Bioscouring of Cotton Fabrics and Fibres by Pectinases from *Penicillium sp.*

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Abstract: The process of bioscouring in the textile industry is an example of white modern biotechnology, which permits the advancement in methodologies of fiber processing to enhance the quality of the final product. The present investigation reports the efficient bioscouring process by pectinases obtained from an indigenous species of *Penicillium*. Prior to this, the production of the enzyme was optimized, enhanced and characterized. Bioscouring performed on samples of cotton fabrics and fibres resulted in overall improvement of sample's quality as compared to control which was evident by decrease in fabric weight, tensile strength, stiffness, and thickness in the enzyme treated samples. Enzyme treatment also resulted in finer, softer and docile yarns.

IndexTerms – Penicillium, Bioscouring, Pectinases, Cotton fibres, fabrics

I. INTRODUCTION

In nature, microorganisms have been enriched with tremendous capabilities of producing a variety of enzymes, which have been used monetarily throughout the years [1]. More than 500 modern industrial products are being made utilizing enzymes [2, 3]. Microbial enzymes are key segments of textile, ethanol and pharmaceutical industries, food and beverage manufacturers [4]. Pectinases are the multiplex and diverse group of enzymes that breakdown the pectic substances [5]. Pectinases (or pectinolytic compounds) holding a share of 25% in the worldwide sales of food enzymes are produced from microbiological sources. Fungi, for example, *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium expansum*, *Penicillium restrictum*, *Trichoderma viridae*, *Mucor piriformis* and *Yarrowia lipolytica* and so forth, which are usually viewed as secure (GRAS) by United States Food and Drugs Administration (USFDA) are utilized for pectinase production [6].Textile chemical processing has frequently been condemned for being most polluting industries. Processors are progressively attempting to supplant as many polluting chemicals as possible with enzymes that go about as biocatalysts. The most settled use of biotechnology in textiles has been in the field of enzymatic pretreatments [7, 8, 9, 10]. Pectinases have been considered as the most reasonable enzymes for cotton scouring by numerous scientists, in light of the fact that the disruption and disposal of pectin encourages the removal of loosened waxes [11, 12]. Though a few researchers have additionally examined the impact of acidic and impartial pectinase on the cotton bioscouring [13]. The selection of productive strains, isolated from the natural habitat, in any case, remains a tedious task, particularly when industrially skillful enzyme yields are to be accomplished. The present study was conducted with a specific goal to acquire exceedingly beneficial strains for investigating their application in enzymatic scouring of cotton based materials.

II. RESEARCH METHODOLOGY

2.1 Collection, isolation and screening of pectinase producing fungal organism

Soil samples were collected from the rhizospheric regions around Chandigarh region. Spread plate method was used with dilution 10^{-4} for the isolation of pectinolytic fungi on the potato dextrose agar (PDA) plates. The qualitative plate assay [14] and quantitative method for pectinase activity [15], was employed for the selection of the best pectinase producing strain.

2.2 Optimization of culture conditions for enhanced enzyme activity

The selected culture was used for the enhancement of the activity by optimizing various physical and biochemical parameters viz. agro industrial wastes as substrate (pomace, sugarcane bagasse, orange peel, mausami peel, pineapple peel), Inoculum size (5, 10, 15, 20 and 25ml), incubation period (24, 48, 72, 96, 120 and 144 hours), pH (4.0, 5.0, 6.0, 7.0 and 8.0), Temperature (23, 28, 33, 38 and 43 ^{o}C), Carbon sources (sucrose, maltose, glucose, galactose, starch, xylose, dextrose, fructose and lactose @1% w/w), nitrogen source (ammonium sulphate, ammonium nitrate, ammonium chloride, sodium nitrate, sodium nitrite and potassium nitrate and yeast extract @0.2% w/w) and C:N (1:1, 2:1, 3:1, 4:1, 5:1).

2.3 Characterization of Pectinase

The characterization of partially purified pectinase was done to determine temperature and pH optima and their metal ion stability. For determining the optima of the studied enzyme, the appropriately diluted enzyme was incubated with substrate at different temperature (30, 40, 50 60 and 70 ^{o}C), pH(4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) and different concentrations of metal ions (1, 5 and 10mM of Fe^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+}), following which pectinase assay was done to find the residual activity. The effect of temperature and pH on stability was studied by incubating the enzyme at temperatures and pH respectively.

2.4 Bioscouring of Cotton fabric

Greige and plain weaved 100 percent cotton fabric and cotton fiber was procured from Ganga Textile Mills, Punjab for the study. The fabric and cotton fiber were desized by boiling in distilled water at 100 ºC and thoroughly washed with cold water and dried in air [16].

