International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 09 , September 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

Production of Xylanase by *Bacillus subtilis* using Agriresidues and Biobleaching of Non woody Pulp of Banana.

Komal Magare[1], Anuja Kurewaru[2].

[1,2]Department of Biotechnology, MGM's Institute of Biosciences and Technology, Aurangabad, [MS] India.

ABSTRACT

Xylanase is an enzyme isolated from microorganisms, it is an alternative to polluting chemicals, and replaces the use of hazardous bio-bleaching agent chlorine in paper and pulp making industries. A cellulose free xylanase was produced from Bacillus subtilis, using cheaper substrate under solid state fermentation. Optimum level of xylanase was produced by agriresidues. The aim aided bio-bleaching results indicate that the xylanase has potential application enhancing the brightness of non woody pulp of banana. The biobleaching results were promising & can be transferred to paper mills which utilize on-woody fiber as a raw material for paper production.

Keywords: Biobleaching, Agri-residues, Non-woody pulp, Xylanase.

I. INTRODUCTION

During past several years, using of enzyme in paper & pulp bleaching has caught attention of researchers and industries over the world .Use of xylanase is cost effective means for paper making industries for different kinds of bleaching benefits. Xylanase is side-cleaving enzyme use for reducing lignin & increase the brightness of pulp. The most important mechanism of xylanase in paper & pulp industries is to degrade the xylan which is responsible for release of lignin from paper pulp & simultaneously reduces the use of chlorine as bleaching agent [1].

Pulpzyme HA, introduced by Novo Nordisk A/S, was extracted from a available xylanase for use in biobleaching of wood pulp. It was extracted from strain of *Trichodermareesei* and was used in first biobleaching stage to reduce the dosage of active chlorine [2]. Several multinational biotech companies are marketing various xylanase preparations, such as Irgazyme (Genencor International), (Cartazyme Sandoz), etc. Enzymatic prebleaching has been successfully demonstrated on mill scale where in pulp with 80%ISO brightness was achieved when used together with chlorine dioxide & hydrogen peroxide.

Lundgren *et al* have reported mill trials on soft-wood pulp using a xylanase optionally active at pH 9.0.They observed that in the pulping sequence where chlorine is totally eliminated, the pulp bleaching& brightness were satisfactory & also consumed lesser quantities of hydrogen peroxide[3]. Current efforts are aimed at process optimization, simplification, & cost reduction of enzyme application in pulp industry. Nisson *et.al* have pointed out that with the xylanase available commercially at present, a pH adjustment of the incoming pulp from pH 10-11 to 6-8 is necessary for its optimal activity. Another promising area for improving bleaching technologies relies on an understanding of the relation between lignin & other cell wall components. Lignin is covalently

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

linked to at least some of cell wall polysaccharides to form lignin-carbohydrate complexes, which are mainly responsible for difficulties encountered in separating liging from wood. Since hemicelluloses results in the mutual dissociation of latter two. Hence hemicelluloses removal leads to enhanced removal of lignin, thus reducing chlorine charge.

Solid state fermentation (SSF): SSF holds tremendous potiential for the production of enzyme. This system offers numerous advantages over submerged fermentation system, including high volumetric productivity, relatively higher concentration of product, less effluent generation, requirement for simple fermentation equipments etc. [4]A number of substrate has been employed for the cultivation of microorganisms to produce enzyme. Substrate like sugarcane, agro-industrial residues are generally bagasse, wheat bran, rice bran, maize bran, gram bran, banana waste etc. Polluting chemical technologies can be replaced with microbial enzymes. Solid state fermentation is well suited for the production of various enzymatic complexes composed of multiple enzymes. Enzymatic compounds generated by SSF find outlets in all sectors where digestibility, solubility is needed.

