CELLULOSE CHEMISTRY AND TECHNOLOGY

EFFECT OF TWO FUNGAL STRAINS OF *COPRINELLUS DISSEMINATUS* SH-1 NTCC-1163 AND SH-2 NTCC-1164 ON PULP REFINING AND MECHANICAL STRENGTH PROPERTIES OF WHEAT STRAW SODA-AQ PULP

C. H. TYAGI, SHALINI SINGH and DHARM DUTT

Department of Paper Technology, Indian Institute of Technology Roorkee, Saharanpur campus,
Saharanpur 247 001, India

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Effect of novel cellulase-poor crude enzymes from two different strains of *C. disseminatus* – SH-1 NTCC-1163 (enzyme A) and SH-2 NTCC-1164 (enzyme B) – produced under solid-state fermentation (SSF) conditions, on both pulp beating and mechanical strength properties of wheat straw soda-AQ pulp bleached by CEHH, TCF and ECF bleaching sequences, was investigated. X^AECEHH and X^BECEHH pulps bleached at 4% chlorine demand required 66.66 and 30.30%, respectively, fewer PFI mill revolutions, to attain a beating level of 40 ^{o}SR, compared to the control. Tensile and burst indexes of X^AECEHH pulp decreased by 10.74 and 6.94%, whereas the number of double folds and tear index improved by 8.63 and 0.73%, respectively, compared to the control. The X^AECEHH and X^BECEHH pulps bleached at 2.5% chlorine demand required more PFI revolutions, while the decrease in mechanical strength properties was lower, compared to X^AECEHH and X^BECEHH sequences bleached at 4% chlorine demand. On the contrary, OX^AEDED, OX^BEDED, OX^AEDEP and OX^BEDEP bleached pulps required more PFI revolutions compared to their respective controls, due to the removal of the low DP xylan content, as a result of xylan removal during oxygen delignification. OX^AEDED and OX^BEDED bleached pulps showed a decrease in tensile and burst indexes and in the number of double folds, as well as an improvement in the tear index, while the decrease in strength properties was observed less in OX^BEDED bleached pulp. The OX^AEDEP and OX^BEDEP bleaching sequences followed the same pattern.

Keywords: wheat straw, soda-AQ pulp, *Coprinellus disseminatus* SH-1 NTCC-1163 and SH-2 NTCC-1164, bleaching, pulp beating, paper properties

INTRODUCTION

The pulp and paper industry is an energy-intensive process, energy contributing with 16-20% to the manufacturing cost.[1] Due to shortage in energy availability and increase in energy cost, energy conservation has become a necessity in paper industry. Consequently, any process that significantly decreases the energy requirements in the pulp and paper manufacture will have a notable beneficial effect on the overall energy input. Pulp refining is an important step in pulp and paper production, applied to modify the structure of fibres for achieving the desired paper-making properties. Refining or beating is a mechanical treatment applied to improve strength properties and sheet formation in the manufacture of paper using pulp fibres. Beating is usually performed in a water medium, where fibres are treated with metallic bars with compositions according to specific designs. Pulp refining is a mechanical treatment of fibres developing their optimum papermaking properties, which depends on the product to be made.[2] The primary effects of beating or refining on fibres are considered to be external fibrillation, internal fibrillation, production of fines and fibre shortening.[3] Refining increases the strength of fibre-to-fibre bonding by increasing the surface area of fibres.[3,4] Dissolution of polysaccharides, mainly xylan, also occurs during refining.[4,5] The significance of the different refining effects varies with the parameters of the refining process and the structure and composition of fibres. Commercial applications of enzyme use xylanase in pre-bleaching of kraft pulps, and various enzymes, in the beating or refining process, as well as recycling paper.[6,7] The use of hemicellulose-hydrolysing enzymes has been found helpful in saving energy, if an enzymatic treatment stage is carried out before beating or refining.[8-10] Enzyme treatment requires additional retention time and sufficient mixing to affect the fibre surface. The desired results can be achieved at optimum pH, temperature and by appropriate mixing.[10] The pulp hydrolyzing enzymes are potential tools for modification of pulp properties. Xylanase enzyme treatments remove less than 2% of the total mass, while improving fibrillation and fibre bonding, and decreasing the beating times. This increases the drainage rate (in °SR) and water retention of the pulp. However, at the same time, it drastically decreases viscosity and breaking length.[11,12] Cellulase/hemicellulases formulation enhances and restores fibre strength, reduces refining energy requirements and increases inter-fibre bonding fibrillation, while increasing the drainage rates and avoiding fibre breakage.[12] The site of the enzyme action depends on the accessibility of the substrate, so that the chemical pulps with larger average pore sizes are generally considered as substrates more accessible to the enzymatic attack.[13]