Conventional scouring of cotton samples

The desized cotton sample was weighed accurately and then steeped in the solution containing 4% caustic soda. The temperature of the solution was increased uniformly to boil the cotton sample for one hour. The cotton sample after scouring was washed first with hot water and then, with cold water thoroughly to remove the impurities and chemicals. It was then, dried in an oven at 80 ºC and weighed accurately to calculate the weight loss from the cotton after scouring.

Bio scouring

Fabric of size 5x5 cm were cut and desized. The desized sample was bioscoured with 2 unit enzyme concentration at pH 5 and 50℃ with agitation for 30 minutes in shaking water bath initially. The fabrics were then rinsed with hot water first, then cold water and was evaluated.

Evaluation of the fabric

Textile testing as a whole refers to the vigorous testing done on textile material which may be inside the laboratory as well as in natural setting.

a) Fabric weight

Fabric weight as the relative weight of the fabric was expressed as the weight of a particular size of piece as the samples of size 5x5 cm were cut and weighed accurately using digital balance having 0.0001 sensitivity. The samples were weighed and the mean values were calculated and recorded before and after enzyme treatment.

For cotton, a bundle of cotton fiber was weighed before and after the treatment of fiber. The samples were weighed accurately using digital balance having 0.0001 sensitivity and the mean value were calculated and recorded before and after enzyme treatment.

The percentage weight loss was calculated using formula:

Weight loss% = ((Initial weight-Final weight)/Initial weight)*100

b) Wicking test

The test was performed by the capillary travel method which measures the rapidity of absorption. For this, a piece of cotton fabric sample were cut, measuring 5 cm length and 1 cm width. One end of the sample strip was pasted with a glass rod which was placed on heavy wooden blocks and, at the other end, two grams weight was attached to keep the sample straight. At the weighed end, the piece was allowed to touch the surface of water in a tray of distilled water. The rise of the water level in the strip was noted by keeping time as constant (30 seconds) [17]. For the test for absorbency was determined by dropping a bundle of cotton onto the surface of water in a beaker. The immediate sinking of the bundle in the water to the bottom of the beaker was considered to represent adequate absorbency [18].

c) Brightness

The brightness test was performed in Nahar Industrial Enterprises Ltd., Mohali, using Datacolor spectrophotometer 650™.

d) FTIR

The FTIR spectra were recorded using a Perkin Elmer, USA instrument in the range of 40-4000 cm^{-1}. The absorption spectra of the fabrics (untreated, bioscoured and conventionally scoured cotton). FT-IR spectroscopy has been used for the characterization of cotton fabric scouring process.

e) SEM

Scanning electron micrograph of cotton fabric and fiber bundle samples was performed to check the surface morphology. The samples were examined at 5 KV under scanning electron microscope (Model JSM6100, JEOL) at various magnifications. Carbon tape was used as a conducting material to analyze the sample in place of conventional metal coating.

Optimization of different parameters for bioscouring

To study the optimum concentration of enzyme, treatment time, optimum pH and temperature, the bioscouring was carried out with different enzyme concentration (1, 2, 3, 4 and 5 units), treatment time (20, 30, 40, 50 and 60 minutes in the shaking water bath)., different pH (4.0, 5.0, 6.0, 7.0 and 8.0), incubation temperatures (30, 40, 50, 60 and 70°C).

III. RESULTS AND DISCUSSION

3.1 Pectinase producing fungal cultures

The collection of diverse pectin rich soils resulted in isolation of a total of 40 different fungal cultures which were obtained into pure form (Table1).

Table 1 Soil sample, collection site and number of fungal isolates obtained into pure culture from each sample

S.No.	Sample Collection Site	Isolates				
1.	Kurali dung site (Punjab)	RSM1	RSM2	RSM3	RSM4	RSM5
2.	Sugarcane Bagasse dump	RSM6	RSM7	RSM8	RSM9	
3.	Bouganvilla Garden, Sector 10, Chandigarh	RSM10	RSM11	RSM12	RSM13	RSM14
4.	Sukhna Lake backside	RSM15	RSM16	RSM17	RSM18	
5.	Dhanas Forest	RSM19	RSM20	RSM21	RSM22	

6.	Golf course	RSM23	RSM24	RSM25	RSM26	
7.	Vegetable market, Sector 26, Chandigarh	RSM27	RSM28	RSM29	RSM30	RSM31
		RSM32	RSM 33			
8.	Sukhna lake wetland, Chandigarh	RSM34	RSM35	RSM36	RSM37	
9.	Orange peel	RSM38	RSM39	RSM40		

All the 40 isolates obtained were first qualitatively screened for pectinase activity. This resulted in 7 positive isolates for pectinases (RSM4, RSM5, RSM7, RSM9, RSM16, RSM18, and RSM26) (**Figure 1; Table 1**).

