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Research Article

# A SUSTAINED GREEN SCOURING PROCESS OF COTTON USING IMMOBILIZED PECTINASE ON IRON OXIDE NANOPARTICLES AND ITS REUSABILITY

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## ABSTRACT

Scouring, a vital process in textile wet processing which removes hydrophobicity nature of the fabrics by removing natural matters and other impurities that imparts hydrophilic property. In this present study, Scouring was performed with free enzyme and pectinase immobilized iron oxide nanoparticles. Iron oxide nanoparticles were synthesized by co-precipitation method and immobilized with pectinase that was characterized by the Scanning Electron Microscope and Fourier Transform Infrared Spectroscopy showed 35 and 86 nm size of control and enzyme immobilized iron oxide nanoparticles and the presence of enzyme functional groups respectively. Scouring parameters were optimized and the scouring was done successfully. Physical and functional properties of the scoured fabrics were analyzed where immobilized enzyme treated fabrics showed higher weight loss 7.5%, 1 sec wettability and 44, 36 lbs tensile strength under optimized condition cycles than conventionally scoured fabrics as control and merely equal to free enzyme treated fabrics. Operational stability of enzyme immobilized nanoparticles was determined and losses its 50% efficiency in $4^{th}$ cycle having 3.2% weight loss with 14 sec wettability. Surface topographical studies of scoured cotton fabrics by SEM had shown that the presence of rough and open primary wall with high protrusions in control whereas presence of lower protrusions in both enzymatic and immobilized enzyme scoured fabrics was found. Absence of impurities was confirmed by functional groups detected by FTIR would strongly affirm the good enough efficiency of treated fabrics.

**Key words:** Scouring, iron oxide nanoparticles, Pectinase, SEM, FTIR, Operational stability

## INTRODUCTION

Textile industry is the main sector of industries where cotton is the top grade raw material in a textile industry still alluded as “Ruler” of fibers[1]. Cotton fibers are naturally having dirt, seed fragments, chemical pesticides residues and impurities such as hemicellulose, pectin, lignin, fats, waxes and protein etc., that solely responsible for hydrophobicity[2]. Scouring, a vital process in wet processing provides hydrophilic nature to the fabrics by removing non-cellulosic natural matters and other impurities stated above[3]. Traditional scouring required high temperature that damage cotton structure, extensive rinsing process, effluent loaded with chemicals, increased oxygen demand levels where environment got affected[4]. As mentioned earlier, the implementations of toxic chemicals used in wet processing of textile goods that contact with the skin causes cancer, hypersensitivity reactions, as a whole it is chemical intensive solely accountable for causing global pollution. To meet this challenge, textile industries now turned to preparation of eco-friendly textile fabrics using enzyme process to give a good solution to the problems of chemical usage.

Enzymes have been remarkably well-known in varied segments in terms of milder, judicious and synchronized manner. Added to that, in scouring it would show substrate specificity, diminished water consumption; evade the use of severe compounds like chemicals, less energy consumption, the reusability of the baths, milder process conditions and environmentally friendly nature. Hence, using of various enzymes in textiles would be regarded as green technology. Among which, microbial pectinase could be widely reported for bio-scouring of gray cotton yarn or fabric[5,6]. From an economical point of view, reusability of an enzyme should be minded for its high cost. For that reason, enzyme immobilization are gaining interest which enables simple operation, easy separation, possibility to reuse enzymes and increased stability[2,7,8,9]. The cost effectiveness that an immobilized enzyme is supposed to provide is not often there is a true sense. Nanotechnology is promptly mushrooming and placed their footpath in several investigations, among other nanoparticles, iron oxide nanoparticles are pulling attention for enzyme immobilization that aids in reusing of unused enzymes for new process thus falling of cost. With the above background information, the current research was designed to explore sustained green scouring process of cotton that switches over the usage of noxious chemicals to an eco-friendly catalyst with iron oxide nanoparticles as a resilient carrier.

## MATERIALS AND METHODS

Pectinase enzyme extracted from *Aspergillus niger* lyophilized powder (9032-75-1) was commercially acquired from SISCO research laboratories, Mumbai, India. Woven cotton desized fabric sample and conventionally scoured fabric sample was obtained from National Textile Corporation Private Limited, Coimbatore.

