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BIOSCOURING OF WOOL USING PROTEASE FROM *BACILLUS SUBTILIS* ISOLATED FROM ABATTOIR WASTE

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ABSTRACT

Bioscouring refers to the enzymatic removal of impurities from fibres/fabrics, which endows it with improved hydrophilicity for further wet processes. Enzymatic scouring preserves the fibre's structure and strength, avoids high energy consumption and severe pollution problems that are associated with conventional alkaline treatments. In the present study, protease enzyme was extracted from *Bacillus subtilis* which was isolated from abattoir (slaughter house) waste. Different medium parameters were optimized for maximal enzyme production. The enzyme was partially purified using ammonium sulphate precipitation followed by dialysis bag method. Partially purified protease enzyme was used in the bioscouring of wool fibres. Different parameters such as pH, temperature, time, enzyme concentration were optimized to achieve an efficient scouring. Comparison of enzymatic process for wool fibre with conventional alkaline soap process in terms of weight loss, whiteness index, tensile strength and FTIR studies confirmed that bioscouring could be as effective as the conventional process.

Keywords: Bioscouring, Protease, *Bacillus subtilis*, Abattoir waste, Wettability, Alkali solubility, Whiteness index

INTRODUCTION

Wool is a natural protein fibre which is obtained from sheep skin. Raw wool fibers obtained from the sheep contain greasy substances referred to as impurities, as high as 40-50 %. Sabaceous glands of sheep's skin secretes oils and fats, often referred to as wool fat/grease, essentially a mixture of higher fatty alcohols and acids **(Saravanan *et al*., 2014)**. The contaminants consist of 5-25% grease, 2-15% suint and 5-20% dirt, moisture, vegetable matter, sand and dirt. All these impurities tend to make the raw wool highly hydrophobic; which has to be removed to make it hydrophilic prior to its further processing. However, entire removal of wool wax is not desirable at fibre stage, in order to facilitate the lubricating actions among the fibers during subsequent spinning processes. The conventional methods of wool scouring utilize high amount of chemicals, detergent, alkali etc which create some serious problems; both, for the environment and the industry while effluent treatment and disposal **(Halliday, 2002)**.

Traditionally, wool scouring is carried out by using alkali and soap at temperature 60ºC - 80ºC. The alkaline scouring treatment emulsifies the waxes and breaks down peptide bonds into water-soluble or water-emulsifiable products that are later washed off from the wool materials. This process effectively removes all impurities that exist in the raw wool fibres but has a high energy requirement and the effluent is ecologically undesirable because of its high alkalinity, biochemical and chemical oxygen demand. These drawbacks in the process led to a consideration of alternatives. Bioscouring of wool with suitable enzymes appears to be most promising in this respect. The necessity to use more environmental friendly processes leads to the replacement of conventional chemicals by enzymatic ones. Bioscouring is a novel process based on the idea of particularly targeting fats, waxes, suints, dirt etc. with specific enzymes.

Zheng et. al have reported a method to optimize the wool-scouring process with bio-enzymes of *Bacillus Subtilis* and *Candida lipolytica*. **(Zheng et. al., 2012).** Enzymatic treatment of textiles has been of great interest because of its effectiveness under mild treatment conditions **(Cardamone *et al*., 2006)**. Enzymes act in the pH range between 5 and 8, at temperatures around 30 to 40ºC at atmospheric pressure while conventional method requires pH 10.8 at temperature around 60ºC - 80ºC. These treatments also enhance many textile properties such as wettability, dye uptake and polymer adhesion **(Negri *et al*., 1993; Brack *et al*., 1999)**.

From an environmental point of view, enzymes are active in small doses and highly biodegradable; hence, the use of enzymes in scouring helps in reduction of the auxiliary agents, which usually are poorly biodegradable.

As per the literature, various enzymes like protease, lipase, pectinase and amylase have been used to carry out treatments on wool **(Saravanan *et al*., 2014; Sayad *et al*., 2010; Hmidet *et al*., 2009)**.

Proteases or proteinases are proteolytic enzymes which catalyze the hydrolysis of proteins. Based upon their structures or properties of the active site, there are several kinds of proteases such as serine, metallo, carboxyl, acidic, neutral and alkaline proteases. Proteases are industrially important enzymes and constitute a quarter of the total global enzyme production **(Kalaiarasi and Sunitha, 2009)**. Proteases are industrially important due to their wide applications in leather processing, detergent industry, food industries, pharmaceutical, textile industry etc **(Deng *et al*., 2010; Jellouli *et al*., 2009)**.

Commercially in wool industry, protease enzyme is used due to its substrate specificity. This enzyme tends to remove desired impurities like wax, suint, sand and vegetable matter etc. from raw wool fibre to make it hydrophilic. Effective removal of wax from raw wool substrate with the enzyme under mild conditions will provide high quality of wool for the subsequent dyeing and finishing processes with less energy consumption under safer conditions. In contrast to drastic alkaline conditions conventionally used, treatment with protein degrading enzymes would not affect the internal structure of wool fibre and thus avoid fibre damage. Wide scale industrial application of protease requires their cost effective production to make the process economically viable **(Shukla, 2001; Karmarkar 1999)**.

Pre-treatment of wool fiber with enzyme leads to increase its hydrophilicity with enhanced swelling properties. Protease can catalyse the degradation of different component of wool fibre **(Hooda 2013)**. Protease enzyme penetrates into amorphous region and causes swelling and it leads to changes in the disulphide region of cystine than amide components during chemical degradation. (**(L. Ammayappan 2013**).