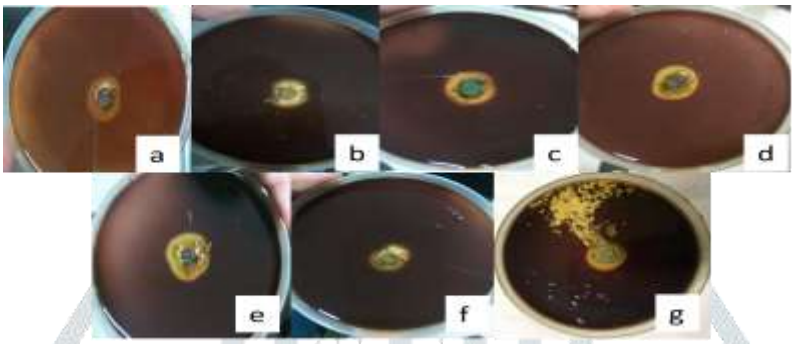

Figure 1 Seven isolates of fungal culture screened to be pectinase positive on the basis of zone of hydrolysis. A) RSM 4 (zone = 2mm excluding colony size; Colony diameter = 4 mm, B) RSM 5 (zone = 1.5mm excluding colony size); Colony diameter = 4mm, C) RSM 7 (zone = 3 mm excluding colony size); Colony diameter = 5 mm, d) RSM 9 (zone = 3 mm excluding colony size); Colony diameter = 5 mm, e) RSM 16 (zone = 2mm excluding colony size); Colony diameter = 4 mm, f) RSM 18 (zone = 1.5mm excluding colony size); Colony diameter = 5 mm, g) RSM 26 (zone = 4 mm excluding colony size); Colony diameter = 5 mm.

All the seven isolates RSM4, RSM5, RSM7, RSM9, RSM16, RSM18, and RSM26 showing the zone of hydrolysis were quantitatively screened for determining the pectinase activity (**Figure 2**).

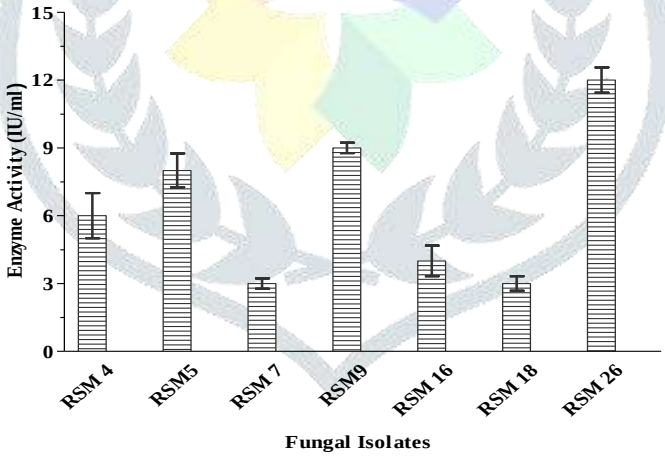

Figure 2 Quantitative screening of the 7 pectinase positive fungal isolates

Out of these seven, the isolate RSM 26 was found to be potential producer of pectinase as judged by the maximum sized zone of hydrolysis (4mm) and maximum enzyme activity (12.51U/ml) by this isolate. Therefore the isolate was selected for identification on the basis of cultural and microscopic features and for optimization studies to enhance its activity.

Identification of the selected isolate

The identification of the selected fungal isolate was done on the basis of cultural and microscopic studies. The isolate was found to belong to genus *Penicillium* (**Figure 3; Table 2**).

Table 2 Cultural and microscopic characteristics of fungal isolates

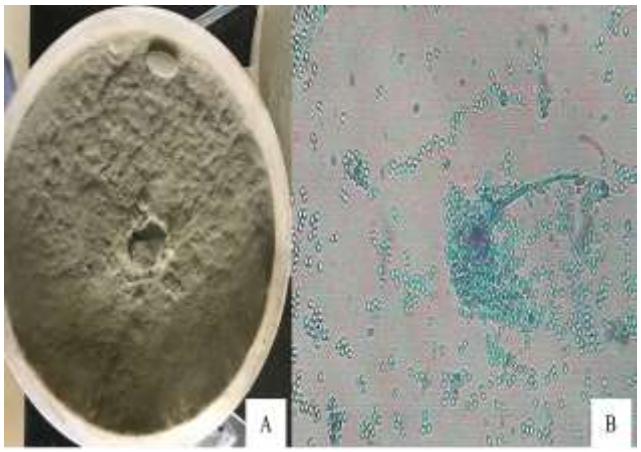

Figure 3 A) Pure culture of RSM 26 identified to be *Penicillium sp*. B) Microscopy of the culture

