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Effect of Xylanases from *C. Disseminatus* SW-1 NTCC-1165 on Pulp and Effluent Characteristics during CEHH Bleaching of Soda-AQ Bagasse Pulp

Swarnima Agnihotri[1], Dharm Dutt[2] and Amit Kumar[2]

[1]NTNU, Department of Chemical Engineering, Sem Saelands vei 4, No-7491, Trondheim, Norway
[2]Department of Paper Technology, I.I.T. Roorkee, Saharanpur campus, Saharanpur, 247 001, India

ABSTRACT

A new thermo-alkali-tolerant xylanase from *Coprinellus disseminatus* SW-1 NTCC-1165 produced under solid-state fermentation, mitigated pulp kappa number of sugarcane bagasse soda-AQ pulp by 29.80 and 36.6% after XE and XO-stages respectively, total chlorine charge by 29.70 and 36.53% with brightness increase by 4.4 and 3.7% for XECEHH and XOCEHH sequences respectively compared to their respective controls. The release of reducing sugars and chromophores was the maximum at a xylanase dose of 8 IU/g, reaction time 120 min, consistency 10%, temperature 55^0C and pH 6.4. The xylanase pretreatment reduced the AOX by 28.16 and 34.65% in combined bleach effluent of CEHH and OCEHH bleaching sequences respectively. In addition, it improved all the strength properties of paper and pulp viscosity by 0.65 (XECEHH) and 2.57% (XOCEHH). Increase in COD and colour in studied bleaching sequences were attributable to hydrolysis of hemicelluloses, release of lignin-carbohydrates complexes after xylanase treatment. Xylanase treatment modifies fibre surface by introducing cracks, peelings, swelling and external fibrillation which facilitates faster penetration of bleach chemicals by extenuating physical barrier as revealed by scanning electron microscopy.

Keywords: *Coprinellus disseminatus SW-1 NTCC-1165, Sugarcane bagasse, Soda-AQ pulp, Bleaching, Bleach effluent, SEM*

1. INTRODUCTION

Stringent laws against pollution generating industries will be helpful to move public perception from 'the pulp and the paper industry is the largest water consumer and the biggest water polluter' to 'the pulp and paper industry is ecologically sound, while producing recyclable products from renewable resources.' For the last two decades, bleaching of pulp has become an issue of great apprehension first and foremost due to increased public attentiveness about environmental hazards caused by the release of AOX. Bleaching of pulp uses large amount of chlorine based and other chemicals which cause several effluent related problems in the pulp and paper industries [1]. Byproducts of these chemicals are chlorinated organic substances, some of which are toxic, mutagenic, persistent, bioaccumulating and cause numerous harmful disturbances in biological systems [2]. The available options for attaining the above objectives are substitution of ClO_2 for Cl_2, use of H_2O_2, dimethyldioxiranes [3], nitrilamine [4] peracetic acid [5] and O_3 [1]. Pollution load can be mitigated by reducing kappa number before bleaching. Various old and up-coming technologies for mitigating kappa factor before pulp bleaching like, oxygen delignification [6], extended modified continuous cooking (EMCC) [7], modified conventional batch cooking (MCBC) [8], isothermal cooking (ITC) and modified conventional batch cooking (MCBC) [9], rapid displacement heating (RDH) [10] and use of cooking aids [11]. Most of these methods involve high capital investment. Thus, an alternative and cost effective method, is the use of xylanases which has provided a very simple and economic way to reduce the use of chlorine and other bleaching chemicals which enables to reduce the amount of toxic compounds (chlorophenols and other forms of organically bound chlorine) in the spent bleach liquor [12]. Cellulase free, thermo-stability and pH stability are the prerequisite characteristics of xylanases for their utility in pulp and paper industry, as the pulp produced after brown stock washing has high temperature (about 70^0C) and is alkaline in nature (pH about 8.5). The use of abundantly available and cost effective agricultural residues, such as wheat bran and other similar agro-wastes to achieve higher xylanase yields using solid-state fermentation (SSF) and immobilized cell systems also provide suitable measures to reduce the manufacturing cost of bio-bleached paper. Alkalo-philic *Bacillus subtilis* ASH produces xylanase in SSF (8,964 IU of xylanase/g dry wheat bran) after 72 h of incubation at 37^0C [13]; while under SmF it produces cellulase-free xylanase using wheat bran in alkaline pH up to 11.0 at 60^0C [14]. *Bacillus circulans* AB 16 isolated from a garbage dump is stable over a wide range of pH (5.0–9.0) and shows good thermal and pH stabilities; at pH 9.0, it retains 67 and 84.5% activities when kept for 1 h at 70^0C and 2 h at 65^0C, respectively. It reduces 20% of total chlorine demand without any decrease in brightness during prebleaching of eucalyptus kraft pulp compared to CEHH bleaching sequence [15]. A cellulase-free, thermo-stable xylanase from a newly isolated strain of *Bacillus pumilus* under SmF in a basal medium supplemented with wheat bran (2%, w/v) at pH 8.0 and temperature 37^0C produced xylanase which showed akalo-stability in neutral to alkaline pH at 70^0C [16].

The present study aims at prebleaching of sugarcane bagasse soda-AQ pulp with new thermo-alkali-tolerant xylanase from *Coprinellus disseminatus* SW-1 NTCC-1165 produced under SSF conditions to mitigate kappa number before CEHH and OCEHH bleaching sequences and investigate its effect on total chlorine demand, effluent characteristics like, colour, COD, AOX and paper properties.

