# Ecofriendly Approach to Improve Pectinolytic Reaction and Process Optimization of Bioscouring of Organic Cotton Textiles

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**ABSTRACT**
The present study focused on improvement of the enzymatic bioscouring performance by using specific mixed enzymes such as alkaline pectinase, protease, lipase and cellulase enzymes. An attempt was made to study the pectinolytic activity of degrading rate of pectin on organic cotton fabric and removal of wax at various enzymatic process conditions such as enzyme concentration, temperature, and time. These process variables were selected based on the artificial neural network (ANN) method. Output of the experiment resulted in fabric physical properties such as weight loss, water absorbency, wetting area, whiteness index, yellowness index, brightness index. The enzymatic scoured organic cotton fabric was tested for wax content and pectin degradation rate on the fabric and their results were optimized with minimum error. The test results were analyzed to predict the optimum process parameters to achieve the required bioscouring fabric properties and removal of pectin degrading rate, and their results were compared with actual trials.

**Keywords:** Organic cotton, Enzyme scouring, Pectin, Wax removal

## INTRODUCTION
Advances in biotechnology and enzymology have brought new lines of research and have accelerated the development of enzymatic applications in textile wet processing for nearly a decade [1-4]. Amongst the various stages of cotton preparation, textile wet processing is a highly energy, water, and chemical consuming processes [5-7]. Enzyme technology can be used to develop a usable, more environmental friendly, economically competitive scouring process. Several attempts were made to develop an enzymatic cotton scouring process [8-12]. Still this process faces several problems like a long incubation time, high enzyme doses, sometimes non-uniform enzyme action, uneven dyeing behavior, high temperature wax removal, and overall slow process speeds [13, 14]. The most important aspect identified was the inability to remove cotton fiber pectins and waxes.

The Global Organic Textile standard (GOTs) emerged as a result of a technical harmonization procedure for organic cotton processing. During the last few years GOTs has become the leading organic textile processing standard [15]. Demand is being driven by apparel and textile companies that are expanding their 100% organic cotton program and developing programs that blend small percentages of organic cotton with their conventional cotton products [16].

The need of the organic cotton fabric for textile wet processing is to process with minimum health-safe chemicals or alternatively way to use enzyme technology, because enzymes are substrate specific bio-catalysts; they operate best at ambient pressures, mild temperatures and often at a neutral pH range. Enzymes are gaining an increasingly important role as a tool in various wet textile pre-treatment and finishing processes [17-20]. Enzymatic scouring has been investigated extensively by various institutes and laboratories for nearly a decade [21-24]. Different enzymes like pectinases, such as lyases (EC 4.2.2.2); polygalacturonase endo acting type (EC 3.2.1.15) and polygalacturonase exo acting type (EC 3.2.1.67), proteases (EC 3.4.21-25), cellulases such as endoglucanases (EC 3.3.1.4); cellobiohydrolases (EC 3.2.1.91), xylanases (EC 3.2.1.8), lipases (EC 3.1.1.3) and recently cutinases (EC 3.1.1.74) have been examined to degrade and subsequently remove the natural component present in the outer layer of cotton fibers [25-27]. These studies incorporated staining tests, scanning electron microscopy (SEM), weight loss analysis, cotton wax residue and nitrogen content analysis.

Pectins are acidic polysaccharides, which are found in fruits, fibers and vegetables [28]. Pectin being a non-cellulosic material in cotton fibers plays several important roles. It contributes to the firmness and structure of cotton fiber, both as a part of the primary cell wall and as a component of the winding layer [29]. Pectin acts as cementing material for the cellulosic network in the primary wall [30, 31]. Pectin has a complex structure and comprised of α-(1, 4)-linked D-galacturonan backbone, occasionally interrupted by α-(1, 2)-linked α-L-rhamnopyranose residue. In cotton fibers, up to 60% of the galacturonic acid residues of the backbone are methyl esterified [32]. The most important reason identified was the inability to remove cotton fiber waxes during enzymatic scouring [33-37].

Pectinases have proved to be the most effective and suitable for cotton bioscouring. The mechanism of pectinase scouring reportedly assumes that the degradation and elimination of pectins makes the loosened waxes more easily removable with help of mechanical agitation. This allows the cotton to achieve superior hydrophilicity without fiber deterioration. A rational approach was adopted to design a new efficient enzymatic scouring process. Several aspects were considered such as the specificity of enzymes, the complexity of the cotton fiber substrate and mass transfer. Different commercial as well as specially produced pectinases were tested for bioscouring performance. Alkaline pectinases (PL and Bioprep 3000L) work better than acidic pectinases (PGs). The pectin removal efficiency of specially produced PL was comparable to commercial Bioprep 3000L. The most important parameters such as enzyme concentration, pH, temperature, ionic strength, chelators etc. for the bioscouring process were evaluated [38, 40]. The aim of this research was to study, the potential of enzyme technology to design an efficient and low-temperature scouring process for 100% organic cotton fabric.

## MATERIALS AND METHODS

### Materials

The grey organic cotton yarns of 2/40s Ne for warp and 40s Ne for weft yarns were procured from M/s. Arm strong mills (P) limited, Tirupur, India. These yarns were taken into weaving of plain organic cotton fabric using power loom. The 100% organic cotton fabric was produced with 64 ends per inch, 60 picks per inch, fabric cover factor of 18.96, and average fabric mass of 120.44 grams per square metre.

### Warp Yarn Sizing and Fabrication

The sizing of warp yarns of 100% organic cotton was carried out using a laboratory model yarn sizing machine using Polyvinyl alcohol starch size (PVA) The average size add-on the warp yarn was 12.21% The sized warp yarns were taken into warp beam preparation for weaving. The average size add-on on the organic cotton fabric was measured with respect to warp and weft yarn mass; it was noticed 8.27%. The aerial density of the organic cotton grey fabric after weaving was found 130.24 grams per square metre.