Characteristics	***Penicillium* sp.**
Morphological characteristics of the colony	
Surface colour	White to green
Margins	Entire
Reverse side	Orange pigmentation
Elevation	Uneven
Growth	Rapid
Microscopic Characteristics	
Hyphae	Septated
Conidiophore shape	Oval
Conidiophore length (µm)	158-280
Conidiophore width (µm)	5-8
Conidia ornamentation	Smooth
Conidia diameter (µm)	3-5
Conidia color	Light green
Sterigmata	Basipetal
Frutification	Cleistothecium

3.2 Optimization of physicochemical parameters for enhancing the pectinase

Fruit peels and sugarcane bagasse are thrown as waste after extracting juice. Utilization of fruit peel waste in one or other form is an immediate necessity from economic and environmental protection point of view. Therefore, the effect of different solid substrates on pectinase production was studied by using a variety of solid substrates like apple pomace, sugarcane bagasse, orange, mausami and pineapple peel. It is evident from the Figure 4a that out of these five substrates, sugarcane bagasse gave the maximum enzyme activity (73±1.5 U /gds) after 72 hours of incubation at 28 °C.

The inoculum size is considered as one of the important parameters in the SSF processes [19, 20]. Therefore, to optimize the amount of inoculum required to achieve maximum activity, the prepared medium was inoculated with varying amounts of active inoculum culture of the test fungus i.e. ranging from 5ml-25ml containing $1x10^6$ spores/gds. The result revealed the maximum enzyme yield in 5 ml seed culture containing $1x10^6$ spores/gds (Figure 4b).

To determine the effect of incubation period on pectinase production, enzyme activity was measured at regular intervals from 48 hours to a period of 144 hours. Highest pectinase activity was measured after 72hours (77.75±2.08 U/gds) followed by gradual decline on either side. Least enzyme production was observed at 144 hours of fermentation time (Figure 4c). Afterwards incubation beyond 72 hours resulted decrease in enzyme activity that could be due to depletion of nutrients available causing a stressed microbial physiology eventually resulting in an inactivation of enzyme [21]. It is well known that each microorganism can grow and sustain its metabolic activities between certain pH values, since the pH of the medium affect the microbial growth through the functioning of cell enzymes and the transport of nutrients into the cell, the suitable pH for fermentation was determined by setting the pH of the growth media between 4 to 8. The result revealed the maximum enzyme activity at pH 5 (74.80±1.8U/gds) (Figure 4d). This implies that the pH of the medium influences the growth of microorganisms and hence the enzyme production.

Temperature is an important parameter for both, the fungal growth and metabolite production, specifically under SSF, therefore to determine the optimum fermentation temperature, the medium was incubated at different temperatures ranging from 23°C to 43°C out of which, the optimum temperature was found to be 28±0.5 ºC (Figure 4e).

The effect of various carbon sources that can induce pectinase was found by testing different carbon sources to the basal medium containing sugarcane bagasse such as (i) monosaccharides: glucose, fructose, xylose and galactose; (ii) disaccharides: maltose, sucrose, lactose; and (iii) polysaccharides – soluble starch and dextrose. Among carbon sources a significant enhancement of pectinase activity was observed in medium incorporated with lactose. The maximum enzyme activity was found to be 97.51±0.11 U/gds, highest among all carbon sources (Figure 4f).

The effect of various nitrogen sources on pectinase was determined by testing different nitrogen sources (ammonium sulphate, ammonium nitrate, ammonium chloride, sodium nitrate, sodium nitrite, potassium nitrate and yeast extract) to the basal medium containing sugarcane bagasse and lactose. The result obtained revealed the maximum activity (99.33±1.44 U/gds) in medium containing ammonium sulphate (Figure 4g).