2. MATERIALS AND METHODS

2.1 Microorganism and Cultural Conditions

A white-rot basidiomycete *Coprinellus disseminatus* SW-1 NTCC-1165 was isolated from the dead and decaying woods and identified at Forest Research Institute (FRI), Dehradun, India. It was grown under solid-state fermentation (SSF) and its physico-chemical variables were optimized like, incubation period 7 days, temperature 37^0C, pH 6.4, carbon and nitrogen sources wheat bran and soya bean meal respectively and substrate to moisture ratio 1:3 for obtaining the maximum xylanase production. *C. disseminatus* SW-1 NTCC-1165 was cultivated at optimized fermentation medium containing 5 g of finely powdered wheat bran as carbon source and 15 ml of nutrient salt solution (NSS) in 250 ml Erlenmeyer flask. NSS contained as g/l, 1.5 KH_2PO_4, 4.0 NH_4Cl, 0.5 $MgSO_4.7H_2O$, 0.5 KCl, and 1.0 soya bean meal in distilled water with 0.04 ml/l trace element solution having as µg/l, 200 $FeSO_4.7H_2O$, 180 $ZnSO_4.7H_2O$ and 20 $MnSO_4.7H_2O$ at pH 6.4. The fermentation slurry in the flask was autoclaved at 15 Psi for 15 min and inoculated with 2 disks of actively growing fungal strain SW-1 of 5 mm diameter and was incubated at 37^0C for 7 days. The enzymes were harvested by crushing the contents of the flask with glass rod in 15 ml of distilled water and were shaken for 30 min. The whole content was filtered through the four layers of cheese cloth and the filtrate was centrifuged (Sigma centrifuge model 2K15) at 5000 g for 10 min at 4^0C. The clear brown coloured supernatant was used as crude enzyme extract in biobleaching studies having 499.60 IU/ml of xylanase activity with negligible cellulase contamination (0.86 IU/ml). Xylanase was biochemically characterized to check its optimum pH and temperature stabilities by incubating the xylanase in buffers of different pH (potassium-phosphate; pH range: 6.0-7.4 and borax-boric acid; pH range: 7.6-9.0) and different temperatures ranging from 55 to 85^0C. Samples were withdrawn after 15 min and analyzed for residual xylanase activity [17] under standard assay conditions.

2.2 Xylanase and Cellulase Assay

Xylanase activity was determined by measuring the xylose units released from birch wood xylan by dinitrosalicylic acid method [17]. Carboxymethyl cellulase (CMCase) activity was determined according to the method of Mandels [18]. The reducing sugars released in the hydrolysis reaction were measured optically at 575 nm by DNS method as described by Miller [17]. Enzyme activities (xylanase and cellulase) were expressed as international units equivalent to micromoles of xylose or glucose released/min/mL of the reaction under standard conditions.

2.3 Pulp Sample

Well depithed sugarcane bagasse was digested in WEVERK electrically heated rotary digester of 0.02 m^3 capacity having four bombs of 1 liter capacity each at optimized pulping conditions like; active alkali dose 12% (as Na_2O), maximum cooking temperature 150^0C, maximum cooking time 60 min, digester pressure 5 kg/cm^2 and liquor to bagasse ratio 4:1 in presence of 0.1% anthraquinone (AQ). After completion of cooking, the pulp was washed on a laboratory flat stationary screen having 300 mesh wire bottom for the removal of residual cooking chemicals. The pulp was disintegrated and screened through WEVERK vibratory flat screen with 0.15 mm slits and the screened pulp was washed, pressed, crumbled and was ready for carrying out further studies. Depithed sugarcane bagasse produces a screened pulp yield of 44. 85% of kappa number 24.26, pulp brightness 34.3% (ISO) and pulp viscosity 26.5 cps [19].

2.4 Optimization of Enzyme Dose, Reaction Time and Consistency for Biobleaching

Enzyme dose was optimized by treating the unbleached pulp sample with different doses of xylanase from *C. disseminatus* SW-1 ranging between 0-25 IU/g at pulp consistency 10%, reaction temperature 55^0C, reaction time 120 min and pH 6.4. The reaction time for enzyme prebleaching was optimized by varying reaction time from 30 to 240 min while keeping other variables constant as mentioned above. Similarly, pulp consistency during enzymatic prebleaching was optimized by varying consistency from 2 to12% while keeping other variables constant. Controls were repeated at the same conditions using buffer in place of xylanase. The treated and untreated pulp samples were filtered through four layered muslin cloth. Enzyme mediated release of chromophoric material was monitored in pulp filtrates by measuring absorption spectra at the wavelength of 237, 254, 280 and 465 nm [20,21]. Reducing sugar concentrations in pulp filtrates were determined by dinitrosalisylic (DNS) acid method [17] and expressed as D-xylose equivalents. Xylanase treated pulp samples were followed by alkaline extraction. The kappa number (TAPPI T 236 cm-85 "Kappa number of pulp") of pulp samples was determined as per Tappi Standard Test Method [20].