### Design of Enzymatic Treatment of Organic Cotton Fabrics

### Enzymatic Desizing

The PVA sized 100% organic cotton fabrics were treated with 3% concentration of alpha-amylase and process treatments at 60 deg C and 45 min reaction time. The process variables were chosen according to the Box-Behnken method of statistical tool for process optimization (Nabil Ibrahim 2004; Tatsuma Mori 1999). A systematic statistical approach was adopted to obtain optimum weight loss of the sized fabric with different process conditions of alpha amylase enzyme concentration, temperature, and treatment time for achieving a required level of 8.20% for efficient level of size removal. The enzymatic desizing process was carried out at pH 6-7 level and then the fabrics was thoroughly rinsed with hot water and cold water and dried at 80 deg C using a hot air oven and weighed using electronic balance with accuracy ±0.01grams.

### Enzymatic Scouring With Mixed Enzymatic System

In this study, the bioscouring of organic cotton fabric was carried out by selecting specific mixed enzymes namely (a) alkaline pectinase, (b) protease, (c) lipase, and (d) cellulase. These enzymes were specially screened, isolated and purified, the selective alkaline pectinase purified from Pectate Lyase was selected for the degradation of cotton pectin. Various experimental setups and techniques were applied in the enzymatic scouring experiments. All experiments were performed with demineralised water. Scouring experiments where performed in 1 L beaker in which three fabric samples of 10 × 10 cm were treated in an enzyme solution of different concentrations of 2-6%, non-ionic wetting agent of 1-2%, treatment time of 30 min, 45min, 60min and adjusted to pH of 8.5-9.0. The beaker was placed in a temperature controlled water bath at 50°C, 55°C and 60°C. After the treatment, the fabric samples were rinsed in 500 mL of water at 90°C for 15 minutes, to inactivate the enzymes. Thereafter the samples were rinsed twice for 5 minutes in water at room temperature. Finally, the samples were dried at 80°C using hot air oven and weigh the fabrics using electronic balance with accuracy ±0.01grams.

## Artificial Neural Network (ANN)

Neural networks are used for modeling non-linear problems and to predict the output values for a given input parameters from their training values. Most of the textile processes and the related quality assessments are non-linear in nature and hence neural networks find application in textile technology. ANNs are typically composed of interconnected "units" which serve as model neurons. The schematic diagram of typical ANN is shown in *Figure 1*. The function of the synapse is modeled by a modifiable weight, which is associated with each connection. Each unit converts the pattern of incoming activities that it reacts with into a single outgoing activity and then broadcasts it to other units. It performs this conversion in two stages. First, each incoming activity is multiplied by the weight on the connection and all these weighted inputs are added together to get a quantity called 'total input'. Secondly, an input-output function transforms the total input into an outgoing activity. The commonest type of ANN consists of three layers of units: (i) a layer of input units connected to (ii) a layer of hidden units, which in turn is connected to (iii) a layer of output units. The activity of the input units represents the raw information that is fed into the networks. The activity of each hidden unit is determined by the activities of the input units and weights on the connections between the input and the hidden units. Similarly, the behavior of the output units depends on the activity of the hidden units and the weights between the hidden and the output units.

## Development of Neural Networks

The software used in this study was backward feed propagation network. In order to carry out prediction, the network was trained with training patterns namely input and output parameters. Input and output parameters used for training the ANN and their selection criteria are given below.

### Input Parameters

- (i) Enzyme concentration
- (ii) Process time
- (iii) Process temperature

### Output Parameters

- (i) Fabric weight loss
- (ii) Fabric water absorbency
- (iii) Fabric wetting area
- (iv) Fabric whiteness index
- (v) Fabric yellowness index
- (vi) Fabric brightness index

## Training of Neural Network

For training, the organic cotton fabrics were treated with various enzyme concentration, time, and temperatures with specific mixed enzyme system. Then the physical characteristics such as fabric weight loss, water absorbency, wetting area, whiteness index, yellowness index, and brightness index of the organic cotton fabrics were evaluated with standard testing procedures and their values were trained by using feed backward propagation algorithm. The logic behind the ANN is to execute the three input variables and six output variables with minimum error and predict the results. For the error back propagation net, the sigmoid function is essentially for non linear function. Training process of the neural network developed was started with 5000 preliminary cycles to optimize the ANN prediction accuracy. The best structure is one that gives lowest training error and it is found to be minimum error percent. The training of the network was further continued in order to reduce the training error. The average training error of 1% was obtained and terminated at this stage since beyond this reduction in training error was not appreciable.

## Testing of Neural Network

For testing the prediction accuracy of the neural network, a known specifications and process parameters were evaluated and their error percentage was compared with predicted sample values. It can be observed that mean absolute error with respect to prediction is around 1%. *Figures 1 and 2* represent schematic diagrams of typical ANN and selective neural network training of specific mixed enzymatic systems and their performance levels respectively.

## Practical Application of the Developed Neural Network

When the target of the physical properties of the organic cotton fabrics is predetermined then we can start the input parameters such as the enzyme concentration, time and temperature of the process condition which will predict the desired quality of the bioscoured organic cotton fabric properties in specific mixed enzymatic systems.

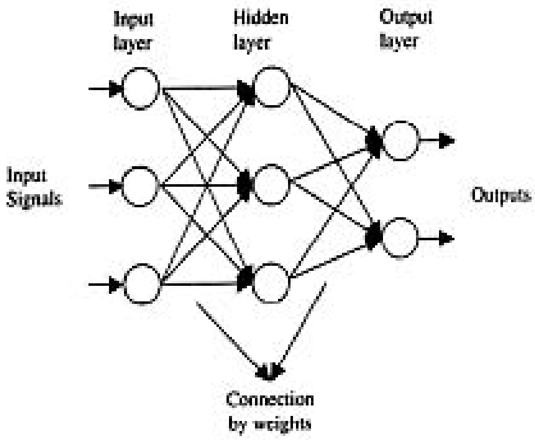



FIGURE 1. Schematic diagram of typical ANN.