Despite its action on pulp cellulose, cellulase can also have positive effects when a limited hydrolysis of fibre carbohydrates is carried out. Cellulases can be expected to act both on the outer fibre surface and inside the fibre wall, similarly to mannanases,[4,14] but, when low enzyme dosages are used, the enzyme action is more pronounced on the outer surface of fibres. By combining the enzymatic treatment with refining, the effects of the treatments on the papermaking potential can be defined. According to Noe *et al.*,[9] Pommier[15] and Stork and Puls,[16] improved beatability of kraft pulps has been obtained by using hemicellulases and cellulases. In such investigations, however, enzyme mixtures are used and hence the effects of individual enzymes are not determined.

This paper discusses pre-bleaching of soda-AQ pulp of wheat straw soda-AQ pulp using xylanases from SH-1 NTCC-1163 (enzyme A) and SH-2 NTCC-1164 (enzyme B) strains of *C. disseminatus*, and its effect on brightness, beating and paper properties.

EXPERIMENTAL

Crude xylanase

Cellulase-poor crude xylanases were obtained from *C. disseminatus* SH-1 NTCC-1163 and SH-2NTCC-1164, under optimum solid-state fermentation (SSF) conditions.[17] Xylanase activity was determined by measuring the release of reducing sugars, with birchwood xylan as a substrate, by the DNS method.[18] Carboxymethylcellulase (CMCase) activity was determined using carboxymethylcellulose (CMC) as a substrate[19] and reducing sugars – by the DNS method.[18]

Under optimized conditions, the xylanase activity was of 727.78 IU/mL, for strain SH-1, and of 227.99 IU/mL, respectively, for strain SH-2. The cellulase and laccase activities in strain SH-1 were of 0.925 IU/mL and 0.640 U/mL, respectively, while those for strain SH-2 were of 0.660 IU/mL and 0.742 U/mL, respectively.

Pulp sample

Wheat straw was produced at an optimum pulp yield of 45.05%, kappa number of 18.25, pulp brightness of 27.41% (ISO), pulp viscosity of 26.04 cps and an active alkali dose of 12% (as Na_2O) and AQ dose of 0.1%, on maintaining a maximum cooking temperature of 150 ºC, maximum cooking time of 60 min and a liquor-to-raw material ratio of 4:1.

Pulp pre-bleaching

The wheat straw soda-AQ pulp was pre-bleached with enzyme A and enzyme B under optimum pre-bleaching conditions, *i.e.* an enzyme dose of 10 IU/g (on oven-dry pulp basis), consistency – 5% for enzyme A and 10% for enzyme B, reaction time – 180 min, pH – 6.4 and temperature – 55 ºC. The pulp samples washed with 1 L tap water were extracted with 2% NaOH (as Na_2O) at 70±2 ºC for 90 min and pH 11.0. The alkali extracted pulps were washed with 1 L tap water, hand-squeezed and analyzed as to kappa number (T236 cm-85), viscosity (T230 om-04) and brightness, % (ISO) (T452 om-02), as per Tappi standard methods.[20]

CEHH bleaching

The enzymatic pre-bleached pulps (enzyme A and enzyme B used under optimum pre-bleaching conditions) were bleached by a CEHH bleaching sequence at full (4.5%) and half (2.25%) chlorine demand. 70% of the total chlorine charge was applied in the C-stage and the remaining 30% in equal amounts, in two distinct hypochlorite Ist stage (H_1) and hypochlorite IInd stage (H_2), respectively. The process conditions for each stage were reported in the tables.