2.5 Effect of Xylanase on Biobleaching of Conventionally Bleached Sugarcane Bagasse Soda-AQ Pulp

50 g of soda-AQ pulp of sugarcane bagasse was bleached by CEHH, XECEHH, OCEHH and XOCEHH bleaching sequences, where 'X' stands for xylanase stage, 'C' for chlorination, 'E' for alkaline extraction, 'H_1' and 'H_2' for hypochlorite I[st] and 2[nd] stages respectively and 'O' for oxygen bleaching stage. The xylanase prebleaching stage was conducted at an enzyme dose of 8 IU/g, pH 6.4, pulp consistency 10%, reaction time 120 min and temperature 55^{0}C. The total chlorine demand was calculated by using the following formula:

Total chlorine demand, % = 0.25 X kappa number of the total chlorine demand, 50% of the molecular chlorine was charged in 'C' stage and remaining 50% was charged in 'H_1' and 'H_2' stages in the ratio of 75:25 respectively. The chlorination stage was conducted in sealed plastic bottles with vigorous mixing at consistency 3%, ambient temperature, pH 1.75 and reaction time 30 min. The pulps were washed and extracted with 1.55% NaOH, at 60 ^{0}C and pH 11.0 for 1 h and at a pulp consistency of 10%. On the other hand, 'H_1' and 'H_2' stages were conducted at consistency 10%, temperature 45^{0}C, pH 11.5 and reaction time 60 min. Pulp was further delignified with O_2 in electrically heated WEVERK rotary digester of capacity 2 liter at consistency 10%, O_2 pressure 5.0 kg/cm^2, temperature 90^{0}C, reaction time 45 min and pH 11.1(1.5% NaOH, O.D. pulp basis) using $MgSO_4$ 0.1% (O.D. pulp basis as carbohydrate stabilizer.

2.6 Preparation of Laboratory Hand-sheets and Evaluation of Paper Properties

The bleached pulp samples were evaluated for pulp yield, viscosity (TAPPI T 206 os-63 "Cupprammonium disperse viscosity of pulp"), and copper number (TAPPI T 430 cm-99 "Copper Number of Pulp, Paper, and Paperboard") [22]. The bleached pulp samples were disintegrated in a PFI mill (TAPPI T 248 sp-00 "Laboratory beating of pulp (PFI mill method)") to attain a reference beating level of 35 ^{0}SR. Laboratory hand sheets of 60 g/m^2 were prepared (TAPPI T 221 cm-02 "Forming handsheets for physical tests of pulp" and tested for various physical strength properties such as tear index (TAPPI T 414 om-98 "Internal tearing resistance of paper [Elmendorf-type method]"), tensile index (TAPPI 494 om-01 "Tensile properties of paper and paperboard [using constant rate of elongation apparatus]"), burst index (TAPPI T 403 om-97 "Bursting strength of paper"), double fold (TAPPI T 423 cm-98 "Folding endurance of paper [Schopper type tester]") [22]. Pulp pad was prepared (TAPPI T 218 sp-02 "Forming handsheets for reflectance testing of pulp [Büchner funnel procedure]") and tested for brightness (TAPPI T 452 om-02 "Brightness of pulp, paper and paperboard [Directional Reflectance at 457 nm]") with Technibrite ERIC 950 from Technibrite Corporation, USA [22].

2.7 Analysis of Combined Bleaching Effluent

Bleach plant effluent collected after each stage of bleaching were mixed in equal amounts (at the end of each bleaching sequence) and were analyzed for COD (closed reflux titrimetric method using Thermo reactor CR 2010) [23], color (204 A) as per standard methods for the examination of water and wastewater, American Public Health Association, 1985 and AOX by column method (User manual ECS 1200 Rev. 3.1.0, Thermo Electron Corporation).

2.8 Scanning Electron Microscopy

The detailed morphological studies of unbleached sugarcane bagasse soda-AQ pulp samples (before and after xylanase treatment) were carried out using scanning electron microscopy (SEM, Leo 435 VP, England). Pulp samples were taken and subjected for fixation using 3% (*v*/*v*) glutaraldehyde-2% (*v*/*v*) formaldehyde (4:1) for 24 h. Following the primary fixation, samples were washed thrice with double distilled water. The samples were then treated with the alcohol gradients of 30, 50, 70, 80, 90 and 100% for dehydration. Samples were kept for 15 min each up to 70% alcohol gradient, thereafter treated for 30 min each for subsequent alcohol gradients. After treating with 100% alcohol, samples were air dried and examined under SEM using gold shadowing technique [24].

3. RESULTS AND DISCUSSIONS

3.1 Optimization of Enzyme Dose

Prebleaching of soda-AQ pulp of sugarcane bagasse with crude xylanase (at a dose of 12 IU/g) releases 2.15±0.1 mg/g of reducing sugars and it increases with increasing up to an enzyme dose of 25 IU/g (Figure 1). The curve can be approximated by two straight lines. The curve with steeper slope is pertaining to rapid release of sugars (up to an enzyme dose of 12 IU/g) whereas the part of curve with gentler slope is pertaining to the slow release of sugars. Release of chromophores is presumably a better indication of enzyme kinetics attacked on the pulp as reducing sugar will continue due to hydrolysis of oligosaccharides. The absorbance of filtrate generated during enzymatic prebleaching at optimum conditions increases (0.449±0.007) up to a xylanase dose of 8 IU/g due to release of phenolic compounds or chromophores and then there is no significant increase in absorbance at a wave length of 237 nm. The absorbance owing to release of hydrophobic compounds at 465 nm increases (0.215±0.015) up to an enzyme dose of 8 IU/g and then there is slight enhancement in absorbance. The increase in absorbance at 280 nm because of release of lignin fragments beyond an enzyme dosage of 8 IU/g supports the observation made by Ziobro [25]. The release of reducing sugars and the release of lignin and phenolic compounds are interrelated phenomenon. When xylan is hydrolysed by the xylanase, lignin and phenolic compounds are also released in addition to xylose from the pulp fibres that ultimately cause the enhancement in absorbance in filtrate compared to control [26]. When sugarcane bagasse soda-AQ pulp is pretreated with crude xylanase, kappa number reduces by 7.9 units (28.85%) at an enzyme dose of 8 IU/g and then there is insignificant decrease in kappa number by increasing enzyme dose after XE-stage. Pulp brightness after XE-stage increases by 9.2 units with increasing enzyme dose from 0.0 to 8 IU/g and beyond that there is an insignificant gain in brightness. The extraction stage after enzymatic prebleaching facilitates the dissolution of lignin-carbohydrate complexes (LCC) in pulp that were previously modified by enzymes but still remains in pulp because of their large molecular weight. In turn, because of the alkaline treatment, the cellulose fibre swells up and results in as an increase in pore size [27].

