Biobleaching of Wheat Straw Pulp using Laccase and Xylanase

Guilong Xu, Xiwen Wang,* and Jian Hu

Wheat straw pulp can be considered to be one of most important raw materials for specialty and functional paper products. Bleaching sequences involving laccase and xylanase were applied to bleach wheat straw pulp. The bleached pulp properties after a sequence of xylanase (X), laccase (L), and extraction (E), *i.e.* XLE, were compared with those of LE and LEX sequences. It was found that the XLE bleaching sequence was the most suitable sequence for laccase and xylanase synergetic biobleaching. The bleaching results of OXLEQP and OQP beaching sequences (where O stands of an oxygen stage, Q indicates a chelation stage, and P means peroxide) were compared. For a specific target brightness level of over 80% ISO, XL pretreatment was found to save 28.6% of the H_2O_2 requirement and increase the viscosity by 6.7% compared with the OQP bleaching sequence. It was also found that synergetic biobleaching could decrease the consumption of refining energy. There were no detectable adsorbable organic halides found in the biobleaching effluents.

Keywords: Wheat straw pulp; Biobleaching bleaching; Bleaching effluent

*Contact information: State Key Lab of Pulp and Paper Engineering, South China University of Technology, Guangzhou, 510640, China; * Corresponding author: wangxw@scut.edu.cn*

INTRODUCTION

Paper consumption has grown substantially in China over the past few decades. Consumption of raw materials for pulp and paper has also grown rapidly. Non-wood fiber is an important raw material for papermaking, particularly in the countries with deficient wood resources, such as China and India. Wheat straw pulp (*Triticum sativum)* has emerged as one of the most important pulp types for specialty and functional paper grades, such as filtration paper. Most of the non-wood pulp mills use a bleaching sequence with successive use of chlorine (C), alkaline extraction (E), and hypochlorite (H), *i.e.* (CEH) to bleach the pulps (Zhao *et al.* 2010). If high brightness is required, an extremely high amount of available chlorine is needed. CEH bleaching leads to the formation of organic chlorine compounds in effluents and the evident loss of pulp strength. For environmental protection and to maintain a high quality of the pulp, new bleaching technologies are urgently required for non-wood pulp bleaching. Totally chlorine free (TCF) bleaching sequences including oxygen-, hydrogen peroxide-, and ozone-based stages have been shown to be suitable for wheat straw pulp bleaching (Roncero *et al.* 2003a,b; Torres *et al.* 2004).

Biotechnology has rapidly developed in pulping and bleaching processes. Enzyme stages involving xylanase (Roncero *et al.* 2000, 2003c) and laccase (Gamelas *et al.* 2005, 2007; Chakar and Ragauskas 2004; Moldes *et al.* 2008; Oudia *et al.* 2007) have provided very promising results in pulp bleaching. The use of xylanase (X) can enhance the bleaching effect of chemical reagents and decrease chemical consumption (Amin 2006; Roncero *et al*. 2005). The use of laccase–mediator systems (LMS) has provided an alternative to xylanase, acting directly on lignin. The mediator is typically a compound of low molecular weight capable of diffusing into cellulose fibers. Laccase–mediator systems react with the phenolic and non-phenolic units of lignin to delignify pulp. However, few references to enzymatic bleaching using both laccase and xylanase appear in the literature (Lian *et al*. 2012; Valls and Roncero 2009).

The aim of this paper was to find an environmentally friendly bleaching process for wheat straw pulp, evaluate the synergetic pretreatment of xylanase and laccase effect, establish an enzymatic pretreatment for nonwood fiber that would be effective in reducing the chemical dosage in following stages, study the effects of enzyme treatment on viscosity and brightness, and compare the results of OXLEQP and OQP bleaching sequences.