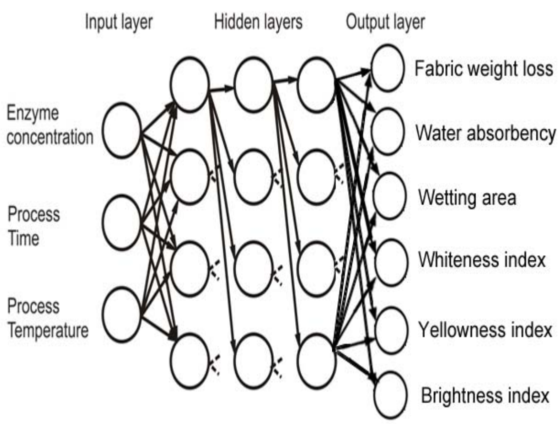



FIGURE 2. Schematic diagram of ANN used in bioscouring of organic cotton fabric.

**Testing**

**Color Spectroscopy Analysis**

The whiteness index, yellowness index, and brightness index values of the bioscoured organic cotton fabric samples were measured using JAYPAK Color Spectroscopy (Model 4800) with CIE 76, observer 10 degree at D65 light source in the range between 400nm and 700nm.

The Brightness Index (ISO-2470-1977) was calculated using the following formula,

$$\text{Brightness Index} = \frac{\text{Reflectance value of the substrate at 457 nm}}{\text{Reflectance value of the standard white tile at 457 nm}} \times 100 \qquad (1)$$

**FTIR-Fourier Transform Infrared Spectroscopy**

The organic cotton fabrics with and without alkaline pectinase enzyme treatment are analyzed using FTIR Spectrometer – Model: 8400S, Make: Shimadzu.

**Ruthenium Red Dye – Pectin Determination**

The pectin removal of the enzyme treated with alkaline pectinase on organic cotton fabrics was carried out as per procedure [39]. K/S was 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 fibers, the lower the K/S value is, the less is the pectic and proteinic substances present in cotton fiber [40].

**Fabric Water Absorbency**

Water absorbency of organic cotton fabric treated with alkaline pectinase was evaluated according to AATCC test method 79-2000.

**Wax Content**

The wax content of the grey organic cotton fabric and alkaline pectinase treated fabrics were carried out as per AATCC test method 97-2009 (revised) by solvent extraction using Soxhlet apparatus.

**Weight Loss**

After the enzymatic treatments, the weight losses of the bioscoured organic cotton fabrics were inspected. The amount of weight losses were calculated according to the following formula:

$$\%WL = (W_1-W_2) * 100 / W_1 \qquad (3)$$

Where

$W_1$ – the weight of fabric before enzymatic treatment.

$W_2$ – the weight of fabric after enzymatic treatment.

**RESULTS AND DISCUSSION**

**Fabric Weight Loss – Effect of Enzymatic Process Variables**

The response surface methodology is an empirical modeling technique, which is used to evaluate the relationship between a set of controllable experimental factors and observed results. Several factors critically influence the enzymatic process of organic cotton such as enzyme concentration; temperature and time. The effect of enzyme concentration and temperature on weight loss of organic cotton fabrics at various time intervals of (a) 30 min, (b) 45 min and (c) 60 min are shown as 3D surface plots in *Figure 3*.

**Effect of Enzyme Concentration and Temperature**

*Figure 3* presents the effect of enzyme concentration and temperature on the weight loss of the alkaline pectinase enzyme treated organic cotton fabrics at various reaction times. With increase in enzyme concentration and temperature there is an increase in fabric weight loss at both lower and higher reaction time intervals but at higher time duration there is higher rate of pectin and wax hydrolysis with increase in enzyme concentration. An interesting observation noticed during the trials is that the organic cotton fabric was having maximum weight loss of 3.2% and above at higher enzyme concentration of 6%, 60 minutes time and 60 $^0$C temperature.

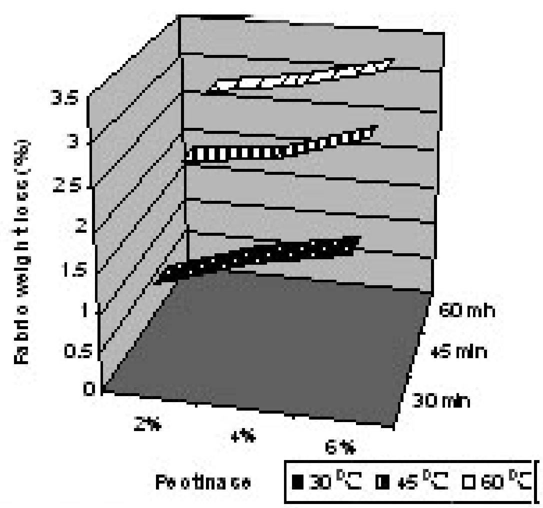



FIGURE 3. Fabric weight loss of bioscoured organic cotton fabric treated with various process conditions of pectinase enzyme.

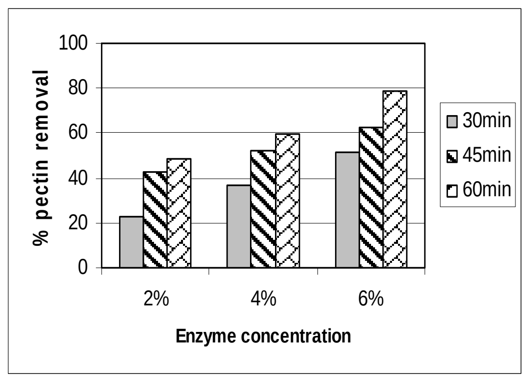
**Improvement of Pectinolytic Reaction Pectin Degradation Analysis**

*Figure 4 (a)* shows that the pectin degradation level of organic cotton fabric treated with various process conditions of time and temperature with 2-6% alkaline enzyme concentration. It was observed that the rate of pectin removal increased with increase in enzyme concentration and higher time and temperature. *Figure 4 (b)* shows the enzyme kinetics of alkaline pectinase treatment on organic cotton fabric at various concentrations with time interval of 30, 45, 60 minutes for pectin removal. It was noticed that the higher pectin removal observed at 60 deg C and 60 min treated pectinase organic cotton fabric was 82.41%, and in addition of efficient wax removal on the organic cotton fabric improves the performance of pectinase in terms of pectin removal and hydrophilicity. The regression equation for evaluating the pectin degradation rate on the organic cotton fabrics is given in *Table I*. From *Table I*, the rate of pectin removal % on the organic cotton fabric depends on the alkaline pectinase enzyme concentration and reaction time to break the pectin components.

