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RESEARCH ARTICLE

Biobleaching of banana fibre pulp with incorporation of xylanase enzyme from *Aspergillus oryzae*

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ABSTRACT : The potential of extracellular xylanase produced by *Aspergillus oryzae* through solid state fermentation was investigated on banana fibre pulp bleaching in association with conventional bleaching with chlorine dioxide. The maximum enzyme production was obtained at 30°C after 48 hrs of incubation using wheat bran substrate. Highest enzyme activity 1136 IU/g dry substrate was found under optimized condition. Banana fibre pulp were pretreated with different dose of xylanase enzyme before the conventional bleaching sequence. Xylanase pre-treatment reduce the kappa no and enhance the optical and physical properties of pulp. The maximum reduction in kappa no were 1.3 unit at the dose of 30 IU/g. Xylanase treatment also improve burst index, tensile index and double fold by11.81 per cent, 5.7 pera cent and 2.8 per cent, respectively.

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Key Words :
Aspergillus oryzae, Xylanase, Biobleaching, Solid-state fermentation

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The continuous consumption of paper is increasing excessively day by day leading to deforestation, to meet the gap in the availability of raw material for paper industry. Deforestation is one of the major causefor environmental pollution and global warming. Agricultural residues such as cotton stalks, wheat straw, rice straw, sorghum stalks, hemp, jute, etccan be prove potential raw materials for paper production. Among them banana is onewhich yields high biomass that may serve as an alternative source in fibre based industries like paper, card board, tea bags, fibre lining for car interiors, high quality dress material, currency notes (Shivashankar *et al.*, 2006). Banana plantation havea significant importance in India. The production of banana in India is about 27.01 million tons from an area of 0.765 million ha in 2011 (Mustaffa, 2011). Banana pseudo stem are good source of long fibre but after the fruit harvesting it were left over in the field itself, which causing environment pollution. Since the availability of banana stem waste is large and its fibre have suitable properties for paper making, it can be used for paper making to overcome the shortage of raw materials in this industry.

Biobleaching, employing enzymatic bleaching techniques, is now considered to be one of the preferred routes, primarily because of number of advantages offered over conventional chemical bleaching. These enzymes could help to lower the consumption of chlorinated organic compounds during chemical bleaching process.Xylanases are the enzymes that have mainly bean used for pre-bleaching of pulps. Xylanases cleave and solubilize precipitatedxylan and lignin located on the surface of the micro fibrils. This

facilitates pulp bleaching and lowers chlorine consumption there by reducing discharge of toxic organo-chlorine compounds in the environment (Senior *et al.,* 1992 and Tolan and Canovas, 1992).

Xylanase is produced by a diverse genera and species of bacteria, actinomycetes and fungi. Several different species of *Aspergillus* have been reported to produce xylanases, including *A. niger, A.ochraceus, A. oryzae, A. awamori A. tamarii* and *A.fumigatus* (Baiely and Pautanen, 1989; Haltrich *et al.*,1996; Kadowaki *et al.,* 1997 and Siedenberg *et al.,* 1998). The present paper discusses the solid state fermentation process for production of xylanase from *Aspergillus oryzae* and its enzymatic pre-bleaching of banana fibre pulp. And later evaluate the optical and physical properties of the bleached banana pulp.

EXPERIMENTAL METHODOLOGY

Fungal strain and its growth conditions, *Aspergillus oryzae*, our own isolate, isbeing maintained on potato dextrose agar (in g/lit: protein infusion form 200.0; dextrose 20.0 and agar 15.0) at 4°C by transferring on to a fresh medium after every 4–6 days.

Solid state fermentation for xylanase production :

Xylanase enzyme production were performed in Erlenmeyer containing 10 g of wheat bran with the addition of 30 ml of Mandel's medium. The Mandel's medium was prepared with the following composition (g/lit) 10.0g; urea, 0.3; peptone, 0.75; yeast extract, 0.25; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2$, 0.3; $MgSO_4.7H_2O$, 0.3 and trace elements (mg/lit): $FeSO_4.7H_2O$, 5; $MnSO_4.4H_2O$, 1.6; $ZnSO_4.7H_2O$, 1.4 and $CoCl_2.6H_2O$, 20.0 (Mandels *et al.,* 1976). The medium and the trace elements were autoclaved separately. The flask was cooled down at room temperature and a known amount of sterilized trace elements was added. The flasks were then inoculated with optimized amount of culture volume and incubated for 48 hrs at the 28±3ºC temperature.

