ECF AND TCF BLEACHING OF *SACCHARUM OFFICINERUM*-CO89003 BAGASSE SODA-AQ PULP WITH ALKALI-THERMO-TOLERANT CRUDE XYLANASE FROM *COPRINELLUS DISSEMINATUS* SW-1 NTCC1165

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An alkali-thermo-tolerant crude xylanase from *Coprinellus disseminatus* SW-1 NTCC1165 produced under solid-state fermentation conditions improves the brightness of sugarcane bagasse soda-AQ pulp by 7.3, 4.7, 6.1, and 8.2% in XODED, XOD(E_{OP})DP, OX(E_{OP})P, and XO(E_{OP})P bleaching sequences, respectively, at an enzyme dose of 8IU/g, a reaction time of 120 min, a consistency of 10%, and a pH of 6.4 at 55 ^{o}C. An improvement in brightness by 2.1% for pulp bleached by XO(E_{OP})P compared to OX(E_{OP})P sequence validates that xylanase treatment is more effective for hydrolysing lignin-carbohydrates complexes before oxygen treatment. AOX after XODED and XOD(E_{OP})DP sequences is reduced by 41.43 and 40%, respectively, compared to controls, but an increase in COD and color in studied bleaching sequences is attributable to the hydrolysis of hemicelluloses and the 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 disrupting physical barriers, as revealed by scanning electron microscopy.

Keywords: Sugarcane bagasse; Coprinellus disseminatus SW-1 NTCC1165; Bleaching; Optical properties; Strength properties; Effluent characteristics

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INTRODUCTION

The pulp and paper industry is the largest water consumer and biggest polluter. Therefore, the industry must look at environmental issues such as nearly zero effluent discharge, minimum releases from the pulping, bleaching, and recovery processes, and adoption of a completely eco-cyclic system; implementing these ideas may encourage our grandchildren to join the pulp and paper industry. The most significant environmental issues are the discharge of chlorine-based organic compounds (from bleaching) and of other toxic organics (Nasman *et al.* 2007). The soluble organic substances removed from the pulp in bleaching stages that use chlorine or chlorine compounds, as well as the substances removed in the subsequent alkaline stages, are chlorinated. Some of these chlorinated organic substances are toxic; they include dioxins, chlorinated phenols, and many other chemicals (Onysko 1993). The prolonged use of papers bleached by molecular chlorine for direct body contact, such as toweling, tissue, napkins, and baby

diapers and for packaging and wrapping of food, with tea bag paper (Dutt *et al.* 2007), bread and biscuits wrappers (Dutt *et al.* 2004), and crimped and curd cups (Dutt *et al.* 2005), is of great importance since it is associated with chlorinated compounds, including the animal carcinogen dioxin (Shoham *et al.* 1992). The modern paper industry reduces pulp kappa number before bleaching by extended cooking and oxygen delignification for mitigating the use of hazardous bleaching chemicals. Wastewater generated during oxygen delignification can be sent to the recovery boiler along with black liquor for steam generation; the steam is then used to generate electricity, thereby reducing the amount of pollutants discharged (Anonymous 1998). Elemental chlorine free (ECF) and total chlorine free (TCF) bleaching sequences not only control the release of toxic substance and non-process elements (potassium and chlorides), but also explore the possibility of reusing the bleaching effluent (Maples *et al.* 1994).

The biotechnological approach to mitigate kappa number prior to pulp bleaching is new and gaining momentum all over the world. Search for novel microbial isolates that are able to produce cellulase-free, thermo-stable, and alkalophilic xylanases for the paper industry is of utmost importance, as the pulp produced after brown stock washing has a high temperature (about 70 ^{o}C) and is alkaline in nature (pH about 8.5). Alkalophilic *Bacillus subtilis* ASH produces cellulase-free xylanase using wheat bran under submerged fermentation at alkaline pH up to 11.0 at 60 °C (Sanghi *et al.* 2007). A cellulase-free, thermo-stable xylanase from a newly isolated strain of *Bacillus pumilus* under submerged fermentation in a basal medium supplemented with wheat bran (2%, *w*/*v*) at pH 8.0 and temperature 37 ^{o}C produces xylanase that is stable in the neutral to alkaline pH region at 70 ^{o}C (Battan *et al.* 2007). Two novel thermo-alkali-tolerant crude xylanases, namely MLK-01 (enzyme-A) and MLK-07 (enzyme-B) from *Coprinellus disseminates*, mitigate kappa numbers of *Anthocephalus cadamba* kraft-AQ pulps by 32.5 and 34.38%, improve brightness by 1.5 and 1.6%, and improve viscosity by 5.75 and 6.47% after ^{A}XE1 and ^{B}XE1-stages, respectively (Lal *et al.* 2012).