In the present investigation, a successful attempt has been made for production of protease using submerged fermentation (SmF) from a newly isolated strain of *Bacillus subtilis* from degraded slaughter house waste material and its application in the bioscouring of wool fibre in textile industry. The parameters essential for effective wool scouring like time, temperature, pH, enzyme concentration, etc were optimized.

MATERIAL AND METHODS

Raw Materials

The raw greasy merino wool fibres of Australian origin were procured from (WRA) Wool Research Association, Thane, Mumbai, India for the scouring experiments being difficult to scour as they contain the high amount of contaminants such as wool wax, dirt, dust and suint. Protease enzyme used for bioscouring was extracted from *B. subtilis* obtained from abattoir waste at our laboratory. Identification of the bacteria was carried out by 16S rRNA sequencing method. Casein was purchased from Sigma Chemicals. Chemicals for microbiological experiments were supplied by Himedia, Mumbai and all other chemicals were procured from S.D. Fine chemicals, Mumbai.

Microorganism and enzyme production

The *B. subtilis* used in this study was isolated from degraded abattoir (slaughter house waste), collected from local market, Mumbai, India. For production of protease enzyme, 100 ml of nutrient medium containing 1% gelatin w/v (as protein source), 0.5% yeast extract and 100 ml distilled water at pH 14 was inoculated with 1% inoculums of 24 h old *B. subtilis* culture (1×10^8 CFU/ ml). As reported earlier, *Bacillus species* has optimum temperature of 40ºC for its growth and its matabolic activity is at highest peak during its growth phase due to which the enzyme works efficently **(Badhe et. al 2016)**. The flasks were incubated at 40º C for 24 h on a rotary shaker maintained at 150 rpm. After 24 h the broth was centrifuged at 5000 rpm at 4º C for 20 min and the cell free supernatant was collected. The supernatant was used as crude enzyme extract. The application of enzyme in the textile industry does not require high grade purity for enzymes and generally requires use of the crude or the partially purified enzyme preparation **(Nerurkar et. al 2013)**. Thus, partial purification of the protease was carried out by ammonium sulphate precipitation since ammonium sulphate is highly soluble in water, cheap, and has no deleterious effect on the structure of protein. Ammonium sulphate was added to the crude enzyme sample to get 30% saturation and was kept overnight at 4ºC. It was then centrifuged at 6000 rpm for 20 min. The precipitate was suspended in phosphate buffer, pH 7.0 and was dialyzed against the same buffer to carry out desalting process.

Protease assay

Protease activity was measured by caseinolytic method, **(Walter, 1984)**. One unit of enzyme was defined as the amount of enzyme that liberates peptide fragments equivalent to 1 mg of bovine serum albumin (BSA) under the assay conditions.

Conventional scouring

Conventional scouring was carried out at pH 10 for 2 h at 35ºC to 40ºC. The solution used for scouring contained 2 g/L Auxipon NP solution (non-ionic surfactant) and 3 g/L sodium carbonate solution (alkali). MLR was kept at 1:30. Sample was given hot wash. The fibres were firstly air dried and then conditioned at 20 ± l ºC and 65 ± 2% RH for at least 24 h in desiccator containing calcium chloride.

Bioscouring

Different parameters of bioscouring namely buffer pH, enzyme concentration, temperature and time period were optimized to examine the efficiency of protease from *B.subtilis* and to further obtain efficient removal of impurities. The raw wool fibres were subjected to four different treatment solutions stated below:

1. Phosphate buffer at pH 7.0 and enzyme (B+E)
2. Phosphate buffer, soap and enzyme(B+S+E)
3. Phosphate buffer, alkali and enzyme (B+A+E)
4. Phosphate buffer, soap, alkali and enzyme (B+S+A+E)

The raw wool fibre samples were subsequently suspended in buffer of pH ranging from 3.0 to 11.0. The material to liquor ratio was set at 1:30 with varying dosage of protease in the range of 2% to 14% on the weight of fabric for treatment time of 30, 60, 90, 120, 150 and 180 min at different temperatures ranging from 30 ºC to 80 ºC. The bioscouring process optimization was carried out by varying one parameter at a time, keeping other factors constant. The experiments were performed in the water bath (Bio Technics India). After the enzymatic treatment; the fibres were given hot wash at boiling temperature for 30 min, air dried and dried in desiccator for 24 h.
Efficiency of bioscouring for each parameter was evaluated by monitoring weight loss of the fibres as the difference before and after the bioscouring treatment. Wool sample, bioscoured using protease enzyme under optimized parameters, was used for further analysis.

Evaluation of fibre properties

Weight loss determination

Enzyme-treated and untreated wool fibers were conditioned at 20 ± l ºC and 65 ± 2% RH for at least 24 h before being weighed. Weight loss was expressed as:

$$\text{Weight loss } \% = \frac{W1-W2}{W1} \times 100$$

Where, W_1 is the conditioned weight of the sample before enzymatic treatment, and W2 is the conditioned weight of the sample after enzymatic treatment respectively.

Wettability (Drop test)

The wettability of raw wool and enzyme treated wool fibres was measured using standard test, BS 4554:1970. According to the method, the time (sec) required by a drop of water to be absorbed by the fibre is defined as the ability of the fibre to get wet.