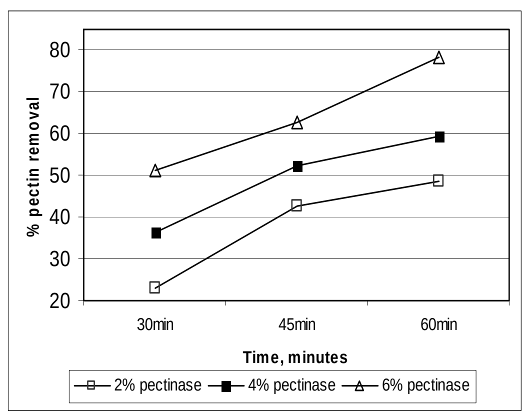
TABLE I. Regression equation of pectin degradation of organic cotton fabrics.

| Enzyme conc. | Regression equation | Correlation coefficient |
|---|---|---|
| 2% | $Y_1=2.465X_1^2-0.515X_1+33.69$ | $R^2=1$ |
| 4% | $Y_2=2.691X_2^2-2.315X_2+46.20$ | $R^2=1$ |
| 6% | $Y_3=2.465X_3^2-0.515X_3+33.69$ | $R^2=1$ |

Note: X-reaction time and Y- pectin removal (%)




(a)




(b)

FIGURE 4. Pectin degradation of alkaline pectinase treated organic cotton fabric (a) various enzyme concentration at 60 deg C and (b) enzyme kinetics – interaction of pectin degradation rate at 60 deg C.

**Wax removal - Alkaline Pectinase**

The wax content of alkaline pectinase enzyme treated organic cotton fabric is shown in *Figure 5*. It was observed that the wax present in the grey organic cotton fabric was 0.81% and subsequent alkaline pectinase treated organic cotton fabric at 2%, 4% and 6% enzyme concentrations at 60 deg temperature and 45 min reaction time noticed 0.52%, 0.42% and 0.37% respectively. It was noticed that the wax removal rate on the organic cotton fabric was 35.80%, 48.15% and 54.32% for corresponding process conditions. It may be due to alkaline pectinase enzyme degrading the pectin component in the organic cotton fiber which hydrolysis the wax component in the fiber.

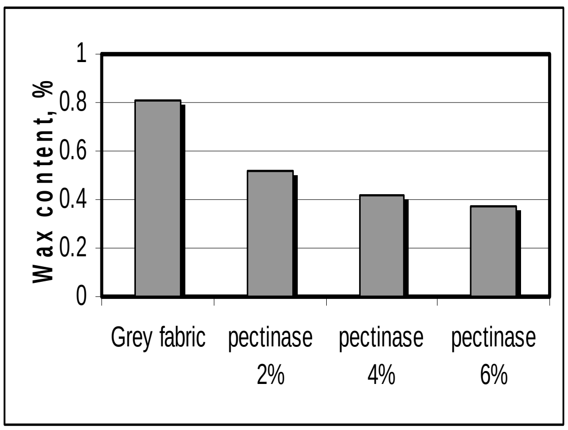



FIGURE 5. Wax content of alkaline pectinase treated organic cotton fabric at 60 deg C and 45 min reaction time.

**FTIR Spectroscopic Analysis**

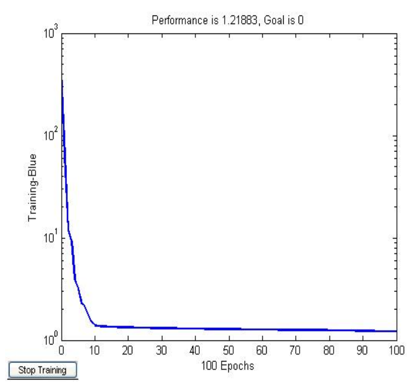
The FTIR spectra of the desized organic cotton fabric and 2%, 4%, 6% pectinase enzyme treated cotton fabrics was carried out using FT-IR Spectrometer – Model: 8400S, Make: Shimadzu. It mainly highlights changes in the non-cellulosic impurities by characterizing the carboxyl acids and esters that are present in pectin and waxes. It can be clearly understood that the presence of cellulose group peaks around 1000-1200cm$^{-1}$ and integrity of the pectin and wax compounds in the organic cotton fabric at 1736cm$^{-1}$ and 1617cm$^{-1}$ respectively [9, 11]. The hydrolysis of the pectin during alkaline pectinase enzymatic treatment (D) at 6% concentration and 45min reaction time of the organic cotton fabric showed the removal of pectin and wax groups in the specimen at 3315 cm$^{-1}$ which was responsible for –OH group stretching, the CH stretching at 2917 cm$^{-1,}$ the asymmetrical COO- stretching at 1617 cm$^{-1}$, and CH wagging at 1316 cm$^{-1}$ [13, 19] The transmittance intensity of the characteristics peaks at around 1736 cm$^{-1}$ varied in the following order: desized fabric > 2% pectinase > 4% pectinase > 6% pectinase fabrics. The test results, the transmittance (%) of the pectinase enzyme treated organic cotton fabrics are noticed lower level when compared to desized fabric which was due to the degradation of pectin, waxes and non-cellulosic compounds while pectinolytic degradation. The residual non-cellulosic components were analyzed after enzyme treatment using FTIR reports by differentiating the transmittance (%) wave length which was given in *Table II.* From the test results, the peaks at 1058cm$^{-1}$, 1112cm$^{-1}$ and 2362cm$^{-1}$ groups are responsible for C-C stretch from phenyl ring, -$CH_2$ symmetric stretching and C-H stretching in the alkaline pectinase treated organic cotton fabrics [25, 40].

TABLE II. FTIR test results of organic cotton fabrics and their transmittance values.