The present study is focused on mitigating the kappa number of soda-AQ pulp of sugarcane bagasse in two distinct prebleaching stages: (i) xylanase treatment and (ii) oxygen delignification. Then, pulp is bleached by ECF (DED and $DE_{OP}DP$) and TCF ($E_{OP}P$) bleaching sequences and its effect on pulp brightness, viscosity, mechanical strength properties, and effluent characteristics like color, chemical oxygen demand (COD), and AOX is determined.

MATERIALS AND METHODS

Enzyme

Coprinellus disseminatus SW-1 NTCC 1165 yielded the highest xylanase activity (362.1 IU/mL) with minimal cellulase contamination (0.64 IU/mL) at an incubation period of 7 days at 37 ^{o}C and pH 6.4 when cultivated on media containing cheap agro-residue wheat bran as a sole carbon source, soya bean meal as nitrogen source, and solid substrate to moisture content ratio of 1:3. The xylanase and laccase activities under optimized conditions were 499.60 and 25.5 IU/mL, respectively, along with negligible cellulase contamination (0.86 IU/mL). Biochemical characterization revealed that optimal xylanase activity was observed at pH 6.4 and 55 ^{o}C, and xylanase is active up to pH 9 (40.33 IU/mL) and 85 ^{o}C (48.81 IU/mL). SDS–PAGE and zymogram analysis indicated

that molecular weight of alkali-thermo-tolerant xylanase produced by *C. disseminatus* SW-1 NTCC 1165 was 43 kDa (Agnihotri *et al.* 2010a).

Pulp

The dry and wet depithed sugarcane bagasse was digested in a WEVERK electrically heated rotary digester of 0.02 m^3 capacity by soda-AQ pulping process using 12% active alkali (as Na_2O), a maximum cooking temperature of 150 ^oC, a cooking time of 60 min, a digester pressure of 5 kg/cm^2, and a liquor to wood ratio of 4:1 in the 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 a WEVERK vibratory flat screen with 0.15 mm slits. The screened pulp yield of sugarcane bagasse is 46.12 ± 2.5% at a kappa number of 24.26 ± 0.38 (Agnihotri *et al.* 2010b).

Pulp Prebleaching Conditions

Sugarcane bagasse soda-AQ pulp of kappa number 24.26 ± 0.38 showed maximum release of reducing sugars and chromophores at a xylanase dose of 8 IU/g, a reaction time of 120 min, a consistency of 10%, a temperature of 55 ^oC, and pH 6.4.

ECF (ODED and OD(E_{OP})DP) and TCF (O(E_{OP})P) Bleaching Sequences

The enzymatic prebleached soda-AQ pulp of sugarcane bagasse was bleached by ODED and OD(E_{OP})DP (ECF) and O(E_{OP})P (TCF) bleaching sequences as per chemical doses and conditions described in Tables 1 and 2. The enzymatic stage in the O(E_{OP})P sequence was kept before and after oxygen delignification in XO(E_{OP})P and OX(E_{OP})P sequences. Oxygen delignification was conducted in a WEVERK rotary digester at a pressure of 5 kg/cm^2 and temperature of 90 ^oC for 45 min using 0.1% $MgSO_4$ as a carbohydrate stabilizer. Pulps obtained after each bleaching stage were filtered through cheese cloth, and filtrate was analysed for residual chlorine except alkali extraction stage and TCF bleaching sequence. The rest of the filtrates were preserved at 4 ^oC for further analysis and the pulps were washed with 2 L of tap water, squeezed, and crumbled.