Enzyme extraction :

After desired growth of the fungi under SSF, the enzyme was harvested. 50 ml 0.05 M citrate phosphate buffer in, was added to each flask. The contents of the flask were crushed with the help of a glass rod, and were then shaken on orbital shaker at 100 rpm, for 45 min at 30ºC temperature. The contents were squeezed through a muslin cloth followed by centrifugation 10,000 rpm for 10 min at 4ºC. The clear supernatant (crude extract) was used for enzyme assay and biobleaching studies. (Deswal *et al*., 2011 and Muthezhilan *et al.,* 2007).

Enzyme assay :

Xylanase activity was determined by bailey method (Baiely *et al.,* 1992). The activity was determined by measuring the release of reducing sugar from birch wood xylan. Appropriate diluted enzyme solution (0.2ml) and 1.8 ml 1 per cent birch wood xylan solution in 0.005 M sodium phosphate buffer (pH 7) were incubated for 30 min at 50ºC. after incubation, the reaction was stopped by adding 3 ml of DNS (Dinitro Salicylic Acid) reagent and solution was boiled for 5 minutes, followed by cooling and adding 20 ml distilled water. The absorbance was measured at 540 nm.

One International Unit of Enzyme activity was defined as the amount of enzyme required to liberate 1 ìmol of glucose from the appropriate substrate per ml per min under the standard conditions of assay.

Pulp production :

Banana pulp were made in cylindrical mini digester. The rawmaterial was cooked with NaOH:Na_2S0_3 in the reactor underthe following conditions: 160 °C, 11 per cent chemical charge as Na_2O, 120 min and1:6 bath ratio. The cooked material was fiberized with disintegratorat 1200 rpm for 10 min and then further used for the enzymatic pre-bleaching.

Enzyme pre-treatment of unbleached pulp :

The oven dried pulp at 10 % (w/v) consistency was pre-treated with *Aspergillus oryzae* xylanase at different dose of IU/g wt pulp in polyethylene bags with pH 9.0 at 50°C for 120 min in a water bath. An untreated pulp sample (as control) was also incubated simultaneously under the identical conditions. The control and xylanase pre-treated pulps were thoroughly washed then subjected to conventional chemical bleaching sequence ($D_0E_PD_1$). The pulp samples were analyzed for kappa number.

Chemical bleaching sequence :

The xylanase pre-treated pulp was exposed to $D_0E_PD_{1.}$(D_0:Chlorine di oxideIst stage, E_P: Alkali extraction peroxide stage D_1 : Chlorine di oxide IInd stage)

Table A : Conditions used during $D_0E_PD_1$ bleaching of banana fibre pulp

Particulars	ClO_2 (D_O) stage	Alkali extraction (Peroxide) stage	ClO_2 (D_1) stage
Temperature, °C	55	70	80
Consistency, %	3.5	10.0	10.0
Treatment time (min.)	45	60	180
pH	2.0-3.0	11.0	3.0-4.0

bleaching sequences to obtain bleach pulp. At each stage of bleaching sequence, the pulp were subjected to different physiological conditions. Process conditions used during $D_0E_PD_1$ bleaching sequence were given in Table A.

Analysis of pulp and paper properties :

The hand sheets prepared from xylanase treated pulps were evaluated for various physical properties following the TAPPI protocols (TAPPI Test Methods, Atlanta, GA, TAPPI Press, 1996). The kappa number (a measure of lignin content), was determined by the treating the pulp samples with acidified potassium permanganate (TAPPI Protocol, T-236 om-85). The brightness (T 452 om-98), yellowness and whiteness of the hand sheets were measured at 457 nm with ISO Colourtech (USA). The strength properties *viz*., tensile strength (T 231 om-96), breaking length (T 404 cm- 92), burstness, burst factor (T 403 om-97), tear factor and tearness (T 414 om-98) were tested according to standard methods of TAPPI. (Gupta *et al.,* 2013).

EXPERIMENTAL FINDINGS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

Xylanase enzyme production :

The direct use of substrate specific enzymes produced by solid substrate fermentation (SSF) without a downstream processing represents an innovative method for modification of pulp and paper properties and reduction of chemical consumption during bleaching that can bring about positive economical and environmental benefits to the industry (Szendefy *et al.,* 2004). *Aspergillus oryzae* produced high activity of xylanase when grown on optimized condition under solid state fermentation. The highest activity of xylanase were found 1136 IU/gds of dry substrate on wheat bran as substrate after 48 hrs. *Aspergillus flavus* ARC-12 produced maximum xylanase production (1345.44 IU/gds) under solid-state fermentation after 48hrs of incubation (Gautam *et al.,* 2015).