Application of xylanase in pulp & technology: Xylanase from different organisms have been evaluated for their interaction with various kinds of pulp. On the laboratory scale xylanase form *Streptomyces roseisclerotius*, actinomycetes, Humicolaspetc. Xylanase used for enzymatic pulp treatment to test their bleach boosting abilities [5]. The biotreatement of bagasse pulp using xylanase from an alkanophilic bacillus sp. & subsequent peroxide bleaching Resulted in a Kappa number by 10 points &an increase in brightness by 2.5 % [6]. Recently xylanase from Thermotogamaritima was compared with commercial pulp enzyme, was found to be efficient in releasing lignin from Kraft pulp [7].

Cloned xylanase expressed in *bacillus cereus* & *E.coli* has also been reported to improve the delignification of unbleached Kraft pulps.The potential application of xylanase include bioconversion of lignocellulosic material & agro waste into fermentive products, the most important biotechnological application of xylanase is use in pulp bleaching ,several chemicals such as cellulose ethers carboxyl-methyl cellulose & methyl & ethyl cellulose),which are all produced by dissolving pulp & purifying fibers from other carbohydrates[8].

II. MATERIALS AND METHODS

2.1 Microorganism

Microorganism *Bacillus subtilis (wild strain)* was revived using nutrient agar plate [10]. Further confirmation of bacteria gram staining was performed. Rod shape, blue colored gram positive bacteria was observed. Therefore, Bacillus species were confirmed by gram staining technique. [11].

2.2 Detection of xylanase activity from B*acillus subtillis*

To detect xylanase activity from *bacillus subtillis, culture* was streaked on nutrient agar containing tree bark powder which is rich source of hemicellulose. The resulting plates were incubated at 37°C for 48 hrs. After the incubation the activity of xylanase enzyme was observed on plates as hemicellulose clearance zone.

2.3 Inoculum preparation

Loopful culture of *B.subtilis* were inoculated in sterile conical flasks containing 100ml of nutrient broth & the flask was kept in rotator shaker for overnight at 120rpm at 37°C.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

2.4 Preparation of fermentation medium

For preparation of fermentation medium by solid state fermentation, moisture content of substrate i.e. wheat bran & sugarcane bagasse was measured. The moisture content ratio was 1:7 for 10gm of substrate was taken in 2 different conical flasks & the moisture content was kept 1:7.then minerals

& salts were added such as KH_2PO_4, K_2HPO_4, $MgSO_4.5H_2O$, and $CaCl_2.2SO_4$ in minute quantity, and flasks were autoclaved for 20min at 121°C. 10% v/v 16hrs old B.subtilis culture was added in both the flasks & the flask were kept in sterile condition for fermentation process.

After 48hrs aeration in aseptic condition was given and allowed further 72hrs fermentation.

After 72hrs of fermentation, enzyme was extracted by filtration by muslin cloth & centrifugation of fermentation medium at 10, 000rpm for 30min.Supernatant was stored in dark place as crude xylanase enzyme.

Filter paper discs were dipped in crude xylanase enzyme solution & placed on sterile hemicelluloses agar medium. After 48hrs clear zone was observed. Hence, it is concluded that the extracted enzyme is active & can be used for biobleaching process.

Pulp making included the typical method of 'open digestion' where raw material was cooked with 8% NaOH for a period of three hours at the boiling temperature. The pulping procedure is commonly used handmade paper industry.

2.5 The enzymatic bleaching

In which pulp were subjected to enzyme treatment in polythene bags. For this; two lots of each pulp were taken. One lot was added with enzyme & the other lot as a control was subjected to the similar condition without adding enzyme, The pulp consistency used was 6%.The initial pH of the pulp to the desired value 4N NaOH & 4N 11284 were used. Enzyme dose of 201U/gm 00pulp was used for all the studies. The pulps were then kept in water bath at a temperature of 55° C for a period of 3 hours. After the retention time, final pH of the pulps was recorded. The pulps were then squeezed to collect the water extract or enzyme filtrates and then washed with hot water and normal tap water. The enzyme filtrates collected were then analyzed for lignin at 280nm, color at 465nm and reducing sugars as xylose to find out the efficacy of the enzyme.