^{A}XECEHH and ^{B}XECEHH bleaching sequences were compared with the CEHH bleaching sequence. The pulps obtained after each bleaching stage were filtered through cheese cloth and the filtrate was analyzed for residual chlorine, except the alkali extraction stage. The pulps were washed with 2 L of tap water after each bleaching stage, including enzyme treatment, for proper impregnation of the subsequent bleaching chemicals.[21]

ECF bleaching

The effect of the enzyme treatment on wheat straw soda-AQ pulp was observed in the ODED and ODEP bleaching sequences. Wheat straw soda-AQ pulp was delignified with O_2 at a pressure of 5 kg/cm^2, 2% NaOH (as Na_2O), 0.1% $MgSO_4$ (as carbohydrate stabilizer), temperature – 110 ºC, time – 90 min, consistency – 15% and pH – 11.0, in a CCL digester (Feronics, Roorkee, India), then evaluated as to kappa number (T236 cm-85),[20] after washing. The enzyme treatment was applied after oxygen delignification.[22] The pulps were washed after the enzyme treatment, for proper impregnation of the subsequent bleaching chemicals.[23] The chlorine dioxide (D) bleaching stage was performed in polyethylene bags at a pulp consistency of 7%, temperature – 70±2 ºC and pH – 2.5. Both D_1 and D_2 stages in the ODED sequence were conducted for a reaction time of 1.5 h each, while the 'D' stage in the ODEP sequence – for 3 h. The peroxide stage was performed in polyethylene bags at a temperature of 90±2 ºC, reaction time of 2 h, pH of 11.8 and consistency of 10%. Similarly, for the sake of comparison, the control pulps were bleached with ODED and ODEP sequences. The pulps obtained after each bleaching stage were filtered through cheese cloth, the resulting filtrate being analyzed for residual chlorine, except the alkali extraction stage. The pulps were washed with 2 L of tap water, squeezed and crumbled.

Characterization of pulps

The pulps were beaten (T248 sp-00) at a beating level of 40 ºSR, in a PFI mill. Laboratory handsheets (60 g/m^2) were prepared on a British sheet former (T205 sp-02), conditioned at a relative humidity of 65%±2 and temperature of 27±1 ºC, and evaluated for burst index (T403 om-02), tensile index (T494 om-01), double fold (T423 cm-98) and tear index (T414 om-04) – as per Tappi standard test methods.[20]

Statistical analysis

Three experimental values were considered in each case, the results obtained representing the mean ± standard deviation (SD) of values.

RESULTS AND DISCUSSION

Effect of enzyme treatment on pulp beating level

The X^{A}ECEHH and X^{B}ECEHH pulps bleached at 4.5% total chlorine charge require 66.66 and 30.30% fewer PFI mill revolutions, respectively, to reach a beating level of 40 ºSR, compared to the control (Table 1). Similarly, the X^{A}ECEHH and X^{B}ECEHH bleached pulps at 2.25% total chlorine charge require, respectively, 64.64 and 28.28%, fewer PFI mill revolutions, as compared to the control, at the same chlorine dose. It means that enzyme A is more effective compared to enzyme B, to achieve the targeted ºSR in fewer PFI revolutions. Some of the most important primary effects are internal fibrillation, *i.e.* replacement of intra-fibre hydrogen bonds by fibre-water hydrogen bonds, and external fibrillation, leading to increased swelling and flexibilization of fibres, as well as improved bonding ability. Fibre cutting and the removal of parts of the fibre wall result in an increased fines content and a decreased fibre length. Dissolution of polysaccharides, mainly xylan, also occurs during refining.[24] The significance of the different refining effects varies, as depending on the parameters of the refining process and the structure and composition of the fibre. The cellulases present in both enzyme preparations, *i.e.* enzyme A and B play an important role in reducing the refining energy of the enzymatically treated pulps.

