International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979
Impact Factor: RJIF 5.72
Received: 13-11-2018; Accepted: 10-12-2018
Volume 5; Issue 12; December 2018; Page No. 127-129

Bio softening and Bio bleaching of *Cleome Viscosa* (L) fibre using crude xylanase from a phyllospheric microorganism-*Aspergillus niger*

Suganya W
Assistant Professor, Department of Botany, Government Arts College, Coimbatore, Tamil Nadu, India

Abstract
The Phyllo sphere represents the habitat provided by the above ground parts of the plants and on a global scale supports a large and complex microbial community. These microbes are the rich source of xylanases which represent one of the largest group of industrial enzymes that have wider applications in food, feed, fuel, textile, paper and pulp industries. In the present study also the phyllospheric microorganism-*Aspergillus niger* is used for the production of crude xylanase. These xylanases are then subjected for the bio softening and bio bleaching of plant fibre procured from *Cleome viscosa.* The enzyme treated fibres enhanced with more brightness, greater whiteness index with reduced yellow index, greater tensile strength. The SEM analysis revealed that the enzyme treated fibres showed more bore cracks, peelings, swelling, and external fibrillation on its surface which enhances the surface topology of the fibre**.** Thus the microrgamisms are ecofriendly and a best alternative for the harmful chemicals used in Industries.

Keywords: phyllosphere, xylanases

Introduction
Enzymes are natural catalysts which are universally found in all living organisms. They may be used for building more complex molecules from simple ones or for selective break down of a mixture of larger molecules. Just one organism can contain over 1000 different enzymes. Enzymes are utilized for environmental purposes in a number of industries like oil, animal feed, detergent pulp and paper, textile, leather, petroleum and chemical industry.

Bio softening aims to achieve a bio polishing effect with the use of specific microorganisms with selected enzyme specificity towards cell wall components. Softening of the fibre without affecting the fibre strength can be accomplished along with the biobleaching treatment, since the component responsible for the color and stiffness are one and the same 'Lignin'. Unlike the weak chemically softened fibre, microbial treatment produced soft brighter fibre having better tensile strength and elongation properties (Rajan *et al*., 2005) [1].

Xylanases are enzymes commonly found in microorganisms, Marine Algae, protozoan's, snails, crustaceans, insects, seeds, plants and other natural sources. Xylanases are able to degrade the hemicellulose present in the fibre without affecting the cellulose. Enzymatic treatment has been shown to enhance various physical properties of the fibre, including the viscosity, tensile strength, breaking length, and tear factor. In addition biobleaching with xylanases softens the fibres allowing them to undergo further chemical bleaching.

There are many fibre yielding plants in our country, which have potential for the use in industries. *Cleome viscosa* (L) belongs to the Family Capparidaceae was taken as the plant source for fibre. It is native to the carribean region. Cleome is a small, erect, profusely branched annual herb, terrestrial, aromatic herb upto 120 cm tall. It is widely distributed around villages and in wastelands and in avenues, Flowers appear in yellow colour in axillary or terminal racemes. In the present study, the plant fibre extracted from Cleome is treated with crude xylanase obtained from the phyllospheric microorganism –*Aspergillus niger* to find out the biosoftening and biobleaching properties.

Materials and Methods
- **Crude Xylanase production:** The method of Bailey *et al* (1992) [3] has been used for estimating the amount of xylanase. Oat spelt xylan was used as the substrate for xylanase assay and the amount of xylose released was measured by DNS method of Miller (1959) [6]. Czapeks dox medium, Potato Dextrose Agar medium, Potato Dextrose broth medium. Miller (1959) [6]. Mandel and Reese medium, Yeast extract medium and Carter and Bull medium were used for standardisation of the medium for crude xylanase enzyme production. Czapeks dox medium was selected as the best and then subjected to different pH conditions using buffers with a pH range of 3.5-9 and it was inoculated with the mycelial spores of *Aspergillus niger* and incubated for 6 days to find out the suitable pH for maximum enzyme production. The medium was incubated at different temperatures of 25ºC, 30 º C, 35 º C, 40 º C, 45 º C. and the organism were then subjected to different incubation periods namely 96hrs, 120hrs, 144hrs, 168 hrs,192 hrs in order to standardize the optimum cultural conditions for the maximum enzyme production.
- **Extraction of Plant fibre:** The plant fibre which are used in the present study were obtained from *Cleome viscosa* (L) belonging to the family Capparidaceae. The stem fibres were cut into suitable pieces and allowed for retting

for 3-4 weeks in stagnant water. After retting the material was cleaned with tap water and allowed to dry at 40˚C.The dried fibres were thrashed by beating to remove the adhering dirt and pulp left on it to loosen the fibres that are stuck together

- **Treatment of Plant Fibres:** The plant fibres were treated with the crude xylanase using the method of Mahjabeen saleem and Muhammad saleem Akhtar, (2012) [5] and the results are tabulated.