**Chemical Synthesis of iron oxide nanoparticles using co-precipitation method**

Iron oxide nanoparticles were synthesized by dropping iron solution into basic (sodium hydroxide) solution under ambient conditions; a brownish black precipitate was formed and allowed to stir vigorously for 30 minutes in a vortex subsequently it was centrifuged for 15 mins at 5000 rpm. Presence of nanoparticles was affirmed by showing attractive property towards an external magnet that moved at the edges of the beaker. Pellet was rinsed a few times with deionized water and centrifuged at 3000 rpm for 10 minutes to evacuate excess ions. At last, the particles were dispersed in 1 ml of ultrapure de-ionized water and dried overnight in an oven at 180ºC[1,10].

**Characterization of iron oxide nanoparticles**

**Absorption spectrophotometer**

The optical absorption attributes of iron oxide nanoparticles were examined by dispersing the solids in the ethanol for the path length of 1 cm under the UV-visible region ranging from 400 to 1100 nm and the measurements were recorded on systronic double beam spectrophotometer by UV-1700 series with the slit width of 1 nm[11]

**Morphology determination of nanoparticles using Scanning electron Microscope**

The morphology, size and crystal structure of the nanoparticles are characterized by High resolution Scanning Electron Microscopy. The SEM specimens were prepared by spreading a thin layer of powder onto a double-side carbon tape. The SEM pictures of iron oxide nanoparticles and immobilized textile enzymes on iron oxide nanoparticles were envisioned and the morphology was resolved[11].

**Fourier transform infrared spectroscopic analysis**

FTIR was used to determine the functional groups of the sample. The iron oxide nanoparticles were mixed with potassium bromide and pressed with the hydraulic pellet press and subjected to FTIR analysis (DTG-60, Shimadzu, Kyoto, Japan)[12].

**Immobilization of enzymes on the magnetic nanoparticles**

Activation of iron oxide nanoparticles were achieved by coating with diethylenediamine followed by addition of 4 ml of 10% glutaraldehyde solution to the nanoparticles solution with continuous stirring of one hour. Centrifugation at 10000 rpm for 15 minutes was performed to remove excess reagents. Activated iron oxide nanoparticles were added with 10 ml of pectinase enzyme and the mixture was incubated overnight with constant stirring in a shaker. Free enzyme was separated from immobilized (pectinase - iron oxide nanoparticles) by centrifuging the solution at 10000 rpm at 4ºC for 15 minutes. The pellet containing the enzyme bounded iron oxide nanoparticles were washed thrice using phosphate buffer, pH-7.0 and redispersed and stored in 1 ml phosphate buffer solution pH- 6.0[13].

**Scouring of cotton with free and immobilized enzyme and its reusability**

Desized fabric samples were subjected for bioscouring at 55°C (pH 8.5) for 1 hr. After the treatment was over, to inactivate the enzymes, the fabric samples were rinsed in 500 ml of water at 90°C for 15 minutes. Thereafter the samples were rinsed twice for 5 minutes in water at room temperature. Finally, the samples were dried at 80°C using a hot air oven. The free enzymatic scouring process has been done for immobilized enzyme treatment by replacing the addition of immobilized enzyme rather than free enzyme. Before washing of the fabrics, enzyme immobilized iron oxide nanoparticles were decanted using a permanent magnet and repeated the same process for three to five times in order to check its efficiency. In each cycle, the recollected enzyme immobilized nanoparticles were checked for its activity.

**Evaluation of scoured fabrics properties**

**Weight loss**

Weight loss of each fabric sample was determined by drying the sample after scouring process was over in oven at 45ºC. Percentage of weight loss of the scoured fabric sample was determined using following formula

% weight loss = (W1 – W2) x 100/W1

where W1 and W2 indicates the weight of the before and after treatment of cotton fabric sample.

**Fabric Water Absorbency**

Water absorbency of scoured cotton fabric was evaluated according to AATCC test method 39-1980 by determination of disappearance of water droplet into the fabrics[14].

**Scanning Electron Microscope analysis**

After scouring process, a small piece of the fabric sample was observed visually using high resolution scanning electron microscope with suitable magnifications that would confirm the surface modification of fabrics after treatment. The magnification of 5000x as applied to visual the image[2].

