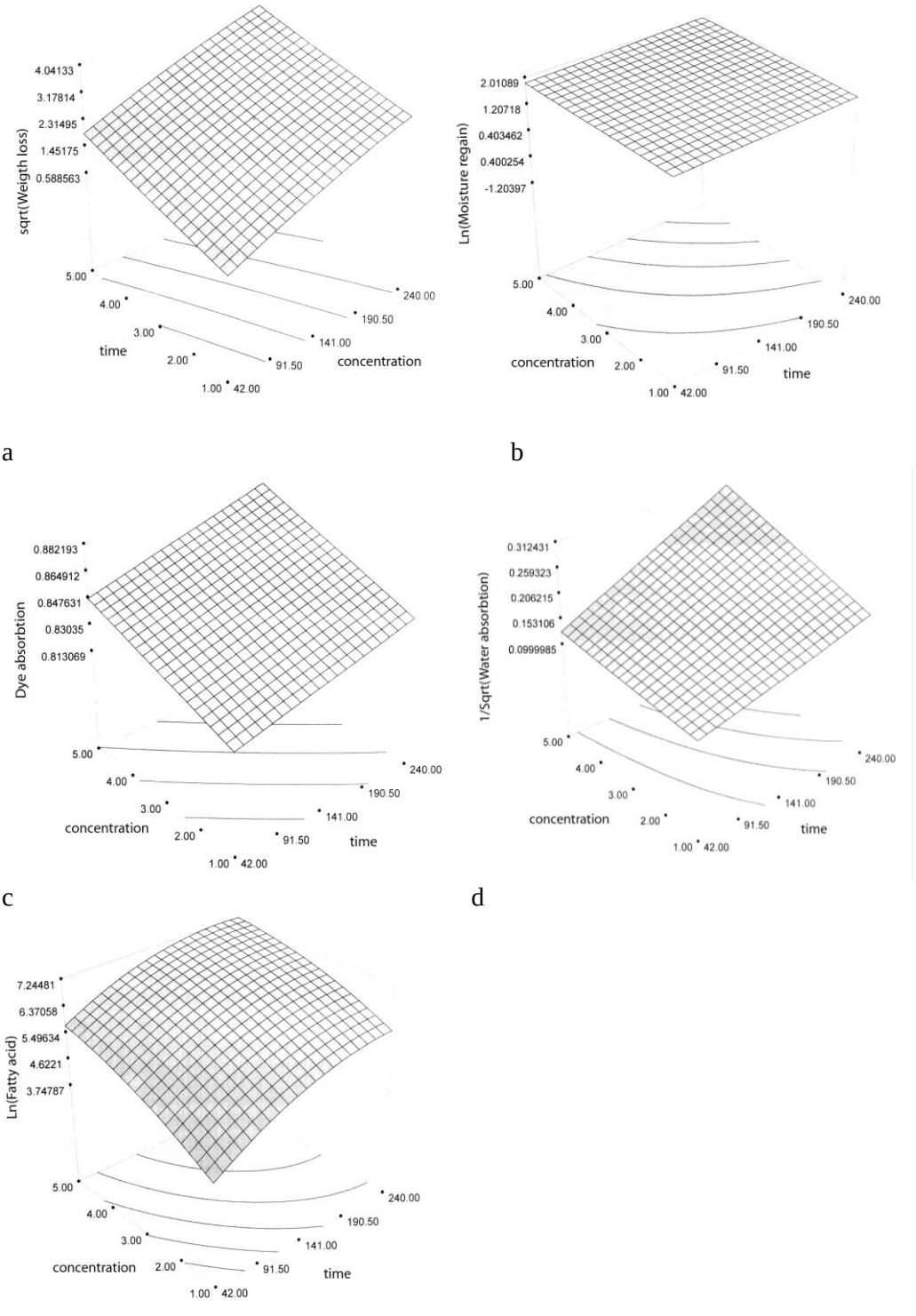


Figure 3. Interaction of treatment time and concentration of lipase enzyme for a) Weight loss, b) moisture regain, c) water absorption on cotton fabric, d) dye absorption, and d) fatty acid.

Increasing time of the treatments and enzyme concentration, weight loss decrease (Fig 2a), and there is a strong interaction between treatment time and enzyme concentration: increasing treatment time coupled with increasing enzyme concentration cause considerable weight loss of samples.
With increasing the time of treatments and enzyme concentration moisture regain of samples increases (Fig 2b), but there is a weak interaction between treatment time and enzyme concentration: with increasing treatment time along with increasing enzyme concentration cause increasing moisture regain of samples.
With increasing the time of treatments and enzyme concentration water absorption decreases (Fig 2c), and there is a strong interaction between treatment time and enzyme concentration: with increasing the treatment time and increasing enzyme concentration cause considerable decreasing water absorption of samples.
With increasing the time of treatments and enzyme concentration dye absorption increases (Fig 2d), and there is a strong interaction between treatment time and enzyme concentration: with increasing the treatment time and increasing the enzyme concentration there is a considerable decrease in water absorption of samples.
With increasing the time of treatments and the enzyme concentration, the amount of produced fatty acid increases (Fig 2e), and there is a strong interaction between the treatment time and the enzyme concentration: with increasing the treatment time and increasing enzyme concentration there is a considerable decrease in production of fatty acid.

Determination of Optimum Condition

After finishing the suitable model for each variable, the optimum condition for system should be determine.
Under the optimum conditions, the principle variable should be determined in such a way that the response variables are as followed:
To have minimum weight loss, maximum dye exhaustion, maximum water absorption and minimum water absorption time.
Three optimum conditions were present considering time factor and concentration.
The calculated amount for each variable is shown in table 6.

Table 6. Optimum condition and amount of calculation for response variable

	time	Conce-ntration	Sqrt (weight loss)	Ln(moisture regain)	1/Sqrt(water absorbtion)	Dye absorption	Ln(fatty acid)
1	138.16	5.00	3.057	1.926	0.216	0.864259	6.935
2	137.54	5.00	3.050	1.926	0.215	0.86415	6.930
3	135.63	5.00	3.029	1.924	0.214	0.863813	6.915

Conclusion

Biotechnology in Textile Processing examines recent trends, techniques, and developments in the finishing and processing of natural fibers. The industry's foremost experts present current research findings on textile biotechnology, bio-treatment, and waste water management, with the emphasis on developing environmentally and friendly production of technologies that use enzymatic processes. In this kind of enzymes utilizing in the textile industry is becoming increasingly popular because of mild processing conditions and the capability for replacing harsh organic/inorganic chemicals.

Lipids removing of cotton fabric by lipase, a well known enzyme cause an increase in water and dye absorption considerably in an environmentally and friendly process, and it could be incorporated or even replace the conventional scouring process which uses chemicals and polluted waste waters. Enzymatic process should be stopped by a deactivation (changing pH and or temperature) process, because it may cause deterioration of physical or mechanical properties for example treating cellulose with cellulase may degrade cellulosic polymeric chains. But in the case of lipase with exhaustion of lipid source hydrolysis terminates. Measuring the amount of produced fatty acid could be considered as a suitable indicator for determining rate and the amount of lipid removed by lipase, it increases with time and after reaching its maximum level, it is reduced gradually due to the exhaustion of lipid source.

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