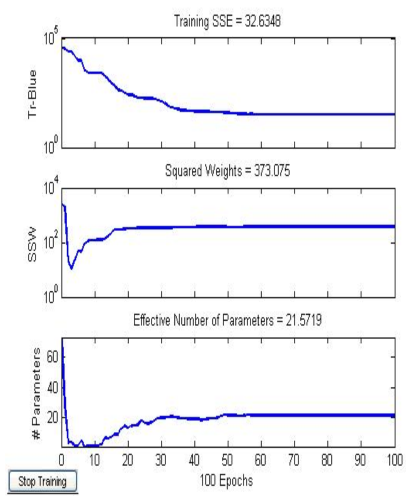
| Wave length, cm-1 | Transmittance, % | | Difference, % |
|---|---|---|---|
| | Desized fabric | 6% alkaline pectinase treated fabric | |
| 559 | 45.8 | 48.2 | 5.24 |
| 617 | 45.2 | 47.2 | 4.42 |
| 667 | 45.8 | 47.8 | 4.37 |
| 898 | 53.4 | 57.4 | 7.49 |
| 1058 | 34.8 | 35.3 | 1.44 |
| 1112 | 35.3 | 37.4 | 5.95 |
| 1165 | 37.5 | 38.2 | 1.87 |
| 1371 | 25.1 | 27.6 | 9.96 |
| 1431 | 44.8 | 46.2 | 3.13 |
| 1617 | 52.2 | 57.2 | 9.58 |
| 1736 | 54.3 | 62.3 | 14.73 |
| 2362 | 46.8 | 47.8 | 2.14 |
| 2917 | 36.3 | 37.8 | 4.13 |
| 3415 | 22.7 | 23.2 | 2.20 |

**ANN Process Optimization - Specific Mixed Enzymatic System**

The various specific enzymes such as alkaline pectinase, protease, lipase and cellulase enzymes with process variables such as enzyme concentrations, temperature and reaction time was optimized using MATLAB 7.0 software with neural network experimental design and their output values are executed. *Table III to IV* represent the selective mixed enzyme concentrations, process variables for training sample of bio scoured organic cotton fabrics of their input values and *Table V and VI* output results of actual fabric properties (trained) and *Table VII and VIII* represents the predicted fabric properties from MATLAB 7.0 respectively. The software was executed to get various options / predicted process parameters to achieve required pectin degradation range of 75-82% and weight loss of the fabric 4.80%. The software was processed for analyzing the performance and desirability of FDS-Fraction of Design Space of design model of process which is shown in *Figure 7 (a)* and (b) respectively for optimized test results. FDS plots are adopted to evaluate designs for generalized linear models for predicting the output results. The output result of the software to achieve the desired bioscouring of organic cotton fabric on their physical properties such as fabric weight loss, water absorbency, wetting area, whiteness index, yellowness index, and brightness index in the specific enzymatic system, out of which the software opted best process conditions of specific mixed enzymes of sample 19, which was treated with 8% alkaline pectinase, 3%protease, 0.8%lipase and 0.8%cellulase process condition at temperature of 55 deg C and reaction time 60 minutes, pH 8.5with 1.0% desirability. From the best opted test results, the actual pectin and weight loss of the bioscoured organic cotton fabric was achieved 78.40% and 4.80% respectively with error of 1.218%. Table IX and X represent the ANN error % of fabric properties compared with actual and predicted values.




(a)




(b)

FIGURE 7. Neural network training of mixed enzymatic system for analyzing (a) their performance level, and (b) FDS –Fraction of Design Space level.

TABLE III. Artificial Neural Network - Training samples (Enzyme concentration).

| S.No | Input data, Specific Mixed Enzymes Concentrations (%) | | | |
|---|---|---|---|---|
| | Pectinase | Protease | Lipase | Cellulase |
| S1 | 4 | 2 | 0.4 | 0.4 |
| S2 | 4 | 2 | 0.6 | 0.6 |
| S3 | 4 | 2 | 0.8 | 0.8 |
| S4 | 6 | 3 | 0.4 | 0.4 |
| S5 | 6 | 3 | 0.6 | 0.6 |
| S6 | 6 | 3 | 0.8 | 0.8 |
| S7 | 6 | 2 | 0.6 | 0.8 |
| S8 | 2 | 2 | 0.4 | 0.4 |
| S9 | 2 | 1 | 0.6 | 0.6 |
| S10 | 2 | 3 | 0.8 | 0.8 |
| S11 | 2 | 1 | 0.4 | 0.4 |
| S12 | 0 | 3 | 0.6 | 0.6 |
| S13 | 0 | 1 | 0.4 | 0.4 |
| S14 | 6 | 0 | 0.8 | 0.4 |
| S15 | 4 | 1 | 0.6 | 0.6 |
| S16 | 6 | 1 | 0.4 | 0 |
| S17 | 4 | 2 | 0.1 | 0 |
| S18 | 0 | 2 | 0.8 | 0 |
| S19 | 8 | 3 | 0.8 | 0.8 |
| S20 | 8 | 1 | 0.2 | 0 |
| S21 | 8 | 3 | 0 | 0.4 |
| S22 | 6 | 2 | 0 | 0.8 |
| S23 | 2 | 2 | 0.6 | 0.4 |
| S24 | 2 | 3 | 0.6 | 0.4 |
| S25 | 2 | 2 | 0.4 | 0.8 |
| S26 | 4 | 3 | 0.6 | 0.4 |
| S27 | 4 | 3 | 0.4 | 0 |
| S28 | 4 | 3 | 0.8 | 0.4 |
| S29 | 8 | 4 | 0.8 | 0.4 |
| S30 | 0 | 3 | 0 | 0.4 |

TABLE IV. Artificial Neural Network - Training samples (Process parameters).

