Bioscouring and Desizing of Textile Fabrics Using crude Enzyme Produced by White Rot Fungus (Basidiomycetes) Isolated from Rotten Wood

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Abstract— With an increasing demand for the reduction of pollution caused by textile industries the exploration of the microbial world has gained a special arena in the field of contemporary research. The usage of microbial enzymes is preferred to chemical processing of fibres and textiles because of their non-toxic and eco-friendly characteristics. The present experimental study circumscribed around the development of an environment friendly and an economical mean of fibre development by desizing and bioscouring with the help of crude enzyme produced from a wild variety of isolated basidiomycetes. Isolation of basidiomycetes was done from wood barks collected from eastern part of India. Plate assay for amylase, cellulase and xylanase, from the isolated organism was performed along with the determination of their respective enzyme activity. Enzyme produced on the 11 day of incubation under SSF condition at 25^{0}C was obtained to show the highest enzyme activity. A comparative study of desizing and scouring has been done subsequently on the basis of biological (with extracted enzyme from the isolate) and chemical processes whereby the biological method has shown a significant level of increase in absorbency and whiteness of the textile fibre. The liquid waste discharged from both the methods were also estimated for COD and BOD and has shown that the usage of crude enzyme for the both desizing and bioscouring was more environment friendly in nature.

Keywords— Bioscouring, Desizing, cellulase, amylase, xylanase, COD, BOD.

I. INTRODUCTION

Fabrics, yarns or raw fibres includes several kinds of impurities like chemical residues, pesticides, dirt, seed coat fragments, pesticides, and metallic salts. The process which aims to improve the whiteness and absorbency of textile materials by discarding such non-cellulosic natural matter from the fabrics is called defined scouring [19, 4]. In nature, non-cellulosic materials create a physical hydrophobic barrier to protect the fibre from the environment throughout its development [22]. This process is one of the most important treatments in textile processing in order to achieve better hydrophilic fabric. Here non-cellulosic materials like waxes, proteins, natural colorants, minerals, fats, non-cellulosic polysaccharides and water-soluble compounds largely found in the primary cell wall are partially or completely unstayed from the native cotton. This method provides cotton fabrics with even wettability such that these fabrics can be dyed and bleached conveniently.

In industries, sodium hydroxide, a highly alkaline chemical is generally used in scouring methods [22]. Along with the removal of the non-cellulosic impurities from the fabrics, these chemicals also attempts to damage the cellulose which results in loss of heavy strand and loss in weight of the fabrics [5]. Additionally, the use of these harmful chemicals leads to high BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand) and TDS (Total Dissolved Solid) in waste water [29].

Desizing is a process which discards a number of adhesive substances from the warp threads which is generally coated for the prevention from thread from breaking during weaving process. This method is generally carried out by treating the fabrics with a wide range of chemicals like alkali, acids or oxidizing agents [5]. In recent years, the bio-catalytic applications for enzymes have evolved immensely because of their specific properties. Enzymes have a high specificity, undergo a wide diversity of reactions, are ecologically correct and additionally present chemo-regio-enantio selectivity. A large variety of enzymes are being produced by a wide range of microorganisms which have been used in textile industry. Some of these microorganisms include: *Bacillus subtillis*, *Aspergillus niger* [8], *Aspergillus oryzae* [8], *Trichoderma reesei* [9], *Phanerochaete chrysosporium*, *Pleurotus ostreatus* [10]. Cellulase, amylase and xylanase are some of the potential enzymes which finds wide spread industrial applications in desizing and bioscouring [21].

Amylase comes from a family of glycoside hydrolase enzymes which acts on alpha 1, 4 glycosidic bonds and finally breaks down starch into glucose molecules. Cellulase are also known as endo-1, 4-beta-glucanase. They play a vital role in the hydrolysis of cellulosic biomass to sugars. Xylanases occurs in a broad range of fungi and bacteria. They are categorized into two families: glycosyl hydrolase family 10 and 11 [21, 11, and 27]. Xylanase which belongs to Family 11 xylanases are found to be of better specificity for xylanase whereas those of family 10 are found to exhibit cellulose activity [11].

It is known that basidiomycetes are capable of breaking down cellulose and lignin. They cause rotted wood to feel soft, spongy, moist or stringy and appear to be yellow or white in colour. While these basidiomycetes break down the lignin in

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moist wood, it causes to leave the lighter-colour cellulose behind. It has come to notice that basidiomycetes are capable of producing enzymes, such as laccase, which is required for the break down lignin and other complex organic molecules; they are being screened for potential use in myco-remediation applications [7].

The alpha amylases cleave at any random site on the starch chain, yielding glucose and maltose. Furthermore, they limit dextrin from amylopectin and amylose. Alpha-amylase is one of the essential enzymes required for digestion in mammals [21]. Cellulases have wide range of industrial uses in the modification of cellulosic materials. They even are used in the degradation of mixed linkage 1, 3; 1, 4-beta-glucans [1].