The optimization of carbon and nitrogen ratio was done by supplementing the original medium by different ratio of optimized carbon and nitrogen source (i.e. 1:1 (control), 2:1, 3:1, 4:1, and 5:1). The result revealed the ratio of 2:1 of carbon source and nitrogen led to the maximum (126±0.07 U/gds) production of enzyme. (Figure 4h)

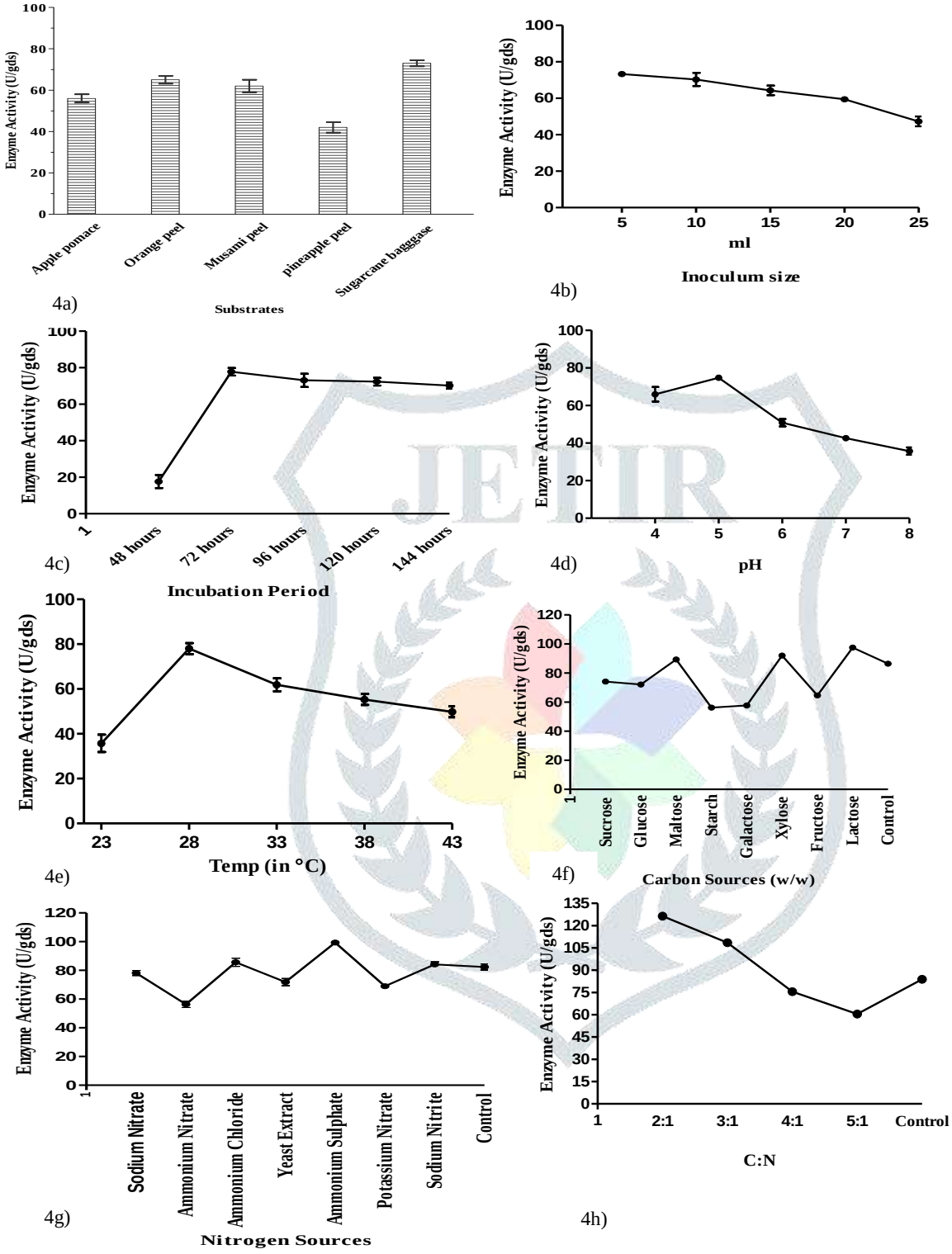

Fig. 4 Effect of different physico-chemical parameters on pectinase activity produced by a sp. of *Penicillium* substrates on pectinase production; 4a) Substrates, 4b) Inoculum size, 4c) Incubation period , 4d) pH , 4e) Temperatures , 4f) Carbon sources, 4g) Nitrogen sources , 4h) Carbon and nitrogen ratio; Values are mean ± SD of triplicates.

3.3 Characterization of enzyme

pH Optima- The partially purified pectinase exhibited a significant enhancement of activity was at pH 5.0. Thereafter a decreasing trend in activity from pH 5.5 to pH 8.0 was observed. The optimum pH for pectinase activity produced was found to be at pH 5.0 (Figure 5).

Temperature Optima- Every enzyme has optimum or apparent temperature at which its activity is highest, above this range, the enzyme is denatured, therefore losses its active site for catalysis. The results obtained revealed the 50ºC as the temperature optima at which the activity of pectinase was found to be the maximum, with further increase of temperature, it decreased (Figure 6).