**Fourier Transform Infrared spectroscopic analysis**

Functional groups were determined using Fourier transform infrared spectroscopy in the wave number range 4000 – 400 $cm^{-1}$ for the detection of impurities present on cotton fabrics and removal of impurities[15].

**RESULTS AND DISCUSSION**

In the present work, we chemically synthesized iron oxide nanoparticles and crushed with a mortar and pestle to make a fine powder were shown in Figure 1. Similar work was done by Rajesh and co-workers[11] and obtained the black precipitate of iron oxide nanoparticles. Madhu and Chakraborty[15] stated that profit-making of immobilized enzymes is motionless but it is a beneficial technology for well-organized recovery method and reclaim of costly enzymes. Hirsh *et al*.,[16] had found out the iron oxide nanoparticles were functionalized with glutaraldehyde that might be responsible for enzyme binding to the available assistance.

Characterization of nanoparticles is a promising tool for the detection of nano sized particles. The optical adsorption properties of iron oxide nanoparticles were measured using UV-Vis Spectrophotometer showed in UV- Visible spectrum at 222 nm demonstrated the presence of iron oxide nanoparticles where electronic changeover between nanoparticles was occurred depicted in Figure 2 which was formerly reported in many research articles. The band at 264 nm indicated the presence of local vacancies in the iron oxide nanoparticles. Similar results were obtained and reported by Rajesh *et al*.,[11] and found the maximum absorbance at 227 nm. The size and morphology of the magnetic nanoparticles were observed by SEM was depicted in

Figure 3. The original magnetic nanoparticles were composed of bunches of nanoparticles with sizes ranging from 35 nm to 50 nm. The average size of the enzyme bounded-MNPs agglomerates ranged from 85 nm to 100 nm. In FTIR analysis, the spectra of immobilized enzyme, pectinase showed the peaks of two functional groups that were missing in the spectrum of activated iron oxide nanoparticles showed in Figure 4. Characteristic peak range of 2000 – 1500cm$^{-1}$ corresponds to absorption caused by the double bond stretching groups such as C=O, C=N and C=C that specifically indicated the presence of ketone, aliphatic compounds which were not found in control nanoparticles. A peak around 3332 and 906cm$^{-1}$ was expected in immobilized pectinase spectrum that is a characteristic band of proteins and corresponds to amide bonds and amine bonds. Several reports confirmed the presence of wider absorption peaks in the range of 3700–3100 cm-1, which is accredited to stretching of O– H and N–H groups in the structure of cellulase that strongly shows the binding of the enzyme on support that correlates with the results of pectinase bounded iron oxide nanoparticles

Scouring of the cotton fabrics (free enzyme and immobilized) were performed. According to Li and Hardin[17] suggested that the pectinase and cellulase combinations may possibly use as enzyme collaboration that could witness in earlier reports. Several research findings described that the pectinase alone was effectively removed 75% of pectin from the cuticle wall of the cotton. An immobilized enzyme on iron oxide nanoparticle was evaluated for its recyclability through repeated scouring of new unscoured fabrics and measuring its enzyme activity. Table 1 represented that the reusability efficiency of immobilized enzyme nanoparticles was lessened, and their enzyme activity was found to be 41.6% of the initial value at 4$^{th}$ reaction cycle mentioned in Table 1. An author Ren *et al*.,[18] had retained immobilized cellulase on magnetic nanoparticles activity for eight cycles while scouring. So far, no pectinase immobilization on magnetic nanoparticles used for scouring was reported. As noted on earlier reports, enzyme loss might be happened due to denaturation, inhibition of product formation or modification of the enzyme structure. Hence, this present study had proven that immobilized pectinase enzyme on magnetic nanoparticles could be used repeatedly up to three cycles efficiently. It should be continued for two more cycles with lesser addition of an enzyme to attain a good quality result. Despite the fabrics was scoured, the efficiency of the treated fabrics could be determined by the physical and functional characterization. In this present study, the weight loss of control fabric, enzyme treated, and immobilized enzyme treated fabrics were tabulated in Table 2. Higher in weight loss of the fabrics evaluated the removal of natural impurities present in the sample. Nisha[19] had been reported that enzymatic scoured fabrics was loss its weight about 4.81%. Similar results were found by Rajendran *et al*.,[20] in their findings. Water absorbency is absolutely a perfect way to determine the efficiency of scoured fabrics since the cotton fabric possess hydrophobicity before scouring. Enzymatic treatment of the fabric with led to a marked improvement on water absorbency of the fabric samples compared with the control of no enzyme treatment was illustrated in the Table 2.