Preparation of Laboratory Handsheets and Evaluation of Paper Properties

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") (Anonymous 2007). The processing of pulp was done by means of a PFI mill to evaluate pulp quality for papermaking (TAPPI T 248 sp-00 "Laboratory beating of pulp [PFI mill method]") at a fixed bating level *i.e.* 35 ^0SR. Laboratory handsheets of 60 g/m^2 were prepared on a British Sheet Former (TAPPI T 220 sp-01 "Physical testing of pulp handsheets"), conditioned at 65% ± 2 relative humidity and 27 ± 1ºC and evaluated for burst index (TAPPI T 403 om-97 "Bursting strength of paper"), tensile index (TAPPI T494 om-01 "Tensile properties of paper and paperboard [using constant rate of elongation apparatus]"), double fold (TAPPI T423 cm-98 "Folding endurance of paper [Schopper type tester]") (135°±2°), and tear index (TAPPI T414 om-98 "Internal tearing resistance of paper [Elmendorf-type method]") (Anonymous 2007). Pulp pads were 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 (Anonymous 2007).

Analysis of Combined Bleaching Effluent

Bleach plant effluent collected after each bleaching stage was mixed in equal amounts, analysed for COD (closed reflux titrimetric method using Thermoreactor CR 2010) (Test method No-508 B), color (Test method No-204A) 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 2006) with AOX Analyser Dextar ECS 1200.

Scanning Electron Microscopy (SEM)

Unbleached sugarcane bagasse soda-AQ pulp samples (before and after xylanase treatment) were analysed by scanning electron microscopy (SEM, Leo 435 VP, England). Pulp samples were fixed using 3% (*v*/*v*) glutaraldehyde-2% (*v*/*v*) formaldehyde (4:1) for 24 hours. Following the primary fixation, samples were washed thrice with double distilled water. The samples were then dehydrated in an alcohol gradient of 30, 50, 70, 80, 90, and 100%. 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 the gold shadowing technique (Gabriel 1982).

RESULTS AND DISCUSSION

Brightness of XODED and XOD(E_{OP})DP bleached soda-AQ pulps of sugarcane bagasse was improved by 7.3% and 4.7% (ISO), respectively (Table 1); and OX(E_{OP})P and XO(E_{OP})P pulps were improved by 6.1% and 8.2% (ISO), respectively, (Table 2) compared to their respective controls at the same chemical charge. Xylanase treatment improved accessibility of the pulps for the bleaching chemicals by decreasing the diffusion resistance to the outward movement of the degraded lignin fragments and allowing the removal of less degraded lignin fragments from the fibre wall, which results in higher brightness (Torres *et al.* 2000). The brightness increase of XO(E_{OP})P bleached pulp compared to OX(E_{OP})P indicates that the xylanase stage prior to oxygen delignification is more effective than after oxygen delignification. The effectiveness of xylan hydrolysis with xylanase prior to the oxygen stage rather than after the oxygen stage is suggested by Roncero *et al.* (2005). The results also substantiate that xylanase pretreatment before oxygen delignification increases pulp shrinkage on account of more xylan removal after XO-stage of XO(E_{OP})P bleaching sequence than that of OX-stage of OX(E_{OP})P bleaching sequence. The effectiveness of xylanase treatment after oxygen treatment is decreased because in addition to the peeling reaction, alkaline hydrolysis (depolymerization) of the polysaccharide chains occurs and is subjected to further degradation reactions (secondary peeling) (Hinrichs 1967; McGinnis and Shafizadeh 1980). The residual chlorine dioxide after XODED and XOD(E_{OP})DP increases by 9.40 and 11.94%, respectively, compared to ODED and OD(E_{OP})DP bleaching sequences at the same chlorine dioxide charge (Table 1). An increased level of residual chlorine dioxide and pulp brightness after XODED and XOD(E_{OP})DP sequences signifies that the treated pulp reaches the chlorine saturation point sooner than the control pulp.