| S.No | Input data, Process parameters | | |
|---|---|---|---|
| | Time | Temp | pH |
| S1 | 30 | 55 | 8.5 |
| S2 | 30 | 55 | 9.5 |
| S3 | 30 | 60 | 8.5 |
| S4 | 30 | 60 | 9 |
| S5 | 45 | 55 | 8.5 |
| S6 | 45 | 60 | 9 |
| S7 | 45 | 60 | 8.5 |
| S8 | 45 | 60 | 9 |
| S9 | 30 | 55 | 8 |
| S10 | 60 | 60 | 9 |
| S11 | 60 | 60 | 8 |
| S12 | 60 | 55 | 8.5 |
| S13 | 60 | 55 | 9.5 |
| S14 | 45 | 60 | 9 |
| S15 | 45 | 60 | 9 |
| S16 | 30 | 60 | 9.5 |
| S17 | 45 | 60 | 8.5 |
| S18 | 60 | 55 | 9 |
| S19 | 60 | 55 | 9.5 |
| S20 | 30 | 60 | 8.5 |
| S21 | 45 | 60 | 9.5 |
| S22 | 60 | 55 | 8.5 |
| S23 | 60 | 60 | 9 |
| S24 | 45 | 55 | 9 |
| S25 | 45 | 60 | 8 |
| S26 | 30 | 55 | 8.5 |
| S27 | 45 | 55 | 8.5 |
| S28 | 45 | 55 | 8.5 |
| S29 | 30 | 60 | 9.5 |
| S30 | 45 | 60 | 8 |

## Fabric Whiteness Index

From *Table III*, the highest whiteness index of bioscoured organic cotton fabric with specific mixed enzymatic system is achieved 52.57% at 8% pectinase, 4% protease, 0.8% lipase and 0.4% cellulase enzyme at 60 min reaction time, 60 deg C and pH 9.0 (Sample no.29). It may be due to better integration and higher concentration of pectinase for removal of pectin up to 83.2% and wax/oil component removal up to 92.4% on the organic cotton bioscoured fabrics which have higher water absorbency and lower yellowness in nature when compared to sample No.18. The mixed enzymes such as pectinase, protease and lipase plays a important role for removal of pectin and wax/oil components and also cellulase enzyme supports the exo and endo partial surface reaction of the organic cotton fabrics.

TABLE V. ANN Output data, Trained Samples Fabric Properties (Actual).

| S.No | Output data, Trained Samples Fabric Properties (Actual) | | |
|---|---|---|---|
| | Fabric Weight loss (%) | Fabric Water absorbency (sec) | Fabric Wetting area ($mm^2$) |
| S1 | 2.8 | 3.2 | 70 |
| S2 | 3.1 | 2.4 | 114 |
| S3 | 4.4 | 1.8 | 240 |
| S4 | 4.1 | 1.4 | 238 |
| S5 | 4.3 | 1.2 | 247 |
| S6 | 4.4 | 1.2 | 251 |
| S7 | 4.7 | 0.8 | 330 |
| S8 | 2.7 | 6.2 | 65 |
| S9 | 2.4 | 5.8 | 64 |
| S10 | 3.8 | 4.8 | 67 |
| S11 | 2.4 | 5.7 | 72 |
| S12 | 2.7 | 7.2 | 45 |
| S13 | 2.9 | 8.9 | 51 |
| S14 | 3.8 | 3.1 | 114 |
| S15 | 4.5 | 3.4 | 120 |
| S16 | 3.1 | 4.2 | 74 |
| S17 | 2.7 | 4.4 | 70 |
| S18 | 2.2 | 8.8 | 61 |
| S19 | 4.8 | 1.2 | 250 |
| S20 | 2.8 | 2.5 | 85 |
| S21 | 4.1 | 1.8 | 264 |
| S22 | 4.2 | 1.4 | 228 |
| S23 | 3.1 | 4.8 | 260 |
| S24 | 3.4 | 4.6 | 268 |
| S25 | 3.3 | 4.2 | 250 |
| S26 | 3.8 | 2 | 300 |
| S27 | 3.6 | 1.8 | 310 |
| S28 | 3.5 | 2 | 280 |
| S29 | 4.9 | 1.6 | 292 |
| S30 | 2.8 | 8.7 | 48 |

TABLE VI. ANN Output data, Trained Samples Fabric Properties (Actual).

| S.No | Output data, Trained Samples Fabric Properties (Actual) | | |
|---|---|---|---|
| | Fabric Whiteness Index (WI) | Fabric Yellow Index (YI) | Fabric Bright Index (BI) |
| S1 | 26.152 | 22.142 | 54.147 |
| S2 | 26.452 | 21.831 | 56.387 |
| S3 | 27.320 | 20.392 | 58.947 |
| S4 | 32.520 | 20.314 | 57.324 |
| S5 | 34.378 | 20.132 | 58.147 |
| S6 | 38.415 | 17.742 | 63.436 |
| S7 | 36.137 | 19.241 | 61.524 |
| S8 | 27.860 | 20.690 | 51.580 |
| S9 | 28.600 | 23.547 | 52.407 |
| S10 | 27.690 | 22.641 | 53.452 |
| S11 | 27.831 | 23.640 | 49.860 |
| S12 | 24.580 | 23.654 | 49.631 |
| S13 | 23.980 | 23.972 | 51.250 |
| S14 | 37.860 | 17.850 | 52.368 |
| S15 | 39.647 | 19.640 | 53.240 |
| S16 | 32.078 | 20.068 | 59.378 |
| S17 | 28.520 | 20.127 | 58.418 |
| S18 | 22.413 | 24.371 | 43.436 |
| S19 | 52.413 | 13.140 | 68.715 |
| S20 | 51.452 | 14.857 | 51.450 |
| S21 | 52.470 | 14.250 | 58.670 |
| S22 | 47.680 | 15.857 | 53.480 |
| S23 | 27.650 | 19.561 | 52.687 |
| S24 | 26.780 | 20.450 | 52.681 |
| S25 | 27.540 | 21.580 | 51.240 |
| S26 | 30.681 | 18.640 | 54.890 |
| S27 | 29.740 | 19.564 | 59.570 |
| S28 | 30.450 | 17.698 | 64.580 |
| S29 | 52.570 | 13.800 | 67.480 |
| S30 | 23.147 | 22.413 | 54.138 |