3.2 Optimization of Reaction Time

Figure 2 reveals that kappa number after XE-stage decreases by 7.06 units (29.1%) on increasing the reaction time from 0 to 120 min and beyond that kappa number remains almost constant. Removal of the reprecipitated xylan by the action of endoxylanases increases the permeability of the fibres and eliminates lignin from the pulp fibre, thus, reducing the pulp kappa number and increasing the concentration of chromophores in filtrate. Likewise, pulp brightness after XE-stage increases by 9.42 units and thereafter an insignificant increase in brightness. The spectrophotometric analysis of filtrates generated during xylanase treatment depicts that the absorbance at wavelengths i.e. 237, 254, 280 and 465 nm increases up to a reaction time of 120 min and then there is no significant gain in absorbance. Similar trend of chromophores release as a result of xylanase pretreatment was reported by Khandeparkar and Bhosle [26].

3.3 Optimization of Consistency

The kappa number decreases by 7.07 units (29.14%) at a pulp consistency of 10% (Figure 3) and beyond that there is an insignificant decrease in kappa number. The cellulosic fibres when merged in water contain mobile and immobile layers surrounding the fibres. The higher pulp consistency provides a close contact between enzymes and pulp fibres due to progressively elimination of mobile layer and leaving only the thin immobile layer enveloping the fibre thus facilitating enzyme adsorption to pulp and the sequential hydrolysis of hemicelluloses. Water layer thickness now becomes the rate-determining step [28]. For the decrease in kappa number above a pulp consistency of 10%, the pulp is to be finely shredded to separate fibre aggregates to the greatest extent possible before contacting the fibre with reactant. The interaction of the enzyme with the pulp is also important, including the effective molecular weight, net ionic properties and specific action pattern [29]. Similarly, pulp brightness increases by 9.6% (ISO) up to a consistency of 10% and beyond that increase in brightness is insignificant. Figure 3 shows that the amount of reducing sugars increases up to a consistency of 10% and beyond that curve becomes nearly stable. The absorbance at 237, 254, 280 and 265 nm also increases with increasing pulp consistency up to 10% and beyond that the increase in absorbance is insignificant.

3.4 Pulp Bleaching

The sugarcane bagasse soda-AQ pulps bleached by CEHH and OCEHH sequences are used as controls and it produces pulp of brightness of 80.1 and 83.2% (ISO) and pulps viscosity of 9.30 and 9.32 cps respectively (Table1). Xylanase pretreatment followed by alkaline extraction before CEHH bleaching sequence reduces kappa number of soda-AQ sugarcane bagasse pulp by 29.80% (Table 2) and total chlorine demand by 29.70% compared to control (Table 3). Likewise, pulp kappa number after XO-stage in XOCEHH bleaching sequence is reduced by 36.6% (Table 2) and total chlorine demand by 36.53% (Table 3) compared to OCEHH bleaching sequence. Brightness of XECEHH, and XOCEHH bleached pulps are improved by 4.4 and 3.7% respectively compared to their respective controls. Xylanase pretreatment improves pulp viscosity of XECEHH and XOCEHH bleached pulps by 0.65 and 2.57% respectively compared to respective controls. Bleaching losses after XECEHH and XOCEHH bleaching sequences are 8.4, and 8.5% (Table 2) compared to 9.0 and 8.78% in case of CEHH, and OCEHH bleaching sequences respectively (Table 1). However, xylanase pretreatment of bagasse pulp before CEHH and OCEHH bleaching sequences reduces the total chlorine demand by 29.70 and 36.53% respectively but still achieving the higher degree of brightness compared to control pulp; which shows the efficiency of crude xylanase produced. Enzymatic pretreatment of wheat straw pulp with xylanase obtained from *A. niger* An76 prior to H, CH or CEH bleaching reduces the chlorine consumption by 20-30% to attain the same brightness level [30]. There is a positive gain in brightness of sugarcane bagasse pulp as xylanase improves the accessibility of bleaching chemicals by disrupting the xylan chain and thus facilitates the easier removal of lignin during bleaching [31]. The increase in viscosity reflects the hydrolysis of low DP xylan in the pulp [32-33]. The crude xylanase extract used in the study is having negligible cellulase contamination because no loss in pulp viscosity is noticed. The nonspecific endoglucanases are reported to reduce the viscosity of softwood kraft pulp, indicating the degradation of cellulose chains [34, 35].