Xylanase systems have been developed to assure selective hydrolysis of hemicelluloses, without loss of fibre strength.[25] The enzyme treatment of pulp modifies the pulp properties, improving both fibre flexibility and (internal and external) fibrillation.[26] Enzyme helps to soften the fibre walls and to increase the access to cellulose fibres, as well as to break the primary wall, which is thin (0.05 micron thick) and relatively impermeable. With the addition of the enzyme, it becomes easier to refine the fibre and to digest the small fibre fraction, reducing fines. It can be seen that the enzyme-treated pulps show varied behaviour to beating. In the case of conventional bleaching sequences, the enzyme-treated pulps required fewer PFI mill revolutions to reach a fixed ºSR, thereby decreasing the energy requirement. Pulp fibrillation by cellulases was recognized as a means to enhance strength properties as early as 1959, by Bolaski and co-workers,[27] being mainly applied to cotton linters and other non-wood pulps. A process patented in 1968 used cellulases from a white-rot fungus, applied at concentrations of 0.1 to 1% (based on the dry mass of pulp), to reduce the refining or beating time.[28] Another researcher[29] also reported that, when applying xylanase from *Aspergillus niger* on an eucalyptus kraft pulp, at a given degree of beating, *e.g.* 30 °SR, the number of revolutions was 1909 for the untreated pulp, whereas the xylanase-treated pulp required only 1595 revolutions. It was also observed that, in the case of enzyme pulp treatment at a lower total chlorine charge, more PFI mill revolutions are required, compared to those treated at a higher (4.5%) total chlorine charge.

Table 1
Effect of enzyme treatment on beating response of wheat straw soda-AQ pulp during conventional and ECF bleaching sequences

Sl. No.	Bleaching sequence	°SR	Revolutions/min (rpm)
1	CEHH	40	990
2	X^AECEHH^a	40	330 (–66.66)
3	X^AECEHH^b	40	690 (–30.30)
4	X^BECEHH^a	40	350 (–64.64)
5	X^BECEHH^b	40	710 (–28.28)
6	ODED	40	900
7	OX^AEDED	40	990 (+10.00)
8	OX^BEDED	40	950 (+5.55)
9	ODEP	40	690
10	OX^AEDEP	40	780 (+13.04)
11	OX^BEDEP	40	750 (+8.69)

[a] chlorine demand = 4.5%; [b] chlorine demand = 2.5% (on o.d. pulp basis)

The OX^AEDED and OX^BEDED pulps require 10.00 and 5.55% more PFI mill revolutions to reach a beating level of 40 °SR, compared to the ODED pulp. Similarly, OX^AEDEP and OX^BEDEP require 13.04 and 8.69% more PFI mill revolutions, compared to ODEP. The increase in PFI mill revolutions after enzymatic treatment may be attributed to a low content of DP xylan, which is removed during oxygen delignification. The enzyme preparation removes the lower DP xylan from the pulp, stuffy, crystalline cellulose fibres remaining in pulp. Secondly, xylan acts as a lubricant during pulp beating. It has been reported that xylanase solubilizes more sugars from the oxygen delignified pulp than the brown stock pulps.[30] The results are also correlated with a higher viscosity after oxygen delignification.