Both the treatment and control pulps were subjected to the peroxide bleaching after enzyme treatment. All the pulps were given an additional EDTA-treatment (0.2% EDTA, pH 4.5, temperature: Ambient, time: 45min. Consistency: 5%), before peroxide bleaching so as to increase the effect of peroxide, because EDTA is a chelating agent which traps the hindering mental ions in the pulps. Peroxide bleaching was carried out at a consistency of 8% NaOH: 1%, hydrogen peroxide 2-3%, temperature: 70°C, time: 2hrs. Color change was observed after peroxide & EDTA treatment. Then washed with hot water & tap water and handmade paper making process was carried out [12].

2.6 Handmade paper making process

The bleaching of non woody pulp was placed on aluminum foil, and uniform layer was made by pressing it by hands & roller. Excess water was removed with the help of sponge. Then the wet sheet of pulp was gently kept in hot air oven for drying. Temperature was maintained at 55°C.After 2 days the water in the pulp was removed by drying and paper was produced.

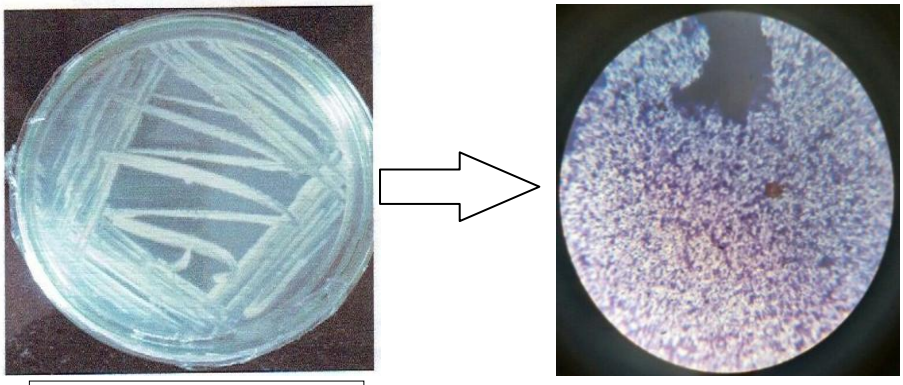
Note: For more effective results instruments like bitter can be use to make handmade paper [12].

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 09 , September 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

III. IDENTATIONS AND EQUATIONS

Not Applicable

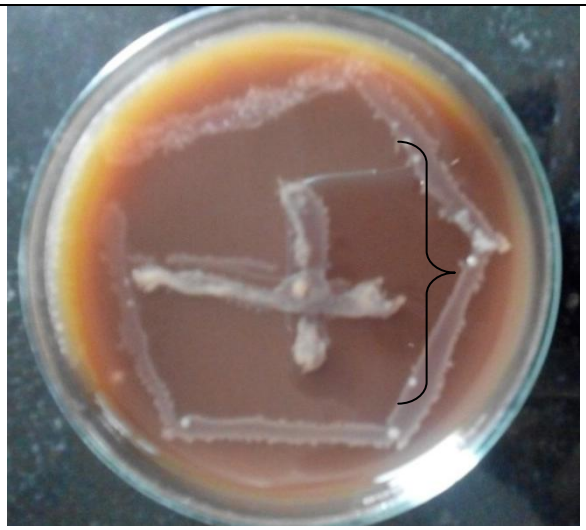
IV. FIGURES AND TABLES: (RESULT)



Nutrient agar plating of *B subtilis*

Confirmation of *B subtilis* by gram staining

3.1 Revival by nutrient agar plating and confirmation of *B subtilis* by gram staining.



Degraded portion Hemicelluloses Agar which is streaked by *B.subtilis*.