Xylanase pretreatment improves tear, burst and tensile indexes by 5.47, 18.98 and 15.99% respectively and double fold numbers by 11.76% of XECEHH bleached pulp compared to CEHH bleaching sequence at the reference beating level i.e. 35 ^{0}SR (Table 3). Similarly, XOCEHH bleached pulp shows an enhancement in burst and tensile indexs by 15.53 and 10.63% respectively and double fold by 8.1% while tear index remains unaltered compared to OCEHH bleaching sequence. Enzymatic treatment shows reduction in copper number by 28 and 15.38% during XECEHH, and XOCEHH bleaching sequences compared to CEHH and OCEHH sequences respectively. Xylanase pretreatment improves pulp viscosity of XECEHH and XOCEHH bleaching sequences which results in an increase in mechanical strength properties of paper when compared with mechanical strength properties of CEHH and OCEHH bleached pulp at the same reference beating level. Clark et al. [36] suggested that xylanase prebleaching increases the fiber swelling which facilitates refining and in turn results in better physical strength properties. The results indicate that xylanase prebleaching facilitates pulp fibrillation, water retention, restoration of bonding and freeness in fibers [16]. Reduction of chlorine demand for xylanase pretreated pulps may also be a possible reason for improved strength properties as higher chlorine charge proves to be detrimental for paper strength as well as for the environment. Enzyme treated wheat straw pulp has a high tear index and breaking length compared to the control pulp [30]. Copper number shows the degree of damage to cellulose in paper [37] which is reduced as a result of reduction in total chlorine demand after xylanase pretreatment of bagasse pulp.

AOX in combined effluent generated XECEHH and XOCEHH bleaching sequences is mitigated by 28.16 and 34.65% respectively compared to their respective controls. On the other hand, introduction of O_2 before CEHH bleaching sequence shows a reduction in AOX by 27.07% compared to CEHH bleaching sequence (Table 3). COD load increases by 8.85 and 8.7% and colour by 11.11 and 16.99% in combined effluent generated during XECEHH, and XOCEHH bleaching sequences respectively (Table 3). Reduction in total chlorine demand after xylanase pretreatment of bleaching sequences, results in lowering the toxicity of the bleach plant effluent also. Therefore, xylanase pretreatment reduces the amount of chlorophenols and other forms of organically bound chlorine (AOX) in the spent bleach liquor [12]. Since the pentosans are released from the xylanase prebleaching, the COD of the bleach effluent is rather high after xylanase pretreatment compared to control [33]. Effluent color is enhanced in xylanase pretreated pulps as xylanase alters the carbohydrate composition of pulps by reducing their xylan content. The dissolution of xylans produced by the xylanase gives rise to an increase in effluent color. This can also be explained by the increased amount of lignin dissolved from enzyme treated pulps [38]. An increase in colour by 27.76% is reported in bleaching effluent when *E. globulus* pulp is pretreated with xylanase in a bleaching sequence ODPD [33].

3.5 SEM Studies

SEM results show that surface of untreated bagasse fibers is smooth and shows no signs of external fibrillation and swelling (Figure 4A) while xylanase pretreated fibers bear cracks, peelings, swelling and external fibrillation on their surface (Figure 4B). SEM studies show that sugarcane bagasse fibers that had undergone xylanase treatment have a rougher surface with striations and splits, i.e. a more open surface. These results confirm that xylanase acts by hydrolyzing the xylans deposited on the surface of fibers during alkaline pulping, which constitute a physical barrier for the penetration of bleaching agents. Their elimination facilitates the flow of bleaching agents, which explains the bleach boosting effect of the xylanases [31, 39]. Xylanase treatment improves accessibility of bleaching chemicals to the pulps, decreases diffusion resistance to outward movement of the degraded lignin fragments and allows the removal of less degraded lignin fragments from the cell wall. As a result, pulps treated with xylanase show lower kappa number and higher brightness and viscosity than pulps not treated with the xylanase [1, 39] have reported that xylanase from *Streptomyces* sp. QG-11-3 introduced greater porosity, swelling up and separation of pulp microfibrils in eucalyptus pulp fibers compared to the smooth surface of untreated pulp fiber.

4. CONCLUSION

It is concluded that the crude xylanase produced from *C. disseminatus* SW-1 has tremendous potential not only for reducing the bleach chemical demand and toxicity of bleach effluents in terms of AOX but also for improving various paper properties. The cost of the biobleaching is satisfactorily low as xylanase is produced using wheat bran as carbon source which is an inexpensive agro residue and used in its crude form as it contains a negligible cellulase contamination and therefore, it does not require any purification step. In addition, the xylanase is found to be thermo-alkali-tolerant which is prerequisite of pulp and paper industry.

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Table 1: Effect of Conventional Bleaching on Pulp Shrinkage, Brightness and Viscosity of Soda-AQ Pulp of Sugarcane Bagasse

Particulars	Bleaching sequences	
	CEHH	OCEHH
Oxygen stage (O)		
O_2 pressure, kg/cm^2		5
$MgSO_4$ applied, % (O.D. pulp basis)	–	0.1
NaOH applied, % (O.D. pulp basis)	–	1.5
Final pH	–	11.1
kappa number of O_2 delignified pulp	–	15.00
Chlorination stage (C)		
Cl_2 applied as available Cl_2, % (O.D. pulp basis)	3.03	1.88
Cl_2 consumed, % (O.D. pulp basis)	3.02	1.878
Amount of Cl_2 consumed, %	99.7	99.9
Final pH	1.75	1.75
Alkali extraction stage (E)		
NaOH applied, % (O.D. pulp basis)	1.55	0.97
Initial pH	11.0	11.0
Final pH	11.2	11.1
Hypochlorite stage (H_1)		
Hypo applied as available Cl_2, % (O.D. pulp basis)	2.27	1.41
Hypo consumed as available Cl_2, % (O.D. pulp basis)	2.18	1.31
Hypo consumed, %	96.03	92.9
Final pH	11.5	11.2
Hypochlorite stage (H_2)		
Hypo applied as available Cl_2, % (O.D. pulp basis)	0.75	0.47
Hypo consumed as available Cl_2, % (O.D. pulp basis)	0.69	0.39
Hypo consumed, %	92.0	82.9
Final pH	11.2	11.0
Total Cl_2 applied, % (O.D. pulp basis)	6.06	3.75
Total Cl_2 consumed, % (O.D. pulp basis)	5.89	3.58
Total Cl_2 consumed on Cl_2 basis, %	97.2	95.5
Total residual Cl_2, %	2.8	4.53
Bleaching losses, %	9.0	8.78
Bleached pulp yield, %	40.81±1.3	40.91±1.5
Pulp brightness, % (ISO)	80.1±0.5	83.2±0.38
Pulp viscosity, cps	9.30±0.021	9.32±0.034