Analytical measurements

- **Brightness:** Fibre brightness is measured by the percentage of light reflected. Brightness was measured using premier color scan SS5000A spectrophotometer using ASTM E313Method, Whiteness Index using ISO2470 method, yellowness index using 1925 method. Untreated fibres were used as a control.
- **Tensile Strength:** The tensile strength of the fibre treated and untreated was tested using Instron Tensile tester using standard ASTM3822-01method.
- **Ultimate Tensile strength:** The ultimate tensile strength of the fibres was calculated by using the formula, Break load / πd^2 where $\pi = 3.14$,d = diameter of the fibre
- **Scanning Electron Microscope (SEM) Analysis:** The surface morphology of the treated and untreated fibres was observed using a Scanning Electron Microscope.

Results and Discussion

Biosoftening is an ecofriendly method to soften the fibre by the selective removal of lignin using specific microorganisms. In Biosoftening process using microbes the removal of lignin is done without the loss of cellulose (Akila Rajan, *et al*., 2005) [1] and it yields a good quality fiber for dyeing (Suganya, *et al*., 2007) [11].

In the present study, the organism was subjected to different cultural conditions like medium, pH, temperature, incubation period – in order to standardise the best cultural conditions for the maximum crude enzyme production. It was inferred that Czapeks dox medium (Table-1) with pH 6.5(Table-2) and temperature 35 ºC (Table-3) with incubation period of 144 hrs (Table-4) showed the maximum crude enzyme production. The enzyme was then subjected for Biosoftening and Biobleaching of fibres.

Softening of the fibre without damaging the fibre strength can be accomplished using the biobleaching treatment. This indeed enhanced the fibre with better tensile strength and elongation properties (Rajan *et al*., 2005) [1]. The fibre extracted from stem of *Cleome viscosa* when treated with crude xylanase enhanced brightness. The fibre were soaked with crude xylanase and tested for its Brightness indices-Whiteness and Yellow indices. It was observed that the yellow index was much reduced and the whiteness index was increased when compared to the control. In the same way, the enzyme treated fibres were tested for its tensile strength using Instron Tensile tester (Materials and methods).It was observed that the fibre treated with enzyme showed greater tensile strength than the control. (Table-5). Our results were in accordance with the results obtained by Ravindranath *et al*, 2007, Anuradha *et al.*, 2012. The Ultimate Tensile Strength (UTS) of the control and the enzyme treated fibres was studied as mentioned in the materials and methods and the results are presented in the Table- 6. It was observed that the enzyme treated fibre showed weight loss than the control which inferred that it enhanced the softness of the fibre. Beg *et al.*, (2001) [4] reported that xylanase from Streptomyces sp.QG-11-3 introduced greater porosity, swelling up and separation of pulp microfibrills in eucalyptus fibres. In the present study also, the SEM Analysis clearly revealed that the enzyme treated fibres showed more bore cracks, peelings, swelling, and external fibrillation on its surface which enhances the surface topology of the fibre (Fig. 1). Similar results were obtained by Poorna and Prema (2007) [7]; Sanghi *et al., (*2009) [10].

Table 1: Crude Xylanase production by *Aspergillus niger* using Different medium

S.No	Name of the Medium	Xylanase Production U/ml
1	Czapeks dox medium	0.22 ± 0.01^{a}
2	Potato Dextrose broth medium,	0.16 ± 0.01^{b}
3	Mandel and Reese medium	0.04 ± 0.00^{d}
4	Yeast extract medium	0.06 ± 0.01^{b}
5	Carter and Bull medium	0.08 ± 0.01^{d}

Values given in each cell is the mean ± SD of three replicates [a-g] Mean values within a column with no common superscript differ significantly ($p<0.05$)

.

Table 2: Crude Xylanase production by *Aspergillus niger* using Different pH conditions

S. No	pH	Xylanase Production U/ml
1	3.5	0.01 ± 0.00^{f}
2	4.0	0.04 ± 0.02^{f}
3	4.5	0.08 ± 0.03^{e}
4	5.0	0.03 ± 0.01^{f}
5	5.5	1.5 ± 0.46^{c}
6	6.0	4.1 ± 0.36^{b}
7	6.5	5.96 ± 0.31^{a}
8	7.0	2.27 ± 0.05^{d}
9	7.5	0.07 ± 0.01^{f}
10	8.0	0.05 ± 0.01^{f}
11	8.5	0.02 ± 0.01^{f}
12	9.0	0.02 ± 0.01^{f}

Values given in each cell is the mean ± SD of three replicates [a-g] Mean values within a column with no common superscript differ significantly ($p<0.05$)

Table 3: Crude Xylanase production by *Aspergillus niger* using different temperature