Effect of metal ions- The effect of selected metal ions on the activity of purified pectinase was studied at an optimum pH and temperature 5 and 50ºC respectively by adding 1 mM, 5 mM and 10 mM metal ions to the reaction mixture. Among the metal ions tested, addition of $CaCl_2$ in the concentrations of 1 mM, 5 mM and 10 mM enhanced the activity of pectinase by 4.79%, 8.87% and 23.7% respectively whereas $FeCl_2$ resulted in the enhancement of the enzyme activity by 12.71% and 3.09% by addition of 5 mM and 10 mM salt. Salts such $MnSO_4$ exhibited a maximum inhibition of around 16 % in 1mM concentration, followed by $CuSO_4$ up to 10% inhibition in 1 mM and 10 mM concentration respectively (Figure 7).

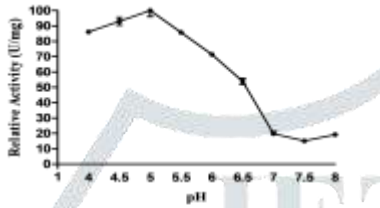

Fig.5 The pH optima of pectinases produced by *Penicillium sp.* Values are mean ± SD of triplicates.

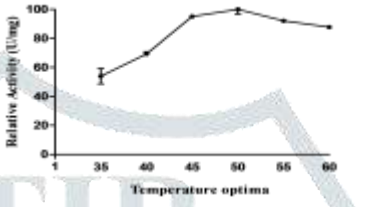

Fig. 6 The temperature optima of pectinases produced by *Penicillium sp.* Values are mean ± SD of triplicates.

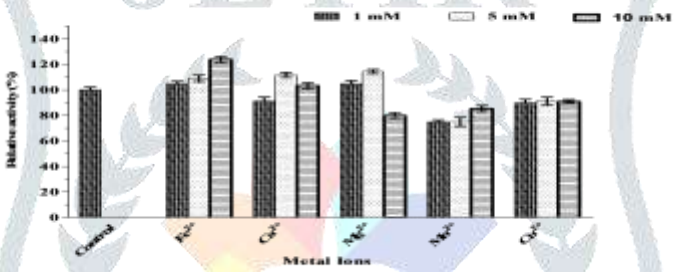

Fig.7. Effect of metal ions on pectinase activity produced by *Penicillium sp.* Values are mean ± SD of triplicates.

3.4 Bioscouring of cotton fabrics and fibers by pectinases

Desizing was performed as a first step in pre-treatment of the cotton fabrics and fibers. After desizing bioscouring was performed on cotton fabrics and fibers. This resulted in remarkable decrease in weight of samples as compared to untreated samples.

The percentage weight loss in conventionally scoured sample was 18.36 percent and in scoured sample was 3.7 percent initially over original (untreated) (**Table 3**).

Table. 3 Initial comparison of untreated, conventionally treated and bioscoured fabric

Cotton fabric	Untreated	Conventionally scoured	Bioscoured
%age weight loss	NIL	18.36	3.7
Wicking test (cm)	1.7	2.7	3.0

Optimization of different parameters for enhancing bioscouring process for cotton fabric

Enzyme dose- Among the different concentrations (1, 2, 3, 4 and 5) units of pectinase used, the minimum weight loss was observed in in the lowest concentration (i.e. 1 unit) (**Table 4**).

Time duration- Among the different time intervals (20, 30, 40, 50 and 60 minutes) the minimum weight loss of 0.9% was recorded for 20 minutes at 1 unit enzyme concentration. Hence, the optimum treatment time was found to be 20 minutes (**Table 4**).

pH- Among the different pH range tested (4.0, 5.0, 6.0, 7.0 and 8.0), at an optimum 1 unit enzyme concentration for 20 minutes of incubation time, minimum percentage weight loss of was noticed at pH 5.0 (**Table 4**).

Temperature- Among the temperature ranges (30, 40, 50, 60 and 70ºC), the minimum weight loss of the fabric was observed at 50ºC (2.4%) (**Table 4**).