Bleaching conditions	**O**	**C**	**H_1**	**H_2**
Consistency, %	10	3	10	10

Temperature, ^0C	90±2	Ambient	45±2	45±2
Time, min	90	30	60	60

Unbleached kappa number 24.26, unbleached pulp brightness 34.3% (ISO), unbleached pulp viscosity 26.5cps and ± refers standard deviation.

Table 2: Effect of Xylanase Pretreatment on Pulp Shrinkage, Brightness and Viscosity of Sugarcane Bagasse Soda-AQ Pulp during Conventional Bleaching

Particulars	Bleaching sequences	
	XECEHH	XOCEHH
Xylanase stage (X)		
Amount of xylanase added (on O.D. pulp basis), IU/g	8	8
Final pH	6.4	6.4
Alkali extraction stage (E)		
NaOH applied, % (O.D. pulp basis)	1.5	–
Initial pH	11.4	–
Final pH	10.2	–
kappa number of xylanase treated pulp	17.03	–
Oxygen stage (O)		
O_2 pressure, kg/cm^2	–	5
$MgSO_4$ applied, % (O.D. pulp basis)	–	0.1
NaOH applied, % (O.D. pulp basis)	–	1.5
Final pH		11.2
Kappa number of xylanase and O_2 delignified pulp	–	9.51
Chlorination stage (C)		
Cl_2 applied, % (O.D. pulp basis)	2.13	1.18
Cl_2 consumed, % (O.D. pulp basis)	2.12	1.177
Amount of Cl_2 consumed, %	99.5	99.74
Final pH	2.5	2.5
Alkali extraction stage (E)		
NaOH applied, % (O.D. pulp basis)	1.09	0.62
Initial pH	11.4	11.4
Final pH	11.2	11.1
Hypochlorite stage (H_1)		
Hypo applied as available Cl_2, % (O.D. pulp basis)	1.6	0.89
Hypo consumed as available Cl_2, % (O.D. pulp basis)	1.51	0.81
Hypo consumed, %	94.4	91.01
Final pH	11.0	11.5
Hypochlorite stage (H_2)		
Hypo applied as available Cl_2, % (O.D. pulp basis)	0.53	0.30
Hypo consumed as available Cl_2, % (O.D. pulp basis)	0.48	0.26
Hypo consumed, %	90.56	86.6
Final pH	11.2	11.0
Total Cl_2 applied, % (O.D. pulp basis)	4.26	2.38
Total Cl_2 consumed, % (O.D. pulp basis)	4.11	2.247
Total Cl_2 consumed, %	96.48	94.41

Total residual Cl_2, %				3.52	5.6	
Bleaching losses, %				8.4	8.5	
Bleached pulp yield, %				41.08±2.1	41.04±1.8	
Pulp brightness, % (ISO)				84.5±0.7	86.9±0.71	
Pulp viscosity, cps				9.364±0.01	9.56±0.026	
Bleaching conditions	**X**	**O**	**C**	**E**	**H_1**	**H_2**
Consistency, %	10	10	3	10	10	10
Temperature, ^{0}C	55±2	90±2	Ambient	60±2	45±2	90±2
Time, min	120	90	30	60	60	60

Unbleached kappa number 24.26, unbleached pulp brightness 34.3% (ISO), unbleached pulp viscosity 26.5cps and ± refers standard deviation

Table 3: Comparison of Pulp Properties and Combined Effluent Generated during Conventional Bleaching

Sl. No.	Particulars	CEHH	XECEHH	OCEHH	XOCEHH
1	Total chlorine demand	6.06	4.26	3.75	2.38
2	Pulp brightness, % (ISO)	80.1±0.5	84.5±0.7	83.2±0.38	86.9±0.71
3	Reference beating level, ^{0}SR	35	35	35	35
4	Tear index, mNm^2/g	4.57±0.22	4.82±0.35	4.82±0. 17	4.82±0.21
5	Burst index, kPam^2/g	2.95±0.25	3.51±0.18	3.22±0. 1	3.72±0. 6
6	Tensile index, Nm/g	48.38±1.9	56.12±1.5	51.34±2.4	56.8±1.7
7	Double fold, number	34±4.2	38±2.2	37±2.1	40±3.9
8	Pulp viscosity, cps	9.30±0.011	9.36±0.009	9.32±0.024	9.56±0.026
9	Copper number	0.25±0.003	0.18±0.005	0.13±0.004	0.11±0.002
10	COD, mg/L	723	787	598	650
11	Color, PTU	2250	2500	1560	1825
12	AOX, kg/T	2.77	1.99	2.02	1.32