Table 1. Results With and Without Xylanase (X) Pretreatment on Pulp Shrinkage, Brightness, and Viscosity of Soda-AQ Pulp of Sugarcane Bagasse during ODED and OD(E_{OP})DP Bleaching

Particulars	Bleaching Sequences			
	ODED	**XODED**	**OD(E_{OP})DP**	**XOD(E_{OP})DP**
Unbleached pulp kappa number	24.26	24.26	24.26	24.26
Unbleached pulp brightness, % (ISO)	34.3	34.3	34.3	34.3
Unbleached pulp viscosity, cps	26.5	26.5	26.5	26.5
Xylanase stage (X)				
Amount of xylanase added, IU/g % (on o.d. pulp basis)	–	8	–	8
Final pH	–	6.2	–	6.5
Oxygen stage (O)				
O_2 pressure, kg/cm^2	5	5	5	5
$MgSO_4$ applied, % (on o.d. pulp basis)	0.1	0.1	0.1	0.1
NaOH applied, % (on o.d. pulp basis)	1.5	1.5	1.5	1.5
Final pH	11.4	11.6	11.6	11.5
Chlorine dioxide stage (D_1)				
ClO_2 applied as available Cl_2, % (on o.d. pulp basis)	1.0	1.0	1.0	1.0
ClO_2 consumed as available Cl_2, % (on o.d. pulp basis)	0.93	0.902	0.91	0.86
ClO_2 consumed, %	93.0	90.2	91.0	86.0
Final pH	4.0	4.1	4.0	4.2
Alkali extraction stage (E)				
NaOH applied, % (on o.d. pulp basis)	2.5	2.5	–	–
Initial pH	11.7	11.7	–	–
Final pH	9.9	9.9	–	–
Extraction stage				
NaOH applied, % (on o.d. pulp basis)	–	–	3.0	3.0
H_2O_2 applied, % (on o.d. pulp basis)	–	–	0.5	0.5
O_2 pressure, kg/cm^2	–	–	5.0	5.0
$MgSO_4$ applied, % (on o.d. pulp basis)	–	–	0.1	0.1
Final pH	–	–	11.3	11.6
Chlorine dioxide stage (D_2)				
ClO_2 applied as available Cl_2, % (on o.d. pulp basis)	0.6	0.6	0.6	0.6
ClO_2 consumed as available Cl_2, % (on o.d. pulp basis)	0.56	0.55	0.57	0.549
ClO_2 consumed, %	93.3	91.6	95.0	91.5
Final pH	4.1	4.1	4.0	4.0
Peroxide stage (P)				
H_2O_2 applied, % (on o.d. pulp basis)	–	–	0.5	0.5
EDTA applied, % (on o.d. pulp basis)	–		0.5	0.5
$MgSO_4$ applied, % (on o.d. pulp basis)	–		0.1	0.1
Final pH			10.4	10.9
Total ClO_2 applied, % (on o.d. pulp basis)	1.6	1.6	1.6	1.6
Total ClO_2 consumed, % (on o.d. pulp basis)	1.49	1.45	1.48	1.409
Total ClO_2 consumed, %	93.1	90.6	92.5	88.06
Total residual ClO_2, %	6.9	9.4	7.5	11.94
Bleaching losses, %	7.5	7.6	7.7	7.7
Bleached pulp yield, %	41.49±2.5	41.44±2.6	41.4±3.3	41.40±1.4
Pulp brightness % (ISO)	79.2±0.47	84.6±0.55	85.5±1.0	90.2±1.3
Pulp viscosity, cps	9.36±0.022	9.40±0.016	9.38±0.034	9.43±0.021

Bleaching conditions	**X**	**O**	**D_1**	**E**	**E_{OP}**	**D_2**	**P**
Consistency, %	10	10	10-12	10	11.5	10-12	10
Temperature, ^{0}C	55±2	90±2	70±2	60±2	75±2	70±2	90±2
Time, min	120	90	180	60	70	180	60

± refers standard deviation

There was an improvement in pulp viscosity by 0.43 and 0.53% after XODED and XOD(E_{OP})DP and 3.37 and 2.55% after OX(E_{OP})P and XO(E_{OP})P bleaching sequences, respectively, compared to their respective controls (Table 3). Increase in viscosity as a result of xylanase pretreatment indicates that crude xylanase only hydrolyses low DP xylan (Vidal *et al.* 1997) and not cellulose chains. This explains why bleaching losses were greater after xylanase pretreatment of ODED, OD(E_{OP})DP, and O(E_{OP})P bleaching sequences.