In this present study basiodiomycetes species has been considered as the model organism for the production of amylase, cellulase and xylanase which will be further studied for their usage in bioscouring and desizing of fabrics. We intend to use them as a good alternative to chemicals used in the textile industries for fibre processing and introduce an economical mean for enhancement of the fibre quality.

II. Methods and Methodologies

2.1 Procurement of standard microbial strain

Standard strain of *Phenerochaetes chrysosporium* 787 was procured from Department of Biotechnology and Biosciences, Lovely Professional University and was subcultured on wheat bran agar for the further study [8].

2.2 Isolation and identification of wild basidiomycetes species

10 samples of rotten wood with white cottony growth of fungus were collected from Dharamshala, Himachal Pradesh. The samples were then inoculated in minimally processed wheat bran for their growth, from which after the arrival of white mycelium they were subcultured for purification on wheat bran agar and were subjected for preliminary identification for the presence of clamp connection by LPCB staining technique.

2.3 Production and profiling of the enzymes produced by the isolate

For enzyme production through fungal cells 50ml of distilled water was taken and added to the fermented matter, after some time solids started to accumulate hence was removed by the process of filtration which was followed by centrifugation at 5000rpm for 5 minutes simultaneously flasks were kept in shaking incubator which was set at 220rpm for 30 minutes at a temperature of 25 °C. After centrifugation was performed the supernatant that was obtained was used as crude enzyme extract [26].

2.4 Estimation of amylase by DNS assay method

To perform DNS assay for amylase activity 0.5ml of enzyme solution is pipetted out and is then incubated for 3 minutes at 25°C. Simultaneously 0.5ml of starch solution was added which was again incubated at ambient temperature for 5 minutes. Once it was done 1 ml of DNS reagent was added which was later heated in boiling water for 5 minutes followed by cooling down under running tap water and later the the volume of the solution was made up to 10ml by addition of distilled water in it. Spectrophotometer was set at 540nm and absorbance was taken [1].

2.5 Estimation of xylanase by DNS assay method

For carrying out xylanase activity by DNS method 1.6ml of diluted crude enzyme was taken and added in a test tube containing 0.4ml of substrate suspension, which was later incubated in shaking incubator set at 100rpm for 15 minutes at 55°C.This was followed by DNS method and absorbance was taken at 540nm in spectrophotometer [8].

2.6 Estimation of Cellulase by DNS assay method

To carry DNS assay for cellulase activity 2ml of crude enzyme was taken and added in 2ml of 2% Carboxymethyl cellulose which was incubated at 50°C for 30 minutes. Followed by DNS Method and absorbance was taken at 540 nm [20].

2.7 Desizing of Raw Fabric

First of all, unprocessed fabrics were washed and then dried respectively. Fabrics were dried and were dipped in aqueous solution which contained our crude enzyme (1 g/l), acetate buffer (pH 5.5) along with EDTA (0.5 g/l) and sodium chloride (0.5 g/l) at a temperature of 40°C. This was carried out for 10 minutes. The material to a liquor ratio was of 1:50. Now, the fabrics undergoing treatment were sqeezed to 100% wet pick up and then batched for 24 hours at ambient conditions. The desized fabrics obtained were washed twice with hot water then cold water. They were then finally dried at room temperature conditions [14].

2.8 Bioscouring of Desized Fabric

Desized fabrics collected and were scoured by harnessing with our crude enzyme in 0.05 M phosphate buffer which contained 0.5 g/l of non-ionic wetting agent at pH 8.5. Temperature maintained during the process was 55°C. Like that of desizing, material to liquor ratio was of 1:50. Four different concentrations of crude enzyme were used in the treatments viz, 0.5 g/l, 1 g/l, 2 g/l and 3 g/l. After the treatments were provided the temperature was raised to 100°C and was kept for 10 minutes to stop the enzyme action. The fabrics were finally washed with hot water and then with cold water. At last they were dried at ambient conditions [14].

III. Result and Discussion

Out of 10 samples 1 has shown positive results for white rot fungus (Basidiomycetes) which has shown characteristics similar to Basidiomycetes showing white cottony growth on wheat bran agar and presence of clamp connection by LPCB technique. The organism was compared with the standard strain for the same.

Enzyme assay was performed for amylase, xylanase and cellulase for the organism which showed positive results for enzyme activity followed by SSF on wheat bran and then spectophotometric analysis for the 3. For amylase the maximum enzyme activity was observed in 12th day of incubation. Refer to Table No. 1. For cellulase the maximum

enzyme activity was seen 12th day. Refer to Table No. 2. For xylanase max activity was seen in 12th day as well. Refer to Table No. 3

Desizing and bioscouring of textile fabrics was done by enzymatic methods showing changes in the whiteness, tensile strength, elongation and pore sizes of fabrics when they were compared with raw fabric. Refer to Table No. 5, 6 and 7.