± refers standard deviation

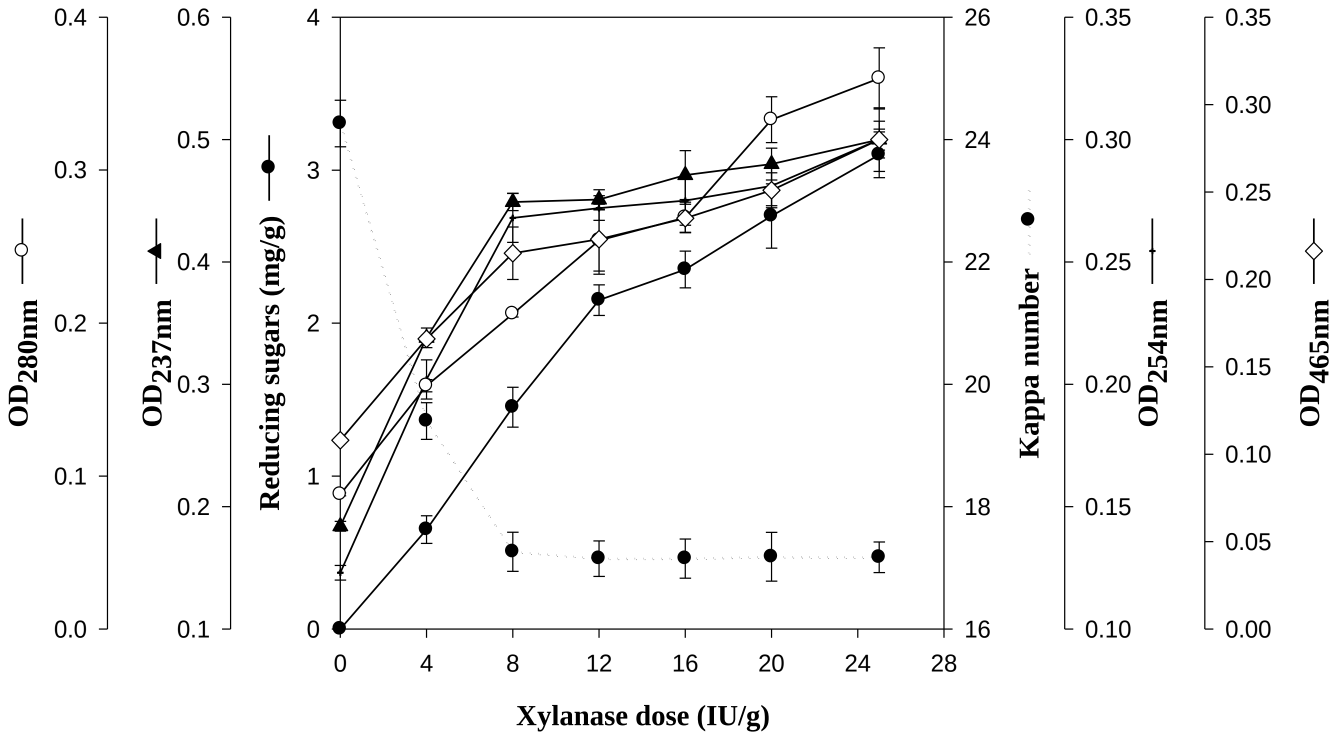


Figure1: Optimization of enzyme dose for xylanase prebleaching of bagasse pulp

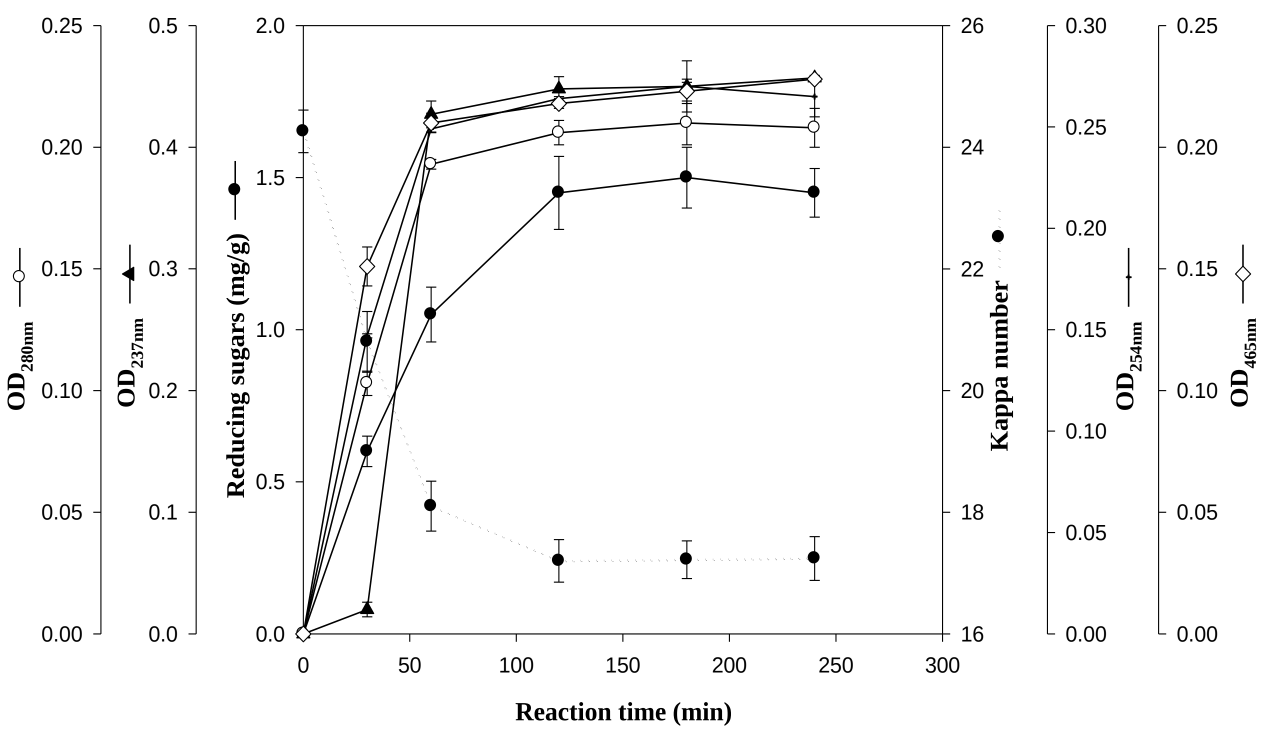


Figure 2: Optimization of reaction time for xylanase prebleaching of sugarcane bagasse pulp