Effect of enzyme treatment on pulp mechanical strength properties

Compared to the control, the tensile and burst indexes were reduced by 11.36 and 8.09%, respectively, for X^AECEHH bleached pulp, and by 10.74 and 6.94%, respectively, for the X^BECEHH bleached pulp, at a 4.5% chlorine charge (Table 2), while the double fold and tear index in X^AECEHH and X^BECEHH bleached pulps improved by 7.52 and 0.59, and 8.63 and 0.73%, respectively, compared to the control, at the same chlorine charge. The reduction in the tensile (6.00 and 4.40%) and burst (3.55 and 3.73%) indexes in X^AECEHH and X^BECEHH bleached pulpsis much lower at a 2.25% chlorine charge than at a chlorine charge of 4.5%, while the double fold (15.71 and 16.50%) and tear index (13.96 and 16.46%) in X^AECEHH and X^BECEHH bleached pulpsare much higher at a 2.25% chlorine charge than at a chlorine charge of 4.5%. The marginally improved mechanical strength properties of X^AECEHH and X^BECEHH pulps at 2.5% chlorine charge may be due to the lower detrimental effect on carbohydrates occurring at a lower chemical dose during bleaching, comparatively with X^AECEHH and X^BECEHH bleaching sequences at 4.5% chlorine charge. The xylanase-treated wheat straw pulps haveon the average longer fibres and a lower fines content than the control, which is disadvantageous for fibre bonding. The average higher fibre length may increase the tear index.[21] The quantity, chemical structure, distribution and DP of hemicelluloses influence paper strength.[31] The removal of hemicelluloses and the concomitant reduction of residual hemicellulose DP might reduce tensile and burst indexes. The encrease in the tear index can be attributed to the fact that, even if excess xylan removal reduced burst and tensile strength by reducing inter-fibre bonding, the fibres themselves were not weakened.[32]

The tensile, burst and double fold indexes of OX^{A}EDED decrease by 16.12, 10.47 and 10.60%, respectively, compared to the control (Table 3). The decrease for the same parameters is lower in OX^{B}EDED pulp. The tear index improves almost to the same extent for both enzyme-treated pulps. The tensile and burst indexes for OX^{A}EDEP decrease by 16.30 and 9.46%, respectively, compared to the control. Compared to OX^{A}EDEP, OX^{B}EDEP presented a slighter decrease in tensile and burst indexes. On the other hand, the double folds and tear index for OX^{A}EDEP pulp are higher by 5.97 and 2.61% than the control (Table 4). The double folds and tear index for OX^{B}EDEP improved by 7.46, and 3.17%, respectively, compared to the ODEP pulp.

Table 2
Effect of enzyme treatment on soda-AQ pulp of wheat straw in CEHH bleaching sequence

Indexes	Bleaching sequences						
	CEHH	^{A}XECEHH	^{A}XECEHH	^{B}XECEHH	^{B}XECEHH		
Pulp beating							
Beating level, °SR	40	40	40	40	40		
Mechanical strength properties							
Tensile index, Nm/g	49.22	43.63 (–11.36)	46.27 (–6.00)	43.94 (–10.74)	47.06 (–4.40)		
Burst index, kPa m^2/g	3.27	3.01 (–8.09)	3.16 (–3.55)	3.05 (–6.94)	3.15 (–3.73)		
Double fold, no.	64	69 (+7.52)	74 (+15.71)	70 (+8.63)	75 (+16.50)		
Tear index, mNm^2/g	5.27	5.30 (+0.59)	6.00 (+13.96)	5.31 (+0.73)	6.13 (+16.46)		
Bleaching conditions	^{A}X	^{B}X	E$_1$	C	E$_2$	H$_1$	H$_2$
pH	6.4	6.4	11.0	2	11.0	11.0	11.0
Consistency, %	10	5	10	3	10	10	10
Retention time, min	180	180	90	45	90	90	90
Temperature, °C	55±2	55±2	70±2	ambient	70±2	70±2	70±2