Copper number, which signifies the brightness reversion after bleaching, decreased by 38.46 and 25% in XODED and XOD(E_{OP})DP, respectively, and 33.3% in both the bleaching sequences, *i.e*. OX(E_{OP})P and XO(E_{OP})P compared to their respective controls. Crude xylanase mitigates hydrolysed or oxidized cellulose by depolymerising low DP xylan and fines that are capable of reducing certain metallic ions to lower valence states, and reactions of this type have served to detect damage to cellulose and to estimate the quantity of reducing groups (Wilson *et al.* 1951).

Tear index in XODED bleached pulp increased by 6.72% while burst index, tensile index, and double fold were reduced by 6.76, 9.1, and 10.34%, respectively, and tear index in XOD(E_{OP})DP was increased by 4.99%, while burst index, tensile index, and double fold were reduced by 14.17, 12.26, and 38.64%, respectively, compared to their respective controls (Table 3). Likewise, tear index in OX(E_{OP})P and XO(E_{OP})P bleached pulps were increased by 5.96% and 3.85% while burst index, tensile index, and double fold were decreased by 16.56%, 8.29%, and 18.51%, respectively, after OX(E_{OP})P and 20.45%, 18.08%, and 11.11% after XO(E_{OP})P bleaching sequences compared to their respective controls (Table 3).

The retention of hemicelluloses is favourable for the mechanical strength properties of papermaking fibres because of their positive effect on the interfibrillar bonding during paper-sheet formation (Clark 1981). The partial hemicelluloses (xylan) removal during the enzymatic stage can therefore adversely affect the pulp strengths (especially tensile and burst index which depend on interfibre bonding of paper). The xylan removal enhances the degree of external fibrillation on fibre surfaces, which improves the tear index after xylanase pretreatment of bagasse pulp (Haarhoff *et al.* 1999).

It was also observed that the mechanical strength properties were inferior after XO(E_{OP})P bleaching sequence compared to OX(E_{OP})P bleaching sequence, validating that xylan removal is more after XO(E_{OP})P bleaching sequence than that of OX(E_{OP})P bleaching sequence. Readsorbed xylan may chemically be bonded to residual lignin in pulp.

The lignin-carbohydrate complexes (LCCs) are thought to be difficult to remove due to diffusion limitations. The enhanced leachability of lignin in the fiber wall has been suggested to be due to hydrolysis of reprecipitated xylan or to the removal of xylan from LCC in fibers. Hemicellulase activity may remove lignin-carbohydrate complexes (LCCs) from the pulp fibers. The extraction stage after enzymatic prebleaching facilitates the dissolution of lignin-carbohydrate complexes (LCC) in pulp that were previously modified by enzymes but still remained in pulp because of their large molecular weight (Lal *et al.* 2012; Singh *et al.* 2010; Paice *et al.* 1992).

Table 2. Results With and Without Xylanase (X) Pretreatment on Pretreatment on Pulp Shrinkage, Brightness, and Viscosity of Soda- AQ pulp of Sugarcane Bagasse during O(E_{OP})P