TABLE VII. ANN Output data, Fabric Properties (Predicted) from MAT LAB 7.0

| S.No | Output data, Trained Samples Fabric Properties (predicted) | | |
|---|---|---|---|
| | Fabric Weight loss (%) | Fabric Water absorb (sec) | Fabric Wetting area ($mm^2$) |
| S1 | 2.874 | 3.087 | 72 |
| S2 | 2.989 | 2.453 | 119 |
| S3 | 4.325 | 1.837 | 220 |
| S4 | 4.090 | 1.472 | 218 |
| S5 | 4.242 | 1.277 | 261 |
| S6 | 4.394 | 1.249 | 248 |
| S7 | 4.636 | 0.907 | 321 |
| S8 | 2.856 | 6.334 | 57 |
| S9 | 2.497 | 5.935 | 61 |
| S10 | 3.863 | 4.478 | 68 |
| S11 | 2.380 | 6.011 | 70 |
| S12 | 2.811 | 7.844 | 51 |
| S13 | 2.994 | 8.284 | 52 |
| S14 | 3.904 | 2.795 | 117 |
| S15 | 4.419 | 3.772 | 121 |
| S16 | 3.176 | 4.009 | 72 |
| S17 | 2.605 | 4.676 | 71 |
| S18 | 2.392 | 8.196 | 63 |
| S19 | 4.888 | 1.299 | 248 |
| S20 | 2.958 | 2.297 | 78 |
| S21 | 4.162 | 1.967 | 248 |
| S22 | 4.334 | 1.390 | 218 |
| S23 | 3.009 | 4.953 | 261 |
| S24 | 3.405 | 4.697 | 270 |
| S25 | 3.491 | 4.612 | 251 |
| S26 | 3.6123 | 2.034 | 298 |
| S27 | 3.437 | 1.846 | 304 |
| S28 | 3.654 | 2.089 | 283 |
| S29 | 4.860 | 1.665 | 290 |
| S30 | 2.504 | 8.496 | 51 |

TABLE VIII. ANN Output data, Fabric Properties (Predicted) from MAT LAB 7.0.

| S.No | Output data, Trained Samples Fabric Properties (predicted) | | |
|---|---|---|---|
| | Fabric Whiteness Index (WI) | Fabric Yellow Index (YI) | Fabric Bright Index (BI) |
| S1 | 28.876 | 21.927 | 57.967 |
| S2 | 28.875 | 21.321 | 58.035 |
| S3 | 28.876 | 21.969 | 57.995 |
| S4 | 33.088 | 19.358 | 57.986 |
| S5 | 34.525 | 17.173 | 57.992 |
| S6 | 38.309 | 17.614 | 58.014 |
| S7 | 35.948 | 18.752 | 57.449 |
| S8 | 28.767 | 22.018 | 57.035 |
| S9 | 28.871 | 21.969 | 50.622 |
| S10 | 27.613 | 20.760 | 57.857 |
| S11 | 29.115 | 22.248 | 48.501 |
| S12 | 24.595 | 23.726 | 47.556 |
| S13 | 23.967 | 24.081 | 51.375 |
| S14 | 38.269 | 17.423 | 52.761 |
| S15 | 34.786 | 20.005 | 55.319 |
| S16 | 31.654 | 19.884 | 55.734 |
| S17 | 28.250 | 20.356 | 54.672 |
| S18 | 22.399 | 24.072 | 46.168 |
| S19 | 52.821 | 15.298 | 58.034 |
| S20 | 51.297 | 16.277 | 53.446 |
| S21 | 52.869 | 13.987 | 55.547 |
| S22 | 47.687 | 17.588 | 53.967 |
| S23 | 28.970 | 20.801 | 56.661 |
| S24 | 29.102 | 20.768 | 57.901 |
| S25 | 23.256 | 22.293 | 56.263 |
| S26 | 28.878 | 19.838 | 58.030 |
| S27 | 29.302 | 19.855 | 58.031 |
| S28 | 29.167 | 17.626 | 57.948 |
| S29 | 51.879 | 13.788 | 58.0546 |
| S30 | 22.983 | 21.973 | 57.1424 |

TABLE IX. ANN Error %, Fabric properties Predicted from MAT LAB 7.0.

| S.No | Output data, Trained Samples Fabric Properties (predicted) | | |
|---|---|---|---|
| | Fabric Weight loss (%) | Fabric Water absorb (sec) | Fabric Wetting area ($mm^2$) |
| S1 | -2.657 | 3.506 | -2.857 |
| S2 | 3.574 | -2.246 | -4.385 |
| S3 | 1.695 | -2.083 | 8.333 |
| S4 | 0.239 | -5.150 | 8.403 |
| S5 | 1.349 | -6.425 | -5.668 |
| S6 | 0.127 | -4.108 | 1.195 |
| S7 | 1.347 | -13.35 | 2.727 |
| S8 | -5.800 | -2.161 | 12.307 |
| S9 | -4.042 | -2.334 | 4.687 |
| S10 | -1.671 | 6.690 | -1.492 |
| S11 | 0.829 | -5.460 | 2.777 |
| S12 | -4.133 | -8.949 | -13.33 |
| S13 | -3.266 | 6.912 | -1.960 |
| S14 | -2.747 | 9.835 | -2.631 |
| S15 | 1.780 | -10.92 | -0.833 |
| S16 | -2.468 | 4.543 | 2.702 |
| S17 | 3.504 | -6.293 | -1.428 |
| S18 | -8.735 | 6.858 | -3.278 |
| S19 | -1.848 | -8.300 | 0.8 |
| S20 | -5.661 | 8.096 | 8.235 |
| S21 | -1.534 | -9.300 | 6.060 |
| S22 | -3.195 | 0.657 | 4.385 |
| S23 | 2.929 | -3.202 | -0.384 |
| S24 | -0.163 | -2.115 | -0.746 |
| S25 | -5.812 | -9.814 | -0.4 |
| S26 | 4.939 | -1.710 | 0.666 |
| S27 | 4.522 | -2.565 | 1.935 |
| S28 | -4.420 | -4.475 | -1.071 |
| S29 | 0.802 | -4.075 | 0.684 |
| S30 | 10.543 | 2.337 | -6.25 |
| Ave | -0.665 | -2.188 | 0.639 |