Unbleached pulp kappa number – 18.25; unbleached pulp brightness – 27.41% (ISO); unbleached pulp viscosity – 26.04cps; A – crude xylanases from *C. disseminatus* SH-1 NTCC-1163; B – crude xylanases from *C. disseminatus* SH-1 NTCC-1164; +/– = % difference compared to control pulp; ± = standard deviation from the mean; experiments were performed in triplicate

Table 3
Effect of enzyme treatment on soda-AQ pulp of wheat straw in ODED bleaching sequence

Indexes	Bleaching sequences						
	ODED	OX^{A}EDED	OX^{B}EDED				
Pulp beating							
Beating level, °SR	40	40	40				
Mechanical strength properties							
Tensile index, Nm/g	49.60	41.60 (–16.12)	43.60 (–12.09)				
Burst index, kPa m^2/g	3.34	2.99 (–10.47)	3.01 (–9.88)				
Double fold, no.	66	59 (–10.60)	60 (–9.09)				
Tear index, mNm^2/g	5.30	5.46 (+3.01)	5.44 (+2.64)				
Bleaching conditions	X^{A}	X^{B}	E$_1$	O	D$_1$	E$_2$	D$_2$
pH	6.4	6.4	11.0	11.0	2.5	11.0	2.5
Consistency, %	10	5	10	15	7	10	7
Retention time, min	180	180	90	90	120	90	120
Temperature, °C	55±2	55±2	70±2	110±2	70±2	70±2	70±2

A – crude xylanases from *C. disseminatus* SH-1 NTCC-1163; B – crude xylanases from *C. disseminatus* SH-1 NTCC-1164; +/– = % difference compared to control pulp; ± = standard deviation from the mean; experiments were performed in triplicate

Table 4
Effect of enzyme treatment on soda-AQ pulp of wheat straw in ODEP bleaching sequence

Indexes	Bleaching sequences						
	ODEP		OX^{A}EDEP			OX^{B}EDEP	
Pulp beating							
Beating level, ºSR	40		40			40	
Mechanical strength properties							
Tensile index, Nm/g	49.97		41.82 (–16.30)			42.61(–14.72)	
Burst index, kPa m^2/g	3.38		3.06 (–9.46)			3.08 (–8.87)	
Double folds, no.	67		71(+5.97)			72 (+7.46)	
Tear index, mNm^2/g	5.35		5.49 (+2.61)			5.52 (+3.17)	
Bleaching conditions	X^A	X^B	E$_1$	O	D	E$_2$	P
pH	6.4	6.4	11.0	11.0	2.5	11.0	11.8
Consistency, %	10	5	10	15	7	10	10
Retention time, min	180	180	90	90	120	90	120
Temperature, ºC	55±2	55±2	70±2	110±2	70±2	70±2	90±2

A – crude xylanases from *C. disseminatus* SH-1 NTCC-1163; B – crude xylanases from *C. disseminatus* SH-1 NTCC-1164; +/– = % difference compared to control pulp; ± = standard deviation from the mean; experiments were carried out in triplicate

CONCLUSIONS

Enzymes A and B, obtained from two different strains of *C. disseminatus*, SH-1 NTCC-1163 and SH-2 NTCC-1164, produced under solid-state fermentation, may reduce PFI revolutions in X^{A}ECEHH and X^{B}ECEHH bleached pulps by 66.66 and 30.30%, respectively. The decrease in PFI revolutions in X^{A}ECEHH and X^{B}ECEHH bleaching sequences at 4.5% chlorine charge is more significant than a chlorine charge of 2.5%. Enzyme A is more effective than enzyme B. The decrease in burst index, tensile index and the number of double folds is lower in X^{A}ECEHH and X^{B}ECEHH bleached pulps, when bleached at a 2.5% chlorine charge, compared to pulps of both bleaching sequences bleached at a 4.5% chlorine charge. The mechanical strength properties of OX^{A}EDED, OX^{B}EDED, OX^{A}EDEP and OX^{B}EDEP bleaching sequences follow the same pattern.

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