Sinking time

Sinking time test AATCC 17-2005 was modified for being used for fibre. Instead of fabric, a bundle of fires was used as indicative test for evaluating absorbency.

Alkali solubility

To quantify the damage to the epicuticle of wool, alkali solubility in terms of weight loss of raw wool and enzyme treated fibres was measured using IWTOTM-4-00 standard test method. The values were calculated as a percentage of the original mass, according to the equation given below,

$$\text{Alkali solubility } (\%) = \frac{M1 - M2}{M1} \text{ X } 100$$

Where, M1 is the mass of oven dry sample before sodium hydroxide treatment, and M2 is the mass of oven dry sample after sodium hydroxide treatment.

Residual grease content

Efficiency of removal of grease content of conventional scoured and enzyme scoured wool sample was evaluated by using the following formula according to standard method IWTO1903:

$$\text{Grease Content} = \frac{W1 - W2}{W1} \times 100$$

Where, W1 is the weight before removal of grease from wool sample and W2 is the weight after removal of grease from wool sample.

Determination of Moisture Regain

Moisture regain was calculated using the following equation according to ASTM method 2654-76:

$$\text{Moisture regain } (\%) = \frac{W1 - W2}{W2} \times 100$$

Where, W1 is the weight of the conditioned sample at standard humidity and W2 is the weight of the sample dried to constant weight.

Determination of Moisture Content

The samples were preconditioned in a desiccator for 24 hr at 65 ± 2 % RH and 27 ± 2ºC. The moisture content was determined after obtaining the weight of wool dried at 105ºC for 3 hr. The oven dry mass was determined according to standard IWTO-34-85-E method.

$$\text{Moisture Content } (\%) = \frac{W1 - W2}{W1} \times 100$$

Where, W_1 is the weight of the conditioned sample at standard RT and temperature and W_2 is the weight of the sample dried to constant weight.

Single Fiber Strength Test

The single fiber strength of raw and scoured wool was measured on Universal Tensile Machine supplied by Aimil Limited, Navi Mumbai according to ASTM D 3822 standard test method. The instrument was based on constant rate of

elongation (CRE) principle. The distance between jaws was 10mm and the travel rate was 6 mm/min.

Fiber Mean Diameter Test

Fiber diameter measurement was carried out using OFDA 100 as per the standard IWTO472011. The fiber samples were cut into 2 mm snippets and spread on a 70 mm square glass slide. The whole slide was scanned with a minimum of 6000 fibers. For each sample, three measurements were taken. The mean diameter and standard deviation of the sample were then calculated.

Whiteness & Yellowness measurement

The ASTM whiteness Index (WI) and yellowness Index (YI) of samples, before and after scouring were determined by using Computer Colour Matching (CCM) System (Spectrascan5100+) according to IWTO3503 standard test method. The Improvement in whiteness and reduction in yellowness are expressed as the percentage change relatively to the original whiteness and yellowness respectively.

FTIR analysis

FTIR study was carried out to analyze the changes in structural groups and impurities on the wool surface of raw wool and enzyme treated wool fibres.

SEM

The surface morphologies of the wool samples were visualized using a JSM-6380L, an analytical scanning electron microscope (JEOL Company, Japan), operated at a typical accelerating voltage of 5 kV. The samples were sputter-coated with Platinum for 30 s at 15 mA prior to the observation.

Statistical Analysis

All the experiments were conducted in triplicate and results were expressed as mean±standard deviation. Student's t-test was used to analyze data, and statistical significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Protease production in submerged fermentation

Glucose was used as sole carbon source for protease production using B. *subtilis* in submerged fermentation. Gelatin was used as protein source as a substrate. Protease so produced showed maximum protease activity of 63 U/ml after optimizing various physical, chemical parameters of fermentation and partial purification using Ammonium sulphate.

Bioscouring

Raw wool fibres were subjected to four different treatment solutions as reported above. It was seen from (Figure.1) that there was maximum weight loss and a less than 2 sec wetting time when raw wool was treated with the solution containing buffer and enzyme (B+E). Solution B+E was found to be optimum and was used for further studies, due to that protease enzyme is stable in buffer solution **(Joshi et al. 2013).**

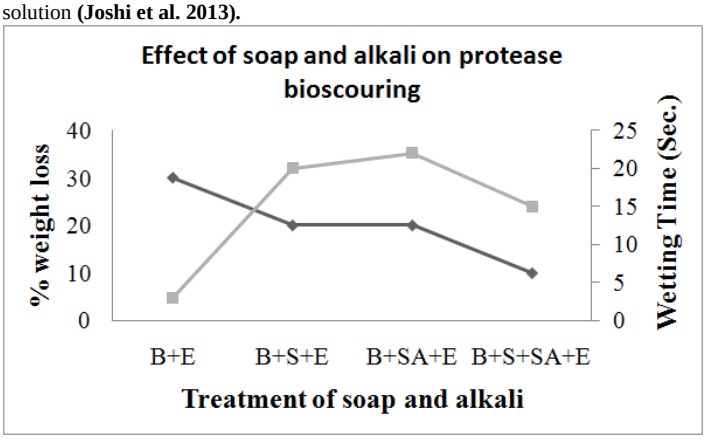

Figure 1 Effect of four different treatment solutions on protease bioscouring.
Protease scouring was carried out at protease dose of 2 % (owf), pH 7.0, at 40ºC keeping MLR at 1:30. Filled squares indicate percentage weight loss of the wool fibre and filled diamonds indicate wetting time of wool fibres in second.
Four different treatment solutions: solution B+E containing 50 mM phosphate buffer and enzyme, solution B+S+ E containing buffer, soap and enzyme, solution B+A+ E containing buffer, alkali and enzyme, solution B+S+A+ E containing buffer, soap, alkali and enzyme.