TABLE X. ANN Error %, Fabric properties Predicted from MAT LAB 7.0.

| S.No | Output data, Trained Samples Fabric Properties (predicted) | | |
|---|---|---|---|
| | Fabric Whiteness Index (WI) | Fabric Yellow Index (YI) | Fabric Bright Index (BI) |
| S1 | -10.41 | 0.967 | -7.055 |
| S2 | -9.160 | 2.332 | -2.923 |
| S3 | -5.696 | -7.733 | 1.615 |
| S4 | -1.747 | 4.705 | -1.156 |
| S5 | -0.428 | 14.697 | 0.265 |
| S6 | 0.274 | 0.719 | 8.545 |
| S7 | 0.520 | 2.540 | 6.623 |
| S8 | -3.256 | -6.421 | -10.57 |
| S9 | -0.947 | 6.698 | 3.404 |
| S10 | 0.274 | 8.307 | -8.242 |
| S11 | -4.614 | 5.886 | 2.724 |
| S12 | -0.061 | -0.307 | 4.179 |
| S13 | 0.054 | -0.455 | -0.244 |
| S14 | -1.081 | 2.388 | -0.751 |
| S15 | 12.259 | -1.858 | -3.905 |
| S16 | 1.321 | 0.916 | 6.136 |
| S17 | 0.946 | -1.139 | 6.411 |
| S18 | 0.058 | 1.223 | -5.112 |
| S19 | -0.780 | -16.42 | 15.543 |
| S20 | 0.299 | -9.561 | -3.880 |
| S21 | -0.761 | 1.839 | 5.321 |
| S22 | -0.016 | -10.92 | -0.912 |
| S23 | -4.777 | -6.342 | -7.543 |
| S24 | -8.671 | -1.559 | -9.908 |
| S25 | 15.554 | -3.305 | -9.803 |
| S26 | 5.875 | -6.429 | -5.721 |
| S27 | 1.472 | -1.489 | 2.582 |
| S28 | 4.213 | 0.403 | 10.26 |
| S29 | 1.314 | 0.083 | 13.96 |
| S30 | 0.704 | 1.961 | -5.549 |
| Ave | -0.242 | -0.609 | 0.143 |

**Fabric Yellowness Index**

From *Table III*, the lowest yellowness index of the bioscoured organic cotton fabric with specific enzymatic system is achieved 13.14% at 8% pectinase, 3% protease, 0.8% lipase and 0.8% cellulase of sample no.19 treated at 60 min reaction time, 55 deg C and pH 9.5. It may be due to higher removal of pectin and wax component in the organic cotton fabric in the enzymatic system which has whiteness index of 52.413. It is noticed that highest whiteness index of organic cotton fabric show lower yellowness index in all the treated fabrics. For sample no.18 which has highest yellowness index of 24.371% due to absence of pectinase and cellulase enzymes, 2% protease and 0.8% lipase. From the test results, the [pectinase plays important role in removal of pectin for lowing the yellowness index of fabric and cellulase plays the better mixed enzyme reaction on the organic cotton fabric during bioscouring.

**Fabric Brightness Index**

From *Table III*, the highest fabric brightness in bioscoured organic cotton fabric was found in the sample no.19 which was treated with 8% pectinase, 3% protease, 0.8% lipase and 0.8% cellulase at 60 minute time, 55 deg C, and pH 8.5. It may be due to higher whiteness of 52.413 and lower yellowness index of 13.14 and fabric treated higher pectinase and cellulase concentrations. It was also noticed that higher concentration of cellulase enzyme treated fabric observed higher brightness index due to surface smoothness of the organic cotton fabric. The lowest brightness index of organic cotton fabric was noticed in sample no.18, it was treated with absence of pectinase and cellulase, 2% protease and 0.8% lipase enzyme conditions. It was noticed that pectinase and cellulase enzymes plays important role in brightness index of the bioscoured organic cotton fabrics.

**Fabric Water Absorbency**

From *Table III,* the water absorbency (sec) of bioscoured organic cotton fabric was noticed better in sample no.7 which was treated with 6% pectinase, 2%protease, 0.6% lipase and 0.8% celluase at 45min reaction time, 60 deg C, and pH 8.5. it may be due to higher removal of wax/oil component in the organic cotton upto 84.2% and presence of cellulase enzyme which improves partial surface hydrophilic nature in the organic cotton fabric. The highest time in sec for water absorbency of the organic cotton fabric was noticed in sample no.18 which has treated with absence of pectinase and protease enzymes. These enzymes are playing important role in the fabric water absorbency by removal of pectin and wax components and also noticed improved water absorbency by adding the cellulase enzyme in the bioscouring process.

## CONCLUSIONS AND PROSPECTIVE RESEARCH

The process optimization of bioscouring of 100% organic cotton fabric through enzyme technology has been studied with selective specific mixed enzymatic system using four enzymes namely alkaline pectinase, protease, lipase and cellulase. The process variables such as enzyme concentration, temperature and reaction time was optimized to achieve the required water absorbency and pectin removal during bioscouring process on the organic cotton fabrics. The alkaline pectinase enzymes are better active and catalyze the degradation of pectin at temperature range of 55-60 deg C and time of 45min to achieve required level of 75-80% pectin degradation. The pH of the process bath is also a major influence for better reaction of enzyme to catalyze the hydrolysis of pectin groups. The higher enzyme concentration at 6% level and higher temperature of 60 deg C took lesser time to achieve required pectin hydrolysis. Process variables are optimized using MATLAB 7.0 and it will pave the way to predict the enzyme kinetics at various concentrations, temperature and reaction time to achieve required degradation of pectin with minimum error %. This study will be helpful to the organic cotton processors for the eco-friendly and sustainable textile wet processing using specific mixed enzymatic system in bioscouring processes.

**Industrial Importance**

This study will provide the industrial bio-scouring technologies an insight into the properties of alkaline pectinase based mixed enzymatic systems and the predictability of their scouring performance while deciding the recipe and process parameters.

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