Effect of enzyme dose on kappa number :

The role of xylanase in decreasing the kappa no and enhancement in brightness were investigated. As the enzyme dose increases the reduction in kappa number was observed. After enzyme pre-treatment step at the enzyme dose of 20, 25 and 30 IU/g, kappa no decreased by 0.6, 0.9 and 1.3 unit as compare to the control (Fig. 1). Biobleaching of rice straw pulp with *Thermomyces lanuginosus* SSBP xylanase enzyme at dosing 1, 5 and 10 IU/ml, gives 0.2, 0.5 and 0.7 unit of reduction in kappa number, respectively (Ziaie-Shirkolaee *et al.,* 2007).

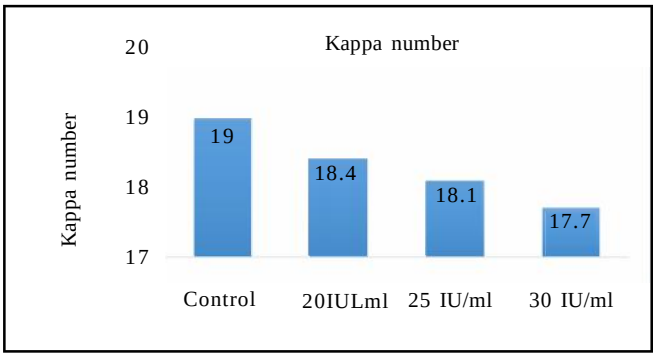

Fig. 1 : Effect of enzyme dose on kappa number

Effect of enzyme on brightness of pulp :

At enzyme dose 20, 25 and 30 IU/ml the brightness of bleached pulp was 81.8, 82.5 and 83.43 per cent ISO, respectively. In all the cases the brightness gain were 1.44, 2.15 and 3.07 unit over control (Table 1). The crude SSF enzymes displayed varying degrees of bleach enhancing ability on pulp depending on the microbial source and enzyme dose. The crude SSF enzymes of *T. lanuginosus* ATCC 36350 appeared as most efficient in biobleaching improving the final brightness by 2 points over control (Christopher *et al.,*2005). The brightness of hardwood and baggase pulp was increased by 2 per cent. Improvement in brightness (0.7 to 1 unit) over the pulp

Table 1 : Effects of xylanase on final bleach pulp

Parameter	Control	Enzyme treated		
		20 IU/g	25 IU/g	30 IU/g
Brightness %ISO	80.36	81.80	82.51	83.43
Brightness gain	-----	1.44	2.15	3.07
Whiteness %ISO	70.96	74.37	72.26	76.85
Yellowness %ISO	5.43	4.3	4.21	4.03

Table 2 : Effects of xylanase on strength properties of banana fibre pulp

Parameter	Unit	Control	Enzyme treated		
			20 IU/g	25 IU/g	30 IU/g
Burst	$KPa.m^2/g$	7.99	8.35	9.06	9
Tensile	(Nm/g)	105.54	107.80	111.92	110.15
Double fold	(No.)	2901	2958	2986	2980
Tear	$mn.m^2/g$	10.24	10.06	9.86	9.80
Porosity	(ml/min)	1.72	1.5	1.35	1.22

was observed with Xyn5B and Xyn11A (Gallardo *et al.*, 2010).

Effects of enzyme on strength properties of banana fiber pulp :

Enzyme treated and untreated, both the pulp were refined in a PFI mill upto 250 ml of CSF. Hand sheets of 90 GSM were evaluated for the strength properties. As the result shows, all the strength properties such as burst index, tensile index, double fold were increase after xylanase enzyme treatment, expect tear index and porosity. The treatment of pulp with xylanase only decrease the tear index, which may explain by the action of xylanases that reduce the intrinsic fibrillary resistance due to removal of superficial hemicelluloses. (Batalha *et al.*, 2011). It was observed that at the dose of 25 IU/ml the strength properties were maximum, but as the dose increase to 30 IU/ml all the properties were decreases slightly, it make sense that enzyme dose play an important role on strength of pulp. The decrease in strength may be due to the removal of hemicellulose, therefore, decreasing the fibre bonding capacity (Ates *et al.*, 2009).

As the result shown in Table 2, it was observed that xylanase treatment improve strength properties such as burst index (11.81%), tensile index (5.7%) and double fold (2.8%).

Conclusion :

The experimental results showed that xylanase from *A. oryzae* have the potential application in bleaching of banana fibre. Banana fibre pulp can be used as raw material for the production biobleached pulps with high optical and physical properties. Xylanase pre-treatment was the most efficient pre-treatment for biobleaching, since it provides pulps with lower kappa numbers, higher brightness and better strength properties.

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