In order to explore the potential of our protease enzyme in bioscouring of wool, various factors like pH, enzyme concentration, temperature and time period were optimized. As seen from (Figures. 2–5), the optimal conditions for the bioscouring of wool sample were 4% protease dosage on the weight of the fibre at 60 ºC, Phosphate buffer pH 7.0 and a treatment time of 120 min.

Effect of Time period

Effective bioscouring using protease was optimized for 120 min in terms of weight loss and wettability. From the (Figure.2), it can be seen that the weight loss remained steady upto 60 min, after that there was a steep rise and the maximum weight loss was observed at 120 min. After that there was a sudden decline in weight loss which can be attributed to protein specific action of protease such that within treatment time of 120 min itself, protein and hence the other loosened impurities get removed.

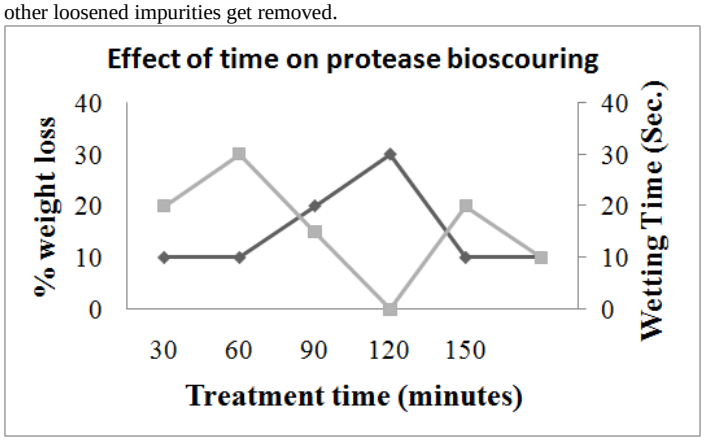

Figure 2 Effect of time period on protease bioscouring.
Protease scouring was carried out at protease dose of 2 % (owf), pH 7.0, at 40ºC for varying period of time keeping MLR at 1:30. Filled squares indicate percentage weight loss of the wool fibre and filled diamonds indicate wetting time of wool fibres in second.

Effect of temperature

Effect of temperature on protease scouring can be seen from (Figure. 3). As compared to 20% weight loss obtained in alkaline scouring, sufficient weight loss is achieved when scouring is carried out between temperature ranges of 30ºC and 80ºC, where as scouring efficiency decreases drastically as temperature of scouring bath is raised to 80ºC. This is because the protease enzyme must be showing less activity at 80ºC as compared to lower temperatures mentioned earlier. Optimum temperature for scouring of wool using protease was observed as 60ºC.

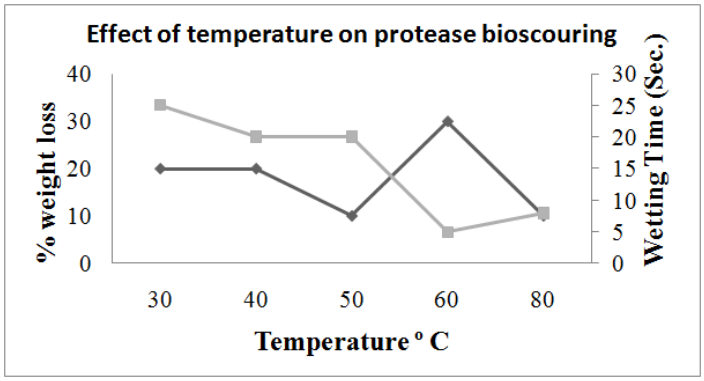

Figure 3 Effect of temperature on protease bioscouring.
Protease scouring was carried out at protease dose of 10% (owf), pH 7.0 for 120min at various temperatures keeping MLR at 1:30. Filled squares indicate percentage weight loss of the wool fibre and filled diamonds indicate wetting time of wool fibres in second.

Effect of enzyme concentration

As can be seen from (Figure. 4), 4 % of protease enzyme (owf) is optimum for scouring as further increase in the protease concentration showed more or less similar weight loss as compared to weight loss achieved when 4% of protease enzyme was used for scouring.

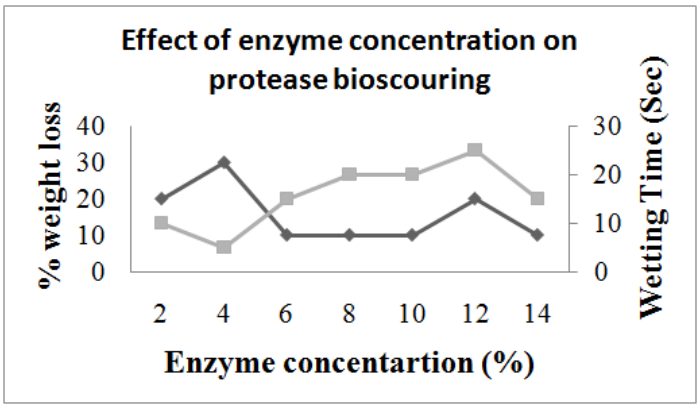

Figure 4 Effect enzyme concentration on protease bioscouring.
Protease scouring was carried out at pH 7.0, 40ºC for 120 min at various concentration of the protease keeping MLR at 1:30. Filled squares indicate percentage weight loss of the wool fibre and filled diamonds indicate wetting time of wool fibres in second.