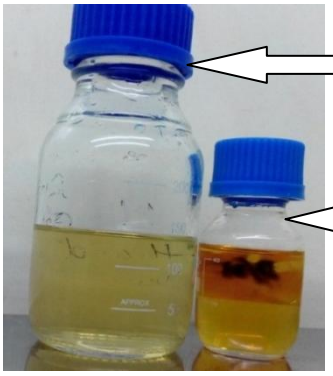
3.2 Present study described that *B subtilis* produced considerable level of xylanase

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 09 , September 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

Sugarcane bagasse | Wheat bran

Solid state fermentation

3.3 Solid state fermentation was successfully carried out using substrate as sugarcane bagasse and wheat bran.



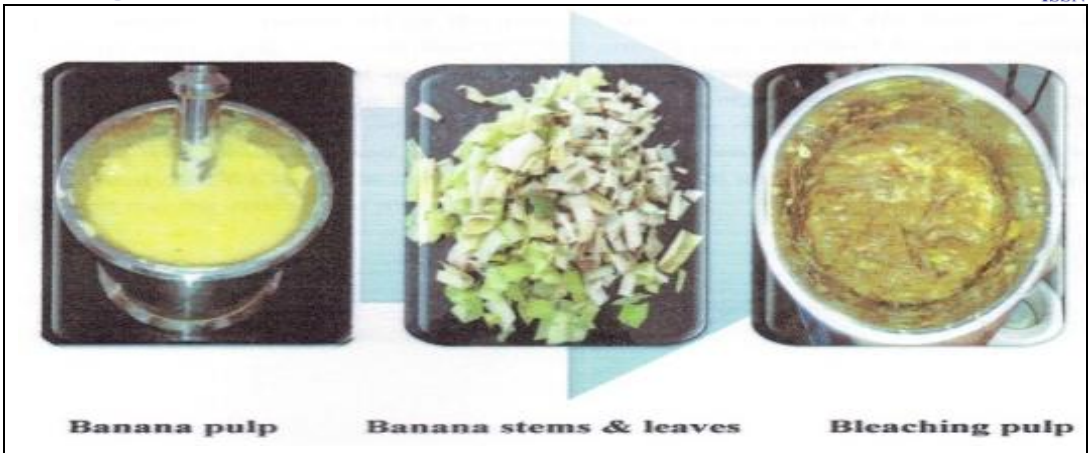
Crude xylanase from sugarcane bagasse

Crude xylanase from Wheat bran

Enzyme assay was carried out by disc diffusion method, Clearance zone Hemicellulose agar degraded by crude xylanase.

3.4 Extraction of crude xylanase by filtration and centrifugation method was done.

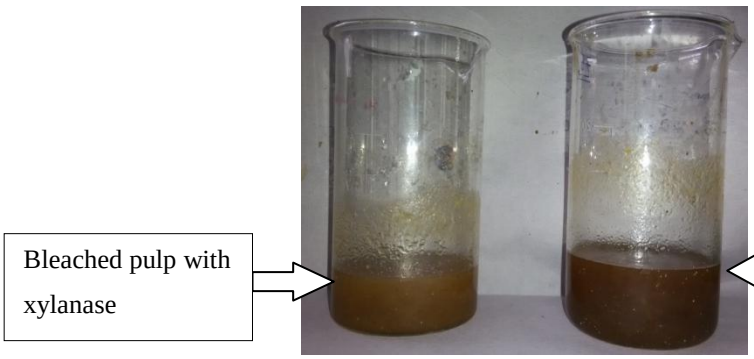
International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 09 , September 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346



3.5 After confirmation of enzyme pulp making process was carried out.

3.6 Produced pulp from banana and banana stem &leaves.

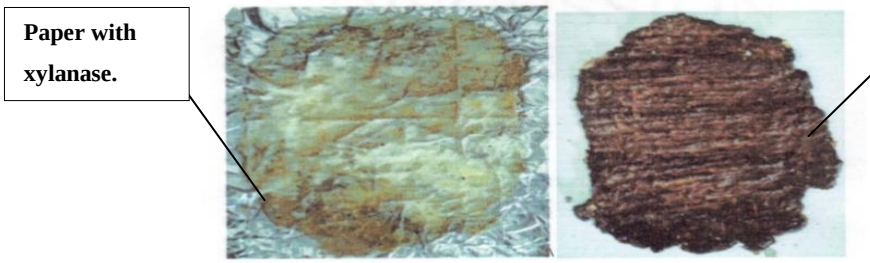
Pulp was ready to biobeached. After biobleaching of pulp by EDTA & peroxide treatment. Handmade paper making process was carried out.