Table 4 Effect of different parameters for the optimization of bioscouring process for cotton fabric

Sr. No.	Different parameters															
	Enzyme Conc.				Treatment Time				pH				Temperature			
	Units	% Weight loss	Wicking Test (cm)	Brightness	Time (min)	% Weight loss	Wicking Test (cm)	Brightness	pH	% Weight loss	Wicking Test (cm)	Brightness	Temp.	% Weight loss	Wicking Test (cm)	Brightness
1	1	2.3	3.1	15.57	20	0.9	3.1	14.75	4	2.3	2.6	13.67	30	3.08	2.6	15.43
2	2	3.2	2.9	12.52	30	1.6	3.0	14.44	5	1.8	2.9	14.01	40	2.98	2.9	15.72
3	3	3.8	2.8	12.95	40	1.7	2.8	13.15	6	1.9	3.1	16.42	50	2.64	3.3	17.40
4	4	3.7	2.6	11.85	50	1.75	2.6	12.49	7	2.8	2.9	14.78	60	3.07	3.1	16.38
5	5	4.7	2.4	9.42	60	2.1	2.5	11.30	8	2.9	2.2	13.39	70	3.5	2.8	14.25

Thus, wicking tests showed that the enzymatic treatments considerablely changed the water absorption capabilities of untreated cotton and unscoured cotton fabrics. The change in the water absorbency of cotton was rapidly catalyzed by pectinases in case of bioscoured cotton.

The overall optimization process resulted in the enhancement of the process of bioscouring. There was a significant difference observed in the weight loss percentage and absorbency of the final bio scoured cotton fabric and the untreated cotton sample (**Table 5**).

Table 5 Final comparison of cotton fabric (Untreated, Conventionally scoured, bioscoured fabric)

Cotton Fabric	Untreated	Conventionally scoured	Bioscoured	% Enhancement
% Weight loss	NIL	18.36	2.38	24.32
Wicking test (cm)	1.3	2.7	3.3	10

FTIR analysis- Fourier-transform infrared (FT-IR) spectroscopy highlighted the changes in the main non-cellulosic impurities by characterizing the esters and carboxyl acids that are present in major amount like pectins, waxes and other minor impurities which do not exist in cellulose structure (Chung *et al.*, 2004).

Figure 8 FTIR analysis of cotton fabrics: A) Conventional treatment, B) Enzyme treated (bioscoured), C) Untreated cotton fabric

SEM analysis of cotton fabrics

From the results of the scanning electron microscopic observations, it was concluded that pectinases penetrate the cuticle in aqueous solutions through cracks or micro pores and make contact with the pectic substances in the substrate. Pectic substances are hydrolyzed with aid of pectinases which resulted in the removal or partial removal of the cuticle or breakdown of the continuity of the cuticle. The parallel ridges and grooves were almost absent in the bioscoured fabric. The bioscoured fabric was smooth and without any cavity. The surface features of the alkali scoured cotton were clearly different from those of the bioscoured fabric.

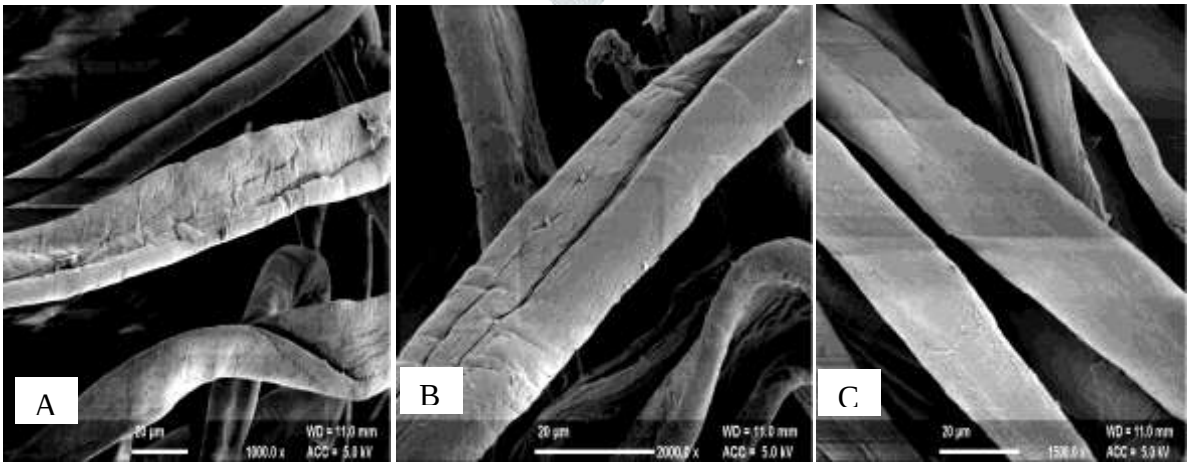

Fig. 9 Scanning electron micrograph of surface features of A) Untreated greige cotton showing roughness of the cotton fabric threads, B) Alkali treated greige cotton showing a bit smoother surface (Conventional scouring), C) Bioscoured greige cotton showing totally a smooth texture when treated with pectinases

Bioscouring of cotton fabric- The experimental results were recorded as the lowest amount of an enzyme concentration and shortest treatment time with pH 5 and temperature 50°C caused the specimen to gain adequate absorbency. The results for the fabric substrate give the lowest concentration and time that produced the adequate absorbency.