Particulars	Bleaching Sequences		
	O(E_{OP})P	OX(E_{OP})P	XO(E_{OP})P
Unbleached pulp kappa number	24.26	24.26	24.26
Unbleached pulp brightness, % (ISO)	34.3	34.3	34.3
Unbleached pulp viscosity, cps	26.5	26.5	26.5
Xylanase stage (X)		–	
Amount of xylanase added, IU/g (on o.d. pulp basis),	–		8.0
Final pH	–	–	6.4
Oxygen stage (O)			
Oxygen pressure, kg/cm^2	5.0	5.0	5.0
$MgSO_4$ applied, % (on o.d. pulp basis)	0.1	0.1	0.1
NaOH applied, % (on o.d. pulp basis)	1.5	1.5	1.5
Final pH	11.8	11.3	11.2
Xylanase stage (X)			
Amount of xylanase added, IU/g (on o.d. pulp basis),	–	8	–
Final pH	–	6.4	–
Extraction stage (E)			
NaOH applied, % (on o.d. pulp basis)	3.0	3.0	3.0
H_2O_2 applied, % (on o.d. pulp basis)	0.5	0.5	0.5
Oxygen pressure, kg/cm^2	5.0	5.0	5.0
$MgSO_4$ applied, % (on o.d. pulp basis)	0.1	0.1	0.1
Final pH	11.6	11.6	11.4
Peroxide stage (P)			
H_2O_2 applied, % (on o.d. pulp basis)	2.0	2.0	2.0
EDTA applied, % (on o.d. pulp basis)	0.5	0.5	0.5
$MgSO_4$ applied, % (on o.d. pulp basis)	0.1	0.1	0.1
Final pH	11.7	11.2	11.1
Total H_2O_2 applied, % (on o.d. pulp basis)	2.5	2.5	2.5
Bleaching losses, %	7.1	7.5	7.8
Bleached pulp yield, %	41.67±0.6	41.49±1.5	41.35±1.2
Pulp brightness, % (ISO)	71.3±1.05	77.4±1.01	79.5±0.5
Pulp viscosity, cps	9.8±0.011	10.13±0.013	10.05±0.023

Bleaching conditions	X-stage	O-stage	(E_{OP})-stage	P-stage
Consistency, %	10	10	11.5	10
Temperature, ^{0}C	55±2	90±2	75±2	90±2
Time, min	120	90	70	90

± refers to standard deviation

AOX in combined effluent of XODED and XOD(E_{OP})DP was reduced by 41.43% and 40%, respectively, compared to their respective controls. The increase in COD and color after XODED and XOD(E_{OP})DP bleaching sequences compared to their respective controls is attributable to the hydrolysis of hemicelluloses, release of xylan, and lignin from pulp after xylanase pretreatment (Roncero *et al.* 2005). The increase in COD and color after XO(E_{OP})P bleaching sequence is more than that of OX(E_{OP})P bleaching sequence, which indicates that the removal of xylan and lignin after the XO stage is more comparable to that of the OX stage (Roncero *et al.* 2005).

The SEM study showed that sugarcane bagasse fibres after xylanase treatment have a rougher surface with striations and splits, *i.e.* a more open surface, confirming that xylanase hydrolyses the xylan deposited on the surface of fibres during alkaline pulping, which constitutes a physical barrier to penetration by bleaching agents. Their elimination facilitates the flow of bleaching chemicals, which explains the bleach boosting effect of the xylanases.

Table 3. Comparison of Strength Properties and Combined Effluent Characteristics during ECF (ODED, XODED, OD(E_{OP})DP, and XOD(E_{OP})DP) and TCF (O(E_{OP})P, OX(E_{OP})P, and XO(E_{OP})P) Bleaching Sequences

Particulars	ODED	XODED	% Difference	OD(E_{OP})DP	XOD(E_{OP})DP	% difference	O(E_{OP})P	OX(E_{OP})P	% Difference	XO(E_{OP})P	% Difference
Pulp brightness, %	79.2±0.47	86.5±0.55	+7.3	85.5±1.0	90.2±1.3	+4.7	71.3±1.05	77.4±1.01	+6.1	79.5±0.5	+8.2
Tear index, mNm^2/g	5.06±0.22	5.4±0.1	+6.72	5.01±0.13	5.26±0.2	+4.99	35	5.51±0.12	+5.96	5.4±0.24	+3.85
Burst index, $kPam^2/g$	3.7±0.12	3.45±0.2	-6.76	3.6±0.1	3.09±0.2	-14.17	5.2±0.1	2.57±0.07	-16.56	2.45±0.13	-20.45
Tensile index, Nm/g	53.14±3.1	48.3±1.25	-9.1	51.47±1.4	45.16±2.27	-12.26	3.08±0.09	47.12±1.02	-8.29	42.09±2.6	-18.08
Double fold, number	58±3.2	52±5.3	-10.34	44±4.7	27±2.2	-38.64	51.38±1.0	22±1.6	-18.51	24±2.5	-11.11
Pulp viscosity, cps	9.36±0.009	9.40±0.005	+0.43	9.38±0.004	9.43±0.011	+0.53	27±3.1	10.13±0.013	+3.37	10.05±0.008	+2.55
Copper number	0.13±0.01	0.08±0.005	-38.46	0.08±0.005	0.06±0.001	-25	9.8±0.011	0.06±0.003	-33.3	0.06±0.001	-33.3
COD, mg/L	551	623	+13.07	570	655	+14.91	0.09±0.001	745	+4.92	776	+9.3
Colour, PTU	1505	1772	+17.74	1750	1884	+7.66	710	3250	+20.72	3580	+32.98
AOX, kg/t	0.42	0.246	-41.43	0.35	0.21	-40	2692	–	–	–	–