Color change observed after EDTA & peroxide treatment.

These substrates were found best inducer of xylanase enzyme production. The pH of extracted xylanase was neutral. Xylanase is stable at room temperature. Xylanase was found to be effective in biobleaching of banana pulp.

3.7 Xylanase treated raw banana fibre pulp. Comparison between control and sample indicate that he xylanase has potential application in enhancing the brightness of nonwoody plant fibre pulp.



IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

V. CONCLUSION

The isolation of xylanase from *bacillus subtilis* is rare. Thus it was confirmed that *bacillus subtilis* produced xylanase enzyme, which replaces the harmful bio-bleaching agent chlorine& it was proved that xylanase enzyme responsible to increase the brightness of paper. Hence it is eco-friendly and convenient method for paper production at industrial level.

VI. ACKNOWLEDGMENT

The authors would like to show immense gratitude towards to the managements and mentors of Mahatma Gandhi Mission's Institute of Biosciences and Technology (MGM's IBT) for motivation and providing research infrastructure and facility.

Author information's: Komal Magare, Anuja Kurewaru, we are undergraduate students of biotechnology completed work under the guidance of Mrs. Anju Agrawal, assistance professor of Mgm's institute of bioscience and technology, Aurangabad.

VII. REFERENCES

[1] Chen *et al.* recently studied xylanase from Thermo toga maritima was compared with commercial pulp enzyme was found to be efficient in releasing lignin from Kraft pulp(1997), *14(3),* 117

[2] Pulpzyme HA introduced by Novo Nordisk A/S, was extracted from a available xylanase for use in biobleaching of wood pulp(2009)

[3] Lundgren *et al* have reported mill trials on soft-wood pulp using a xylanase optionally active at pH 9.0. (2012) *3(4),*37

[4] Ashok Pandey *et al* This system offers numerous advantages over submerged fermentation system, (2002)*13(2-3),*81-82

[5] P.Bajpai, M.B jauhari Xylanase from different organisms have been evaluated for their interaction with various kinds of pulp. *(*1994), *38(1),*2-3

[6] Kulkarni & Rao xylanase from an alkanophilic *bacillus sp*. & subsequent peroxide bleaching Resulted in a Kappa number by 10 points & an increase in brightness(1999),*23(4)*422-423

[7] Chen *et a.l* recently studied xylanase from Thermotogamaritima was compared with commercial pulp enzyme was found to be efficient in releasing lignin from Kraft pulp. (1997), *14(3),* 118

[8] Ian D. Reid & Michael G Paice, cloned xylanase in bacillus cereus & E.coli Paiseet et al.(1998)has been reported to improved the delignification of unbleached Kraft pulp. (1993),*60(5),*1397-1399

[9] F.L. Motta, C.C.P Andrade & M.H.A.Santana, The most important biotechnological application of xylanase is use in pulp bleaching ,several chemicals such as cellulose ethers(carboxyl-methyl cellulose & methyl & ethyl cellulose),which are all produced by dissolving pulp & purifying fibers from other carbohydrates. (2013), By INTECH, 258

[10] Agar plate method by walter hense (1881).Wikipedia Gram staining method by Hans Christians' gram, WikipediaM Ayyachamy, T M Vatsala, Production and partial Characterization of cellulose free xylanase by Bacill subtilis C 01 using aggriresidues and its application in biobleaching of nonwoody plant pulps (2007),*45(5),* 467 472.