Effect of pH

As can be observed from (Figure.5), pH of scouring bath greatly affects scouring action of the protease enzyme. Optimum pH for scouring of wool using protease was observed as 7.0.

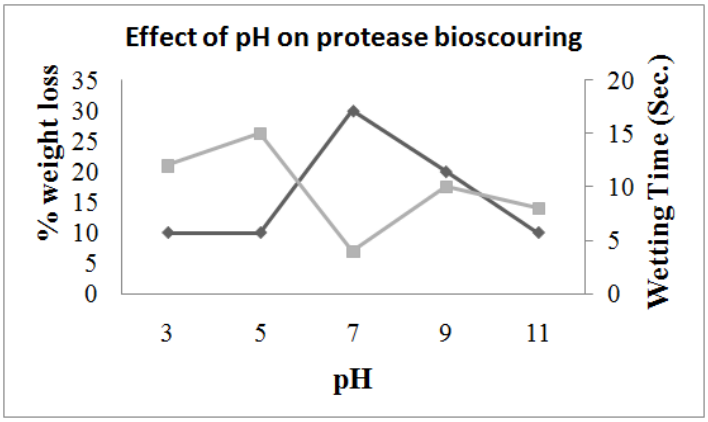

Figure 5 Effect of pH on protease bioscouring.
Protease scouring was carried out at protease dose of 10% (owf), pH 7.0 for 120 min at 60ºC for varying pH, keeping MLR at 1:30. Filled squares indicate percentage weight loss of the wool fibre and filled diamonds indicate wetting time of wool fibres in second.

Wettability of fibre

The raw wool fibers were tested for wetting time after it was subjected to scouring. The comparison of wetting time for B+E solution, conventional i.e. B+S+A+E solution and raw wool fibers. It was found that after treating raw wool with B+E solution, there is increase in its hydrophilicity and wettability was found to be less than 2 s. The raw wool fibres were not able to absorb the water droplet; it was observed that, after more than 30 min also the water drops remain as it is and can be rolled off easily from the fiber surface without wetting the surface.

Sinking time

Sinking time of both, conventionally scoured and protease scoured wool sample was found to be less than 2 s. Optimum temperature for bioscouring was found to be 60ºC. At this temperature, though some of the fats or waxes from wool fibre get loosened up, it still adheres to the fibre surface. When hot wash is given, they leach out completely in the bath; allowing the fibre to sink analogous to alkaline scoured fibre.

Alkali solubility

The alkali solubility of the enzyme treated fibers was tested and compared with the raw wool fibres. The alkali solubility values for wool fibres reported in literature are between 9% and 15% for undamaged wool **(Atav and Yurdakul, 2011)**. The alkali solubility for raw wool and enzyme treated wool was found to be 9.10% and 11.48%.

Residual grease content

Efficiency of removal of grease content of raw wool which was 11.90% for conventional scoured, (B+S+A+E) scoured was 0.16% and enzyme scoured (B+E) wool sample was 0.36 %.

Moisture Regain and moisture content

Removal of hydrophobic greasy substances from the surfaces of the raw wool is expected to increase the moisture regain values and higher weight loss in the scouring treatment translates to higher moisture regain values. The highest moisture regain values were observed in the case of enzyme scoured i.e. B+E samples at 42.85 %, while the moisture regain values of conventional i.e. B+S+A+E scoured, B+S+E scoured samples, or B+A+E scoured wool samples were in the range of 25 %. According to standard method, enzyme and buffer showed maximum moisture regain and content as compared to other.

Effect on Physical properties

Fiber Mean Diameter Test and Single Fiber Strength Test

There was no significant effect on mechanical properties of wool fibers on bioscouring. Little difference in the tenacity and elongation of wool fibers is attributing of high degree of variability in fiber dimension and non uniformity in wool. It has also been found that there is no difference in fiber diameter of all the samples as shown in (Table. 1). The fiber diameter ranges between 19.2-19.5 micron (Table. 2) **(Kalantzi et al., 2008)**.

Table 1 Effect of Protease and alkaline scouring on tensile strength of wool fibers

Serial no.	Sample	Breaking strength	Extension (%)
1	Raw wool	5.80	27
2	Alkaline scouring (B+S+A+E)	6.00	37
3	Protease Scouring (B+E)	4.10	38
4	B+ S+ E	5.30	34
5	B+A+E	4.80	35
6	B+S+A+E	4.10	50

Table 2 Effect of protease scouring on the properties of wool fiber.

Sr. no	Fibre sample	Raw wool	Alkaline scoured (B+A+S+E)	Protease Scoured (B+E)	(B+S+E)	(B+A+E)	(B+S+A-
1	Average fibre fineness (micron) (IWTO 47)	19.3	19.2	19.2	19.2	19.2	19.2
2	SD	1.13	1.27	1.59	1.11	1.33	1.46
3	CV%	21	31	27	26	22	30

SD- Standard Deviation, CV- Coefficient Variation.