Beating level 35^0SR, ± refers standard deviation

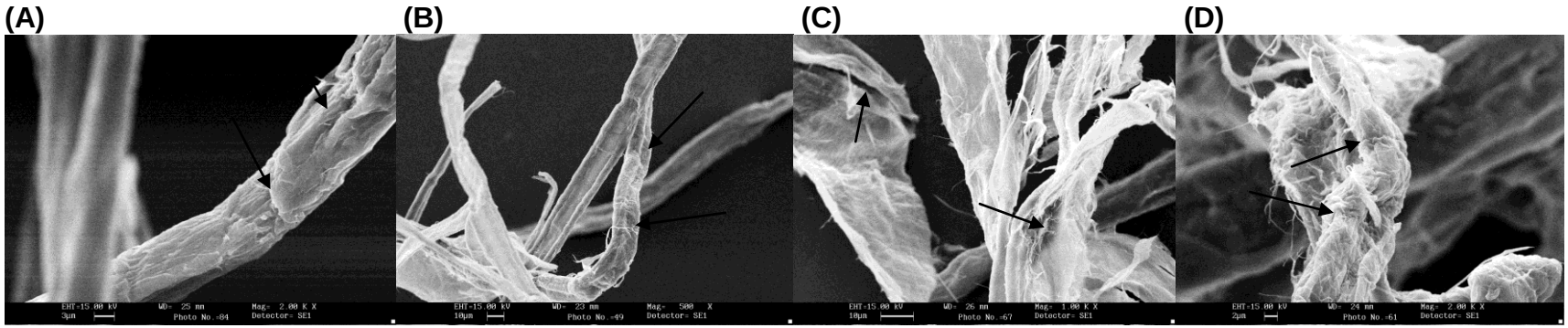

Fig. 1. (A) Fibers after xylanase pretreatment at a magnification of 2.00 KX, (B) at a magnification of 500 K, arrows show cracks on fiber surface, (C) Unbleached sugarcane bagasse pulp fibers beaten at 35 ^{0}SR at a magnification of 1.00 KX, arrows show fibrillation, and (D) Xylanase pretreated fibers beaten at 35 ^{0}SR at a magnification of 2.00 KX, arrows show profuse fibrillation and cracks on fibers.

CONCLUSIONS

The crude xylanase from *Coprinellus disseminatus* SW-1 NTCC1165, produced under SSF, was effective not only in reducing the pollution load during XODED, XOD(E_{OP})DP, OX(E_{OP})P, and XO(E_{OP})P bleaching sequences, but improved the pulp brightness compared to their respective controls. Xylanase treatment was more effective for removing lignin-carbohydrates complexes before oxygen delignification compared to keeping the xylanase treatment stage after the oxygen prebleaching stage. Xylanase treatment facilitated faster penetration of bleach chemicals, as shown by SEM; it reduced the overall chemical charge for bleaching while directly reducing toxicity of effluents generated during XODED and XOD(E_{OP})DP bleaching sequences in terms of AOX. However, color and COD of bleached effluent increased due to an increase in concentration of lignin-carbohydrates complexes. It also mitigated brightness reversion of bagasse pulp without affecting pulp viscosity.

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