Table 9 Final comparison of cotton fabric (Untreated, Conventionally scoured, bioscoured fabric)

Fabric	Untreated	Conventionally scoured	Bioscoured
%age weight loss	NIL	3.88	2.64
Absorption test	57 min	35 min	49 min

SEM analysis of cotton fibers- The scanning electron microscopic observations of untreated, conventionally treated and enzyme treated cotton fiber.

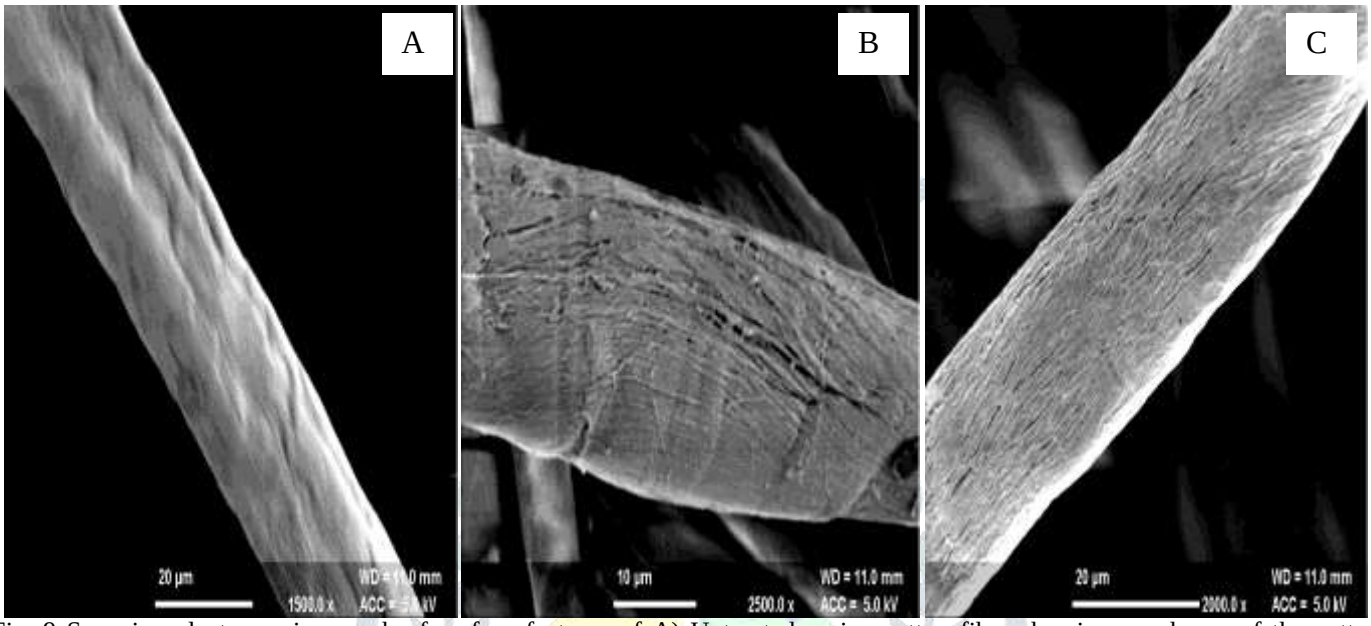

Fig. 9 Scanning electron micrograph of surface features of A) Untreated greige cotton fibre showing roughness of the cotton thread surface, B) Alkali treated cotton fibre showing a bit smoother surface (Conventional scouring), C) Bioscoured cotton fibre showing totally a smooth texture when treated with pectinases

III. CONCLUSION

The present investigation is to brighten the possibilities of assessing the efficiency of the pectinolytic fungi, *Penicillium sp.* in the production of pectinase. The study also explored the potential application of its crude pectinase in textile industry. The use of the agro industrial pectin enriched residues would be beneficial to textile industry is not only for providing value added commercially important pectinase at a low cost but also giving a commercial application to waste thus preventing environmental contamination due to its disposal. The information generated from the study might be very useful for the cotton manufacturers, weaving industry, textile finishing firms and consumers. It can be suggested that the textile processing units can safely apply these finishes to the organic handloom cotton fabric to improve the hand feel and texture without altering their inherent mechanical properties.

IV. Acknowledgment

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