Effect on Whiteness and Yellowness

Enzymatic removal of wool impurities by the process of bioscouring resulted in whiteness improvement. Though, compared to alkaline scouring yellowness index of the bioscoured wool was less, whiteness index of both alkaline (B+S+A+E) scoured and protease (B+E) scoured wool was almost similar (Table. 3). Protease being substrate specific, it only attacks peptide bonds and does not remove colouring matter in wool whereas caustic soda removes colouring matter being non-specific in action.

Table 3 Wool fiber properties at different stages of textile wet processing

Serial no.	Fibre sample	WI	YI	BI
1	Raw wool	8.71	10.31	38.51
2	Alkaline scouring	21.29	20.46	49.57
3	Protease Scouring (B+E)	20.76	11.37	48.27
4	B+S+ E	12.19	16.96	50.96
5	B+A+E	16.29	15.56	52.43

6	B+S+A+E	17.21	14.31	48.01

B= Buffer, S= Soap, A= Alkali (sodium carbonate), E= Enzyme, WI- whiteness index, YI- yellowness index, BI- brightness index.

FTIR Spectroscopic Analysis

The FTIR spectra of the raw wool, alkaline-scoured, protease-scoured fibers were shown in (Figure. 6 to 10) indicating characteristic absorption bands assigned mainly to the peptide bonds (CONH) which represent the fundamental structural unit of the polypeptide chain **(Zoccola *et al*., 2009)**. The results confirm that no new chemical bonds are produced in wool fibre. The two sharp peaks in the range of 2935–2915/2865–2845 cm^{-1} for raw wool fibre samples corresponds to Methylene C-H asymmetric/symmetric stretch and similarly at 1485–1445 cm^{-1} for Methylene C-H bend **(Meyers, 2000)**. Intensity of these peaks in raw wool fibre is high, while after protease treatment the intensity of all these peaks (Methylene C-H stretch and bend) reduces, indicating the removal of 18-MEA acid covalently bonded to epicuticle. The peak at 1737 cm^{-1} for raw wool fibre corresponds to covalent bond of 18-MEA acid to epicuticle through the (C=O) sulfoester. After B+E solution (protease) treatment, this bond breaks and it can be seen from Fig.7 that, peak at 1737 cm^{-1} disappears. The five spectra reveal that the bands near 1600cm^{-1} assigned to amide I and II vibrations are shifted. They reveal a combination of amide C=O and N-H modes. The frequency is sensitive to protein conformation, i.e., alpha helix, random, beta-sheet, etc. The intensity is proportional to the concentration of amide linkage, i.e.,-C (=O)-N (-H)-. Yet in this case, it is suspected that the differences are ascribed to the differences in the water content of fibers. There is an H-O-H bending mode at 1635 cm^{-1}. This is supposed to push up the intensity of the amide I peak after B+E solution (protease) treatment **(Mori and Inagaki, 2006)**. After protease treatment the additional peak appears at 1076 cm^{-1} which corresponds to the S-S oxidation in the surface of wool after treatment **(Meyers, 2000; Hocker, 2002)**.

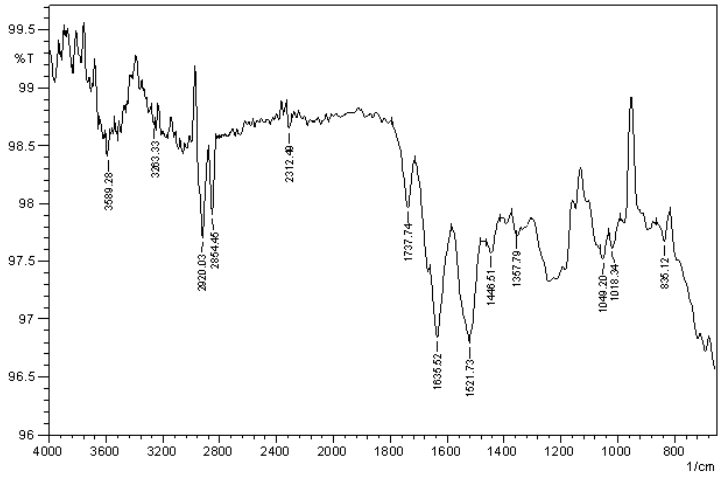

Figure 6 FTIR spectra of raw wool fibre
Analysis of functional group of raw wool fibre at wavelength ranging from 750 cm^{-1} to 3600 cm^{-1}.

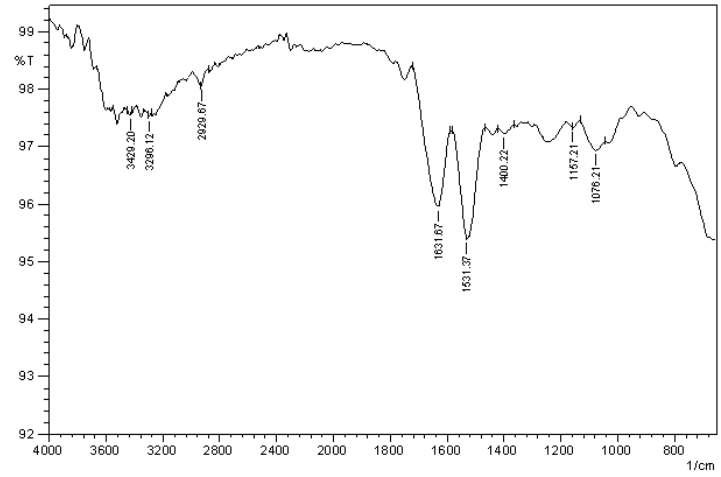

Figure 7 FTIR spectra of protease scoured (B+E) wool fibre.
Analysis of functional group of protease scoured wool fibre at wavelength ranging from 750 cm^{-1} to 3600 cm^{-1}.