EXPERIMENTAL

Materials

Wheat straw pulp was supplied by Yin'he Paper Corpration of Shandong province China and cooked in an electrically heated rotating digester (PL1-00, Shaan xi, China) by a NaOH－AQ process. Oxygen delignification (O) was done in a pressure vessel. Conditions of oxygen delignification were: consistency 10%, NaOH 2%, $MgSO_4$ 1%, oxygen pressure 0.6 MPa, temperature 100 ^oC, and time 1 h.

Laccase (Denilite IIs) produced by submerged fermentation of a genetically modified *Aspergillus* was supplied by Novozymes (China) Corporation. Xylanase (HC 51088) was also provided by Novozymes (China) Corporation. The method to assay the activity of enzymes was according to He *et al.* (2003) and Li *et al.* (2001).

Xylanase Treatment (X)

The X treatment involved using 3 U /g odp (oven-dried pulp) xylanase at 10% consistency and Tris-HCl buffer at pH 7 at 60 ^oC for 2 h. These conditions were selected based on the work of Valls and Roncero (2009). The resulting pulp was washed with deionized water three times and distilled water.

Laccase Treatment (L)

Laccase activity was measured by spectrophotometer. One mL of laccase (diluted 5×106 times) was added with 1.0 mL buffer into the colorimeter tube. Then 1.0 mL of 0.5 $mmol.L^{-1}$ABTS was added to start the reaction, at which point a timer was started. The absorbency was recorded every minute at the wavelength of 420 nm. The definition of the unit laccase activity (U) is the quantity of the laccase which can oxide 1 µmol ABTS within one minute (ε=3.6×104 $cm^{-1}M^{-1}$). The pulps were washed with sodium acetate buffer solution at pH 4 before laccase treatment. Laccase was dissolved in the buffer. The mediator HBT (1-hydroxybenzotriazol) and the laccase solution were added into the pulp. Pulp consistency was adjusted with sodium acetate buffer. Then these pulps were transferred into a steel autoclave pressurized with oxygen. The mixing stocks were heated and kept at a desired temperature with continuous stirring. The conditions were: pulp consistency 10%, HBT 1%, laccase dosage 20 U/g, pH 4.0, temperature 40 ^oC, time

3 h, and oxygen pressure 0.4 MPa. These conditions were selected based on the work of Garcia *et al.* (2003).

Alkali Extraction (E) of Pulps

An alkali extraction stage (E) is usually used after the delignification. The pulps were extracted by alkali with 2% NaOH at 60 °C for 1 h after LMS treatment. The pulps were washed with distilled water after alkali extraction.

Pulp Bleaching

The pulps were bleached with OQP, OXLEQP, and CEH sequences (where Q, P, C, and H denote chelating treatment, hydrogen peroxide bleaching, chlorine, and hypochorite, respectively).

Beating, Sheet Making, and Evaluation of Physical Strength Properties

Pulps were beaten at fixed beating level (40 °SR) in a PFI (LABTECH, Canada) mill. Laboratory handsheets (60 g/m^2) were prepared on a sheet former (TAPPI method T205 sp-02), conditioned at relative humidity 50% ± 2 and temperature 23 ± 1 °C and evaluated for burst index (T403 om-02), tensile index (T494 om-01), and tear index (T414 om-04) as per TAPPI test methods. Thick pulp pads were prepared (T218 sp-02) and evaluated for kappa number (T236 cm-85), brightness (ISO) (T452 om-02), and viscosity (T230 om-04).

Determination of Bleaching Effluent

The 5-day BOD of a sample was determined at 20 °C using the standard dilution technique according to the American Public Health Association (APHA). COD_{Cr} tests with the open reflux method and suspend solids (SS) method were according to APHA (Greenberg *et al.* 1995). Adsorbable organic halgons (AOX) was measured by AOX Analyzer Dextar ECS 1200.

Scanning Electronic Microscope (SEM) Observation

Samples of OQP, OXLEQP, and CEH bleached pulps were first prepared by suspending fibers in water placed on a cover glass, and then allowed to dry. The samples were examined under SEM using the gold shadowing technique (Gabriel 1982). Electron photomicrographs were taken at 15 kV using detector SE1 at desired magnifications with a LEO1530VP instrument from Germany.