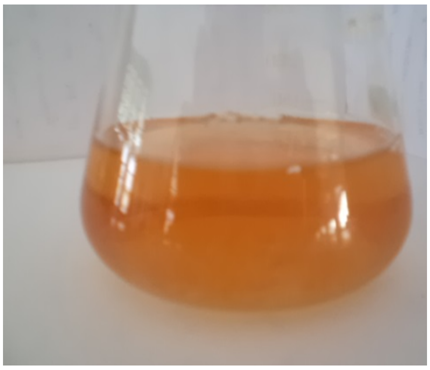
Hydrolysis of pectin and other impurities removal in cell wall of cotton would cause changes in the outline structure of cotton fabrics. The scoured cotton samples were visualized under Scanning electron microscope shown in Figure 5. Morphological analysis of the scoured fabrics revealed the presence of protrusions and grooves on parallel in the fabric however the surface of untreated fabrics was identified with smooth and waxy layer that has been already conveyed by previous reports. Rajendran *et al*.,[20] also acknowledged that enzyme treated fabrics were absence with smooth and waxy layer of cotton fabrics with his findings. Figure 6 obviously shows the spectrum of FTIR of untreated, enzymatically treated fabrics. The characteristics peaks appear in the 3200 – 2800 cm$^{-1}$ discloses the presence of alkanes and carboxylic acids through broad C-H and O-H stretch band. The two absorption peaks obtained in scoured fabrics in the range of 2800 – 2700 cm-1 corresponds to presence of aldehyde group that found missing in scoured fabric instead the absorption band near 1743cm-1 specified for C=O stretching mode denoted the presence of carbonyl groups such as carboxylic acids, esters that might responsible for intermediate compounds traces after pectin degradation. The peaks obtained at the region of below 2800, 1700 – 1500 cm$^{-1}$ could exploited the asymmetrical and symmetrical stretch mode of the $–CH_2$ groups of long alkyl chains. Similar reports had been gained by Rajendran and co-workers[20]. Henceforth, the present study reveals the successful scouring with the enzymatic agents, which has been considered as fruitful application in textile bioscouring.

**Table 1 – Reusability of immobilized enzyme**

| Scouring cycles | Weight loss (%) | Water absorption (Seconds) | Enzyme activity (%) |
|---|---|---|---|
| First | 7.7 | 1 | 100 |
| Second | 6.1 | 2 | 79.2 |
| Third | 5.3 | 4 | 68.8 |
| Fourth | 3.2 | 6 | 41.6 |

**Table 2 – Physical properties of scoured fabrics**

| Fabric tested | Weight loss (%) | Water absorption (Seconds) |
|---|---|---|
| Control fabric | 0 | ≥ 60 |
| Enzyme scoured | 6.9 | 1 |
| Immobilized enzymatic scoured | 7.5 | 1 |



1a. Iron solution

1b. INP formation

1c. Separation of INP

**Fig 1 Synthesis of iron oxide nanoparticles (INP)**

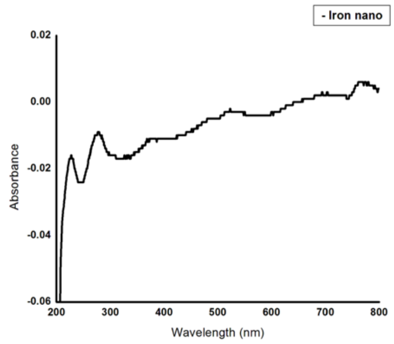
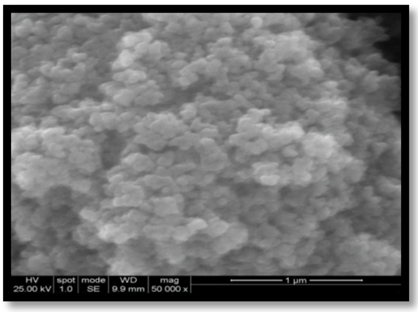
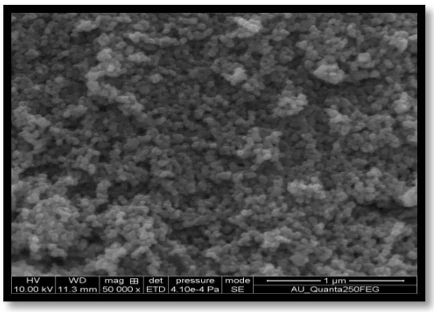