IV. Calculation

Set 1:

Weight of pre-desized fabric = 1.75 g (w1)

Weight of desized fabric = 1.62 g (w2)

Weight of the fabric after treatment with 35% HCl = 1.56 g (w3)

Residual weight = w1 – w2
= 1.75 gm – 1.62 gm
= 0.13 gm

Total weight = w1 – w3
= 1.75 gm – 1.56 gm
= 0.19 gm

Weight of pre-desized fabric = 1.72 g (w1)

Weight of desized fabric = 1.58 g (w2)

Weight of the fabric after treatment with 35% HCl = 1.52 g (w3)

Residual weight = w1 – w2
= 1.72 gm – 1.58 gm
= 0.14 gm

Total weight = w1 – w3
= 1.72 gm – 1.52gm
= 0.20 gm

Set 2:

Weight of pre-desized fabric = 1.72 g (w1)

Weight of desized fabric = 1.58 g (w2)

Weight of the fabric after treatment with 35% HCl = 1.52 g (w3)

Residual weight = w1 – w2
= 1.72 gm – 1.58 gm
= 0.14 gm

Total weight = w1 – w3
= 1.72 gm – 1.52 gm
= 0.20 gm

Set 3

Weight of pre-desized fabric = 1.74 gm (w1)

Weight of desized fabric = 1.63 gm (w2)

Weight of the fabric after treatment with 35% HCl = 1.57 gm (w3)

Residual weight = w1 – w2
= 1.74 gm – 1.63 gm
= 0.11 gm

Total weight = w1 – w3
= 1.74 gm – 1.57 gm
= 0.17 gm

Mean Residual weight = (0.13 gm + 0.14 gm + 0.11 gm) ÷ 3
= 12.6 gm

Mean Total weight = (0.19 gm + 0.20 gm + 0.17 gm) ÷ 3
= 18.6 gm

Enzyme activity = V (µl) × O.D. / ε × Incubation time × Enzyme (µl)

V = volume of water added

ε = standard value (60 µl)

V. List of Tables and Photographs

List of Tables

Table Number 1: Comparison between the Spectrophotometric analysis by measurement of Ab Absorbance of Amylase with Amylase for standard

No. of days	Absorbance at 540 nm	Absorbance at 540 nm for *Phenerochaetes chrysosporium 787*	Enzyme activity (µmoles/min per µg)
1	0.012	0.17	3.2×10^{-4}
2	0.072	0.22	3.77×10^{-5}
3	0.085	0.25	3.95×10^{-5}
4	0.089	0.34	4.26×10^{-5}
5	0.096	0.37	4.88×10^{-5}
6	0.11	0.40	6.22×10^{-5}
7	0.14	0.47	7.5×10^{-5}
8	0.17	0.49	8×10^{-5}
9	0.18	0.54	8×10^{-5}
10	0.21	0.59	9.3×10^{-5}
11	0.22	0.62	9.77×10^{-5}
12	0.24	0.67	1.0×10^{-4}

Table Number 2: Depiction of pore size variation in fabrics processed by Desizing and Bioscouring

Fabric Types	Length (micrometers)	Width (micrometer)
Raw Fabric	8	3.5
Desized Fabric	7.7	3.4
Bioscouring Fabric	7.4	3.2

TABLE NUMBER 3: RESULTS OF TENSILE STRENGTH AND ELONGATION ACHIEVED IN VARIOUS FABRICS AFTER DESIZING AND BIOSCOURING

Fabric Types	Force required to (kg/inch2)	Elongation (cm)
Raw Fabric	3.11	4
Desized Fabric	4.2	5.7
Biscouring Fabr	5.21	14.2

TABLE NUMBER 4: DEPICTION OF WHITENESS OF THE FABRICS WHEN COMPARED WITH THE COLOUR SCALE

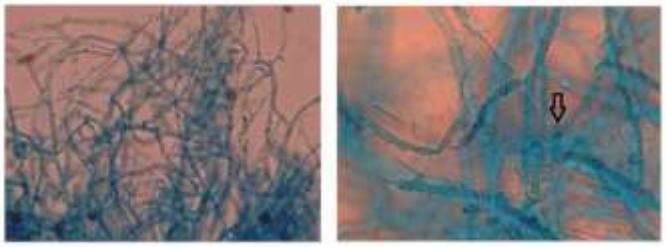
Fabric Types	Whiteness Level (code)
Raw Fabric	3/4
Desized Fabric	4/5
Bioscouring fabric	4/5

List of Photograph

Photograph 1: Mycelial growth on wheat bran agar (8th day)

Photograph 2: Fungal fruiting bodies on wheat bran agar (29th day)

Photograph 3 (a) and (b): Microscopic identification of Basidiomycetes (clamp)



VI. CONCLUSION

Desizing and scouring of textile fabrics with biological enzymes in industries is an example of white industrial biotechnology which will facilitate eco-friendly technologies in fiber treatment technology and strategies to improve the final product quality.

There are certain potential factors which promote the use of biological enzymes in processing of textile fabrics. Out of many, few principal reasons to choose enzymes are the consumption of energy and requirement of raw materials. One noticeable point lies in the increased awareness of environmental concerns related to the use and disposal of harmful chemicals into water, landfills, or release into the air during chemical processing of textiles which also plays an important role for the application of biological enzymes in finishing textile materials.

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