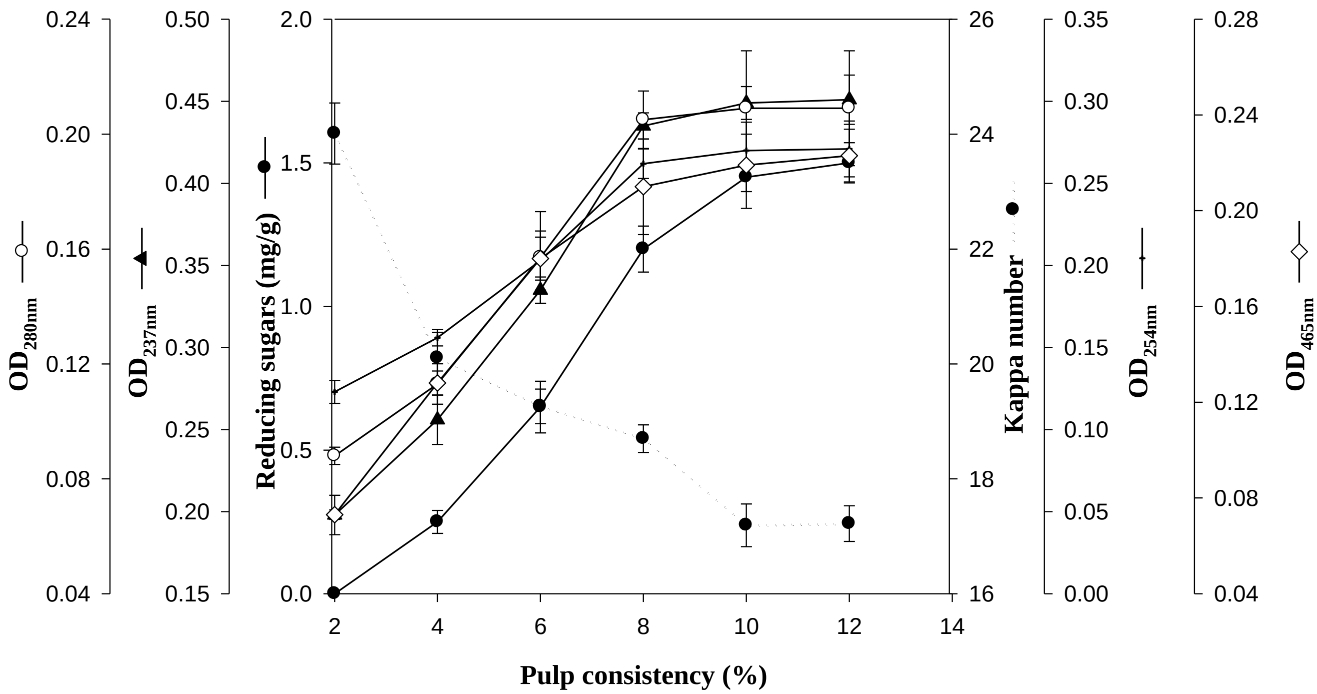
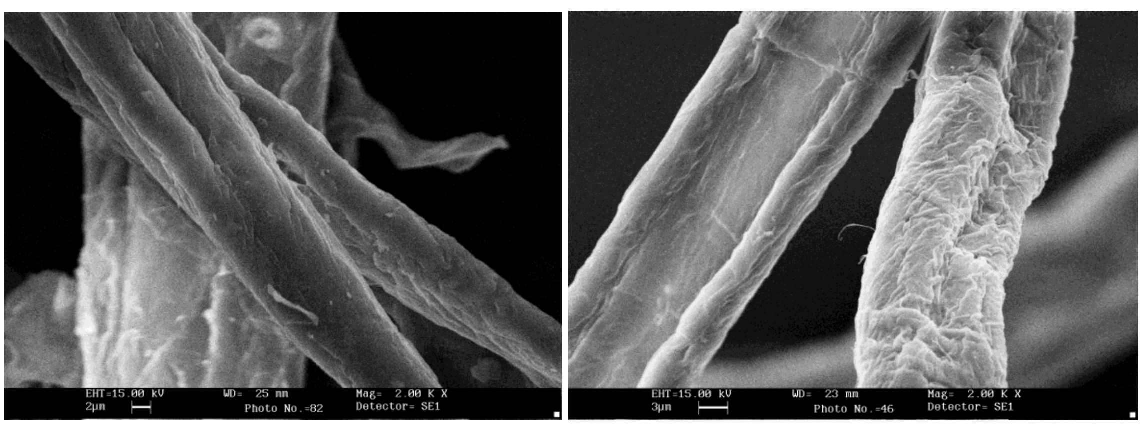


Figure 3: Optimization of pulp consistency for xylanase prebleaching of sugarcane bagasse pulp



(A) **(B)**

Figure 4: (A) Unbleached fiber of sugarcane bagasse, arrow shows the smooth surface of fiber (B) fibers after xylanase pretreatment, arrows show rough surface