**Fig 2 UV absorption spectra of iron oxide nanoparticles (INP)**



3a. Control INP (86nm)



3b. Pectinase immobilized INP (136nm)

**Fig 3 Scanning electron microscope image of INP and enzyme immobilized INP**

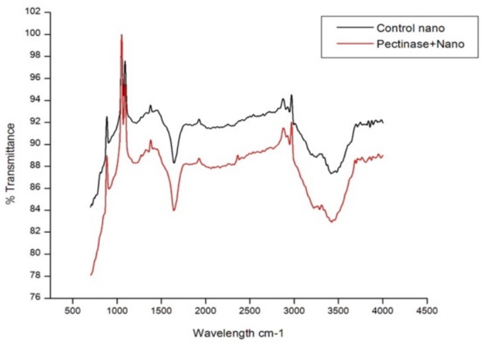
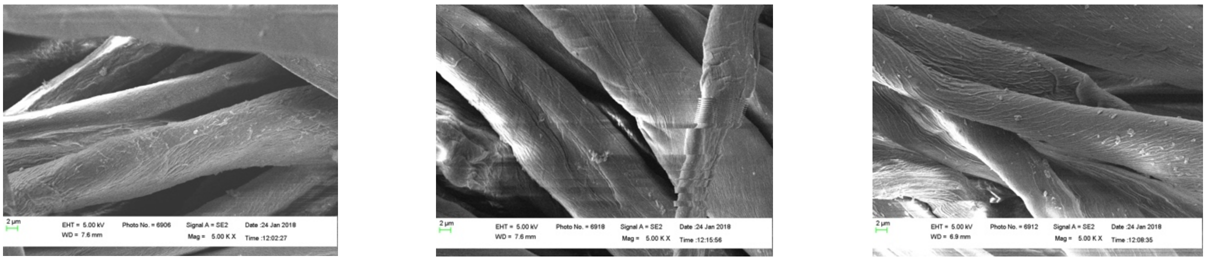



**Fig 4 Determination of functional groups using FTIR spectroscopy**



5a. Control fabric    5b. Enzymatic scoured (ES)    5c. Immobilized ES

**Fig 5 Surface topographical analysis of scoured cotton fabrics**

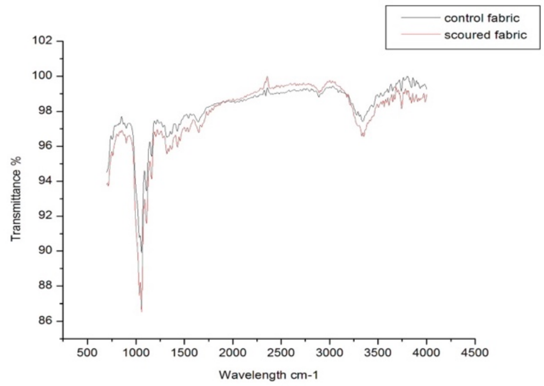



**Fig 6 Analysis of functional groups in scoured cotton fabrics**

## CONCLUSION

Today the growing concern about the environment is giving rise to technological changes towards the environmentally acceptable and friendly processes. In avoidance of using of harsh and high alkaline material, scouring with enzymes could be likened alternative probably might be even more economic if industrialized. Development of cotton fabrics manufacturing by making use of nanotechnology could be innovatively achieved through intensive studies. Therefore, the fabrics treated with the enzyme immobilized iron oxide nanoparticles displayed a better maintainable after it got repeated washing cycles. Bioscouring of cotton fabrics by means of pectinase enzyme has shown as a thought-provoking approach for the reduction of lesser energy and chemical consumption in textile wet processing.

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