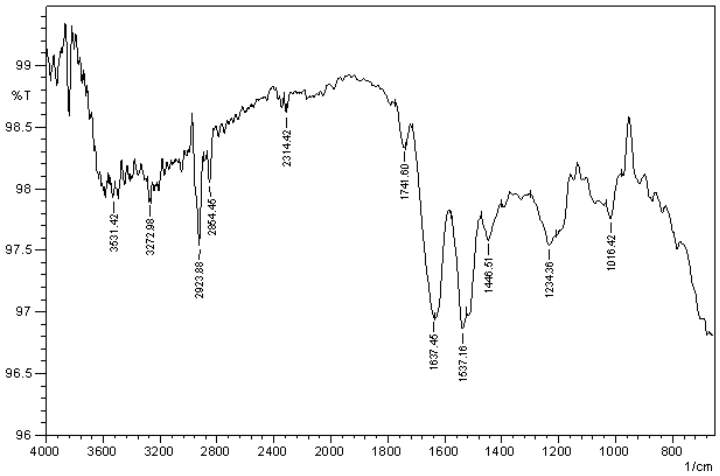

Figure 8 FTIR spectra of buffer, soap, soda ash and enzyme (B+S+S+E) scoured wool fibre.
Analysis of functional group of B+S+S+E scoured wool fibre at wavelength ranging from 750 cm^{-1} to 3600 cm^{-1}.

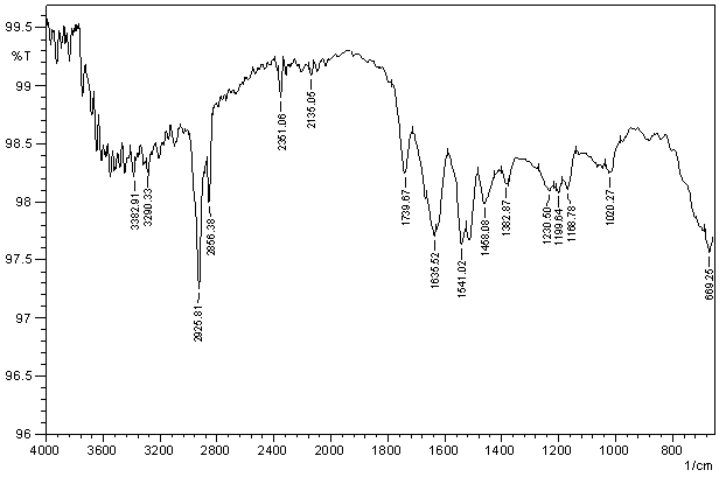

Figure 9 FTIR spectra of buffer, soda ash and enzyme (B+S+A+E) scoured wool fibre.
Analysis of functional group of B+S+A+E scoured wool fibre at wavelength ranging from 750 cm^{-1} to 3600 $cm^{-1.}$

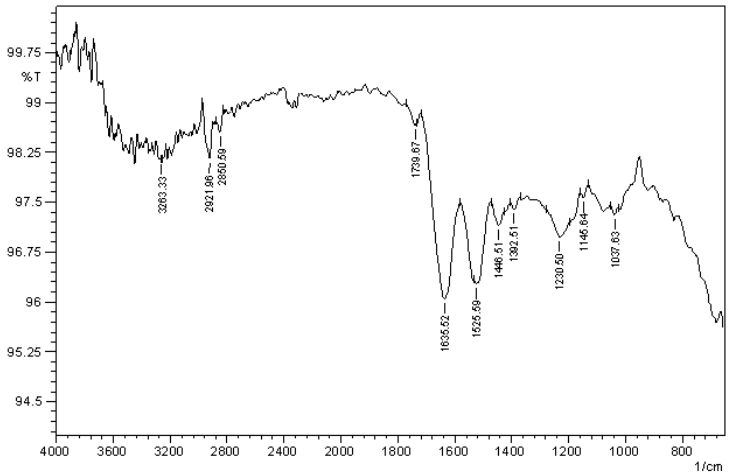

Figure 10 FTIR spectra of buffer, soap and enzyme (B+S+E) scoured wool fibre.
Analysis of functional group of B+S+E scoured wool fibre at wavelength ranging from 750 cm^{-1} to 3600 cm^{-1}

SEM ANALYSIS

The clarity of wool surface was observed with scanning electronic microscope (SEM), as shown in (Figures 11 to 15). It could be seen that, the wool surface scoured using protease enzyme (B+E solution) was very clean and smooth as compared with those scoured using conventional process, B+S+E solution, B+A+E solution and B+S+A+E solution. There was partial removal of cuticles

from wool fibre due to substrate specificity of protease enzyme. Prominent scales were observed on wool fibre surface when treated with B+E, as it got damaged with protease enzyme treatment. There was though a rise in surface performance and capillarity effect of wool fiber under this bio-scouring.