S.No	Temperature (Degree celsius)	Xylanase Production U/ml
1	25	0.23 ± 0.01^{d}
2	30	1.82 ± 0.41^{b}
3	35	4.85 ± 0.42^{a}
4	40	0.87 ± 0.48^{c}
5	45	0.13 ± 0.02^{e}

Values given in each cell is the mean ± SD of three replicates [a-g] Mean values within a column with no common superscript differ significantly ($p<0.05$)

Table 4: Crude Xylanase production by *Aspergillus Niger* using Different Incubation Period

S.No	Incubation period (hrs)	Xylanase Production U/ml
1	96	0.36 ±0.05d
2	120	1.79 ±0.33c
3	144	3.86 ±0.42a
4	168	0.78 ±0.46b
5	192	0.13 ±0.02e

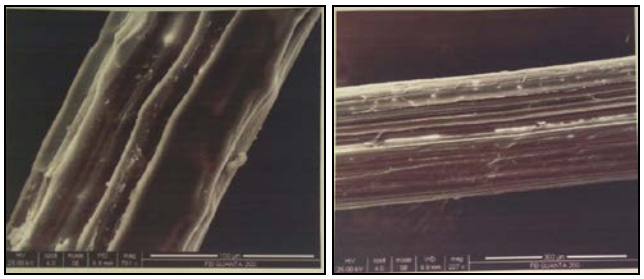
Values given in each cell is the mean ± SD of three replicates [a-g] Mean values within a column with no common superscript differ significantly ($p<0.05$)

Table 5: Effect of Crude Xylanase on *Cleome Viscosa* Stem Fibre

S.No	Parameters	Control (Without enzyme)	Enzyme treated fibre
1	Whiteness index	54.42	55.29
2	Yellow index	47.52	43.93
3	Tensile strength	159.85	371.66

Table 6: Ulitimate Tensile strength of the plant fibres

S.No	Parameters	Tensile strain at break (Standard) (%)	Tensile stress at break (Standard) (Mpa)	Time At Break (Standard) (sec)	Modulus (Automatic youngs) (gf/den)	UTS Breakload/πd^2
1	Control (without enzyme)	3.14	0.38	2.62	14531.54	0.009
2	Enzyme Treated	2.74	0.15	2.28	5580.58	0.003



Control **Enzyme treated**

Fig 1: SEM analysis of stem fibre of *Cleome viscosa*

Conclusion

Thus the crude xylanase obtained from the phyllospheric microorganism proved to be the best alternative for bio softening and bio bleaching of plant fibres there by reducing environmental pollution.

Acknowledgement

I gracefully acknowledge UGC-SERO, Hyderabad for their financial Support under UGC Minor Project. I am also thankful to "The Principal, LRG Govt Arts College for Women, Tirupur to carry out my project work successfully.

References

1. Akila Rajan, Jayalakshmi Gopinadhakurup, Tholath Emila Abrahqm. Bio softening of Arecanut fibre for value added products. Bioactive Polymer Engineering Section. 2005; 3:29-33
2. Anuradha S, ValliNachiyar C. Optimization for the biopolishing of jute fibres with cellulases from Aspergillus nidulans AJSU04.Int.J.Bioscience. 2012; 2:12-16.
3. Bailey MJ, Biely P, Poutanen K. Interlaboratory testing of methods for assay of xylanase activity. J. Biotechnol. 1992; 23(3):257-270
4. Beg QK, Kapoor M, Mahajan L, Hoondal GS. Microbial Xylanases and their industrial applications: A review. Applied Microbiology and Biotechnology. 2001; 56:326-338.
5. Mahjabeen Saleem, Muhammad Saleem Akhtar. Bio bleaching of Kraft pulp by xylanase produced by Bacillus subtilis. International journal of Agriculture and Biology. 2002; 2:242-244
6. Miller GL. Use of Dinitro Salicylic acid reagent for determination of reducing sugar. Analytical chemistry, 1959.
7. Poorna, Prema. Production of cellulose free endoxylanase from novel alkalophilic thermo tolerant *Bacillus pumilus* by solid state fermentation and its application in waste paper recycling. Bioresour Technol. 2007; 98:485-490.
8. Rajan A, Senan RC, Pavithran C. Bio softening of coir fibre using selected microorganisms. Bioprocess Biosyst Eng. 2005; 28:165-173.
9. Ravindranath AD, Sarma US. Economically and ecofriendly wet processing of coir using Trametes versicolor and Coir ret. Central Coir Research Institute, Alleppey, 2007, 1-7.
10. Sanghi A, Garg N, Sharma J, Kuhar K, Kuhad RC, Gupta VK. Enhanced production of cellulose free xylanase by alkalophilic *Bacillus subtilis* ASH and its application in biobleaching of kraft pulp. Bioresources. 2009; 4:1109-1129.
11. Suganya DS, Pradeep S, Jayapriya J, Subramanian S. Biosoftening of mature Coconut husk for facile coir recovery. Indian J.Microbiol. 2007; 47:164-166.