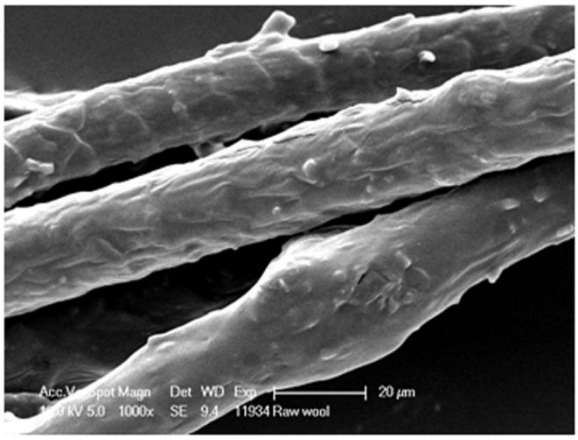

Figure 11 SEM images of raw wool fibre (1000x). Surface analysis of raw wool fibre.

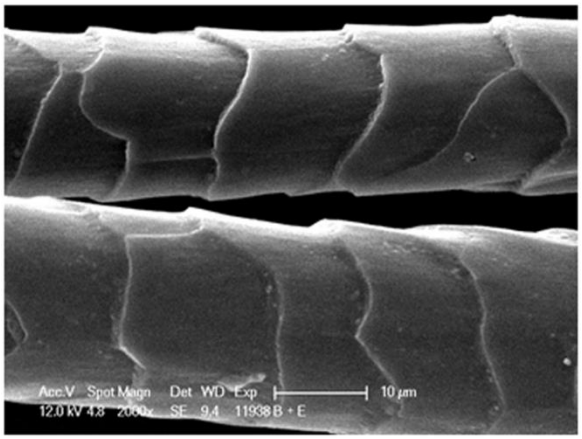

Figure 12 SEM images of protease (B+E) scoured wool fibre (2000x). Surface analysis of protease (B+E)scoured wool fibre.

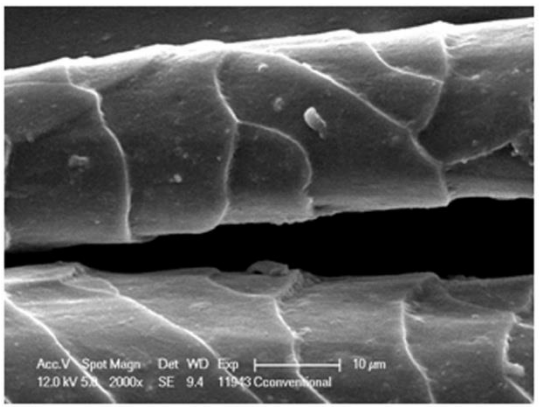

Figure 13 SEM images of alkaline(B+A+S+E) scoured wool fibre (2000x). Surface analysis of alkaline (B+A+S+E)scoured wool fibre.

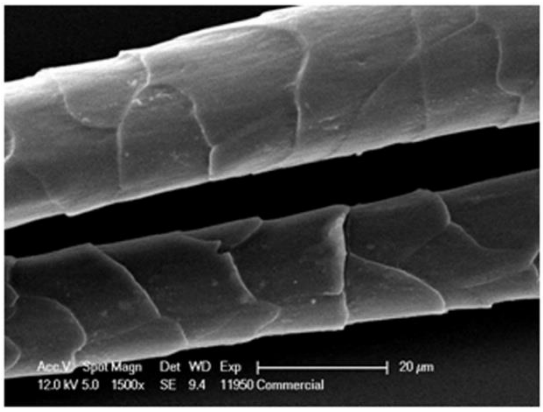

Figure 14 SEM images of commercial scoured wool fibre (1500x). Surface analysis of commercial scoured wool fibre.

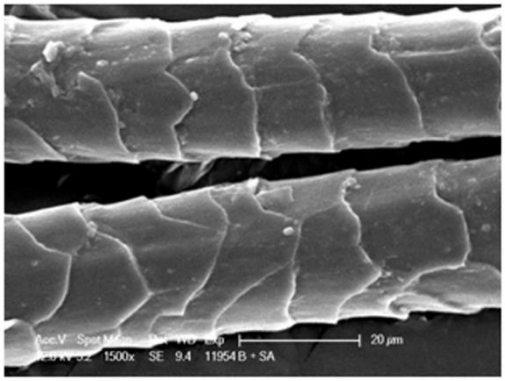

Figure 15 SEM images of B+A+E scoured wool fibre (1500x). Surface analysis of B+A+E scoured wool fibre.

CONCLUSION

The bioscouring studies indicated that protease from *B. subtilis* is capable of removing waxes from the raw wool fibre and impart hydrophilicity to the fibre. In addition, the experimantal studies of bioscouring parameters clearly states that under alkaline conditions at 60ºC for 120 min with a protease dosage of 10% on the weight of fibre, better than required hydrophilicity is achieved. The sharp peaks obtained from the FTIR data and smooth surface of wool revealed by the SEM images show the efficiency of protease in the removal of waxes from wool fibre indicating no damage to the wool fibre. Thus, the protease is a potential candidate to be used in bioscouring of wool. The approach described in the present work seems to be convincingly reproducible and environment friendly which can be easily adapted by the textile industry.The further work can be undertaken to check dyability of such bioscoured wool fibers vis-a vis conventionally scoured wool and also combine bioscoring and dyeing to acheive shortening of overall processing cycle for dyed wool.

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