RESULTS AND DISCUSSION

Synergetic Biobleaching Sequence

The results of different synergetic biobleaching sequences are listed in Table 1. It was shown that xylanase pre-treatment had a significant effect on kappa number. For example, for the LE and XLE pulps, the kappa number was reduced 6%. But xylanase had no effect on delignification by itself. After an E stage followed an L stage, the Kappa number of pulp was decreased by 0.2, but the brightness increased to 44.5%ISO. The results indicated that there were some chromophores formed in the pulp during the L treatment. An alkali extraction treatment following L treatment was beneficial for the removal of lignin fragment from the pulp, leading to the reduction of Kappa number and improvement of the brightness (Ferrer *et al.* 2011). In a comparison between the synergetic biobleaching sequences XLE and LEX, it was found that the Kappa number following a XLE sequence was 0.2 lower than that of the LEX sequence without adversely affecting the pulp viscosities. The brightness of XLE bleached pulp was 1.5%ISO higher than that of LEX bleached pulp. It was shown that the X pretreatment could improve delignification of L treatment. So it was judged that the most suitable synergetic bleaching sequence was XLE. The previous work of Valls and Roncero (2009) reported the same result for eucalyptus kraft pulp.

Table 1. Properties of Pulps with Different Treatments

Pulps	Kappa number	Brightness (%ISO)	Viscosity (mL/g)
Original pulp	10.5	33.8	1285
X	10.4	36.7	1301
L	6.5	39.8	976
LE	6.3	44.5	984
XLE	5.9	47.2	961
LEX	6.1	45.7	975

Pulp Properties Following TCF Bleaching

The TCF bleaching sequences of OXLEQP (where Q, P, C, and H denote chelating treatment, hydrogen peroxide bleaching, chlorine, and hypochorite, respectively) and OQP were compared with traditional CEH bleaching. Properties of bleached pulps are listed in Table 2.

Table 2. Properties of Bleached Pulps

Bleaching Sequences	Yield on Unbleached pulp (%)	Kappa number	Brightness (%ISO)	Viscosity (mL/g)
Unbleached	100	10.5	33.8	1285
O	93.1	6.9	45.2	1009
OQ	91.4	6.6	50.3	932
OQP1	86.2	1.8	76.3	851
OQP2	85.7.	1.4	78.6	825
OQP3	84.9	1.0	80.5	922
OX	92.5	6.8	54.4	1052
OXL	88.7	3.4	57.2	978
OXLE	87.6	3.3	58.1	961
OXLEQ	87.1	3.2	58.3	951
OXLEQP1	85.3	1.8	80.6	858
OXLEQP2	84.7	1.6	83.3	835
OXLEQP3	82.5	1.2	83.8	804
C	91.6	4.7	54.3	752
CE	89.7	3.9	58.1	703
CEH	85.6	1.3	79.1	612

Q: consistency 10%, EDTA 0.5%, 60 ^{o}C, 1h
P_1: consistency 10%, H_2O_2 2.5%, $MgSO_4$ 0.05%, NaOH 1.5%, 90 ^{o}C; 4h.
P_2: H_2O_2 3%, the others were the same as P_1.
P_3: H_2O_2 3.5%, the others were the same as P_1.
C: consistency 3%, active chlorine 4%, room temp., 1h
H: consistency 10%, active chlorine 2%, 40 ^{o}C, 2h

Table 2 shows that CEH bleached pulp had the lowest viscosity (612 mL/g) and brightness (79.1%ISO). These results indicated that cellulose was drastically degraded in the CEH bleaching process. The results from Table 2 indicate that the TCF bleaching sequence including the XL treatment was able to achieve higher brightness with better pulp properties. The brightness values of OXLEQP bleached pulps were all over 80% ISO. Especially, the viscosities of OXLEQP bleached pulps (>800 mL/g) were much higher than those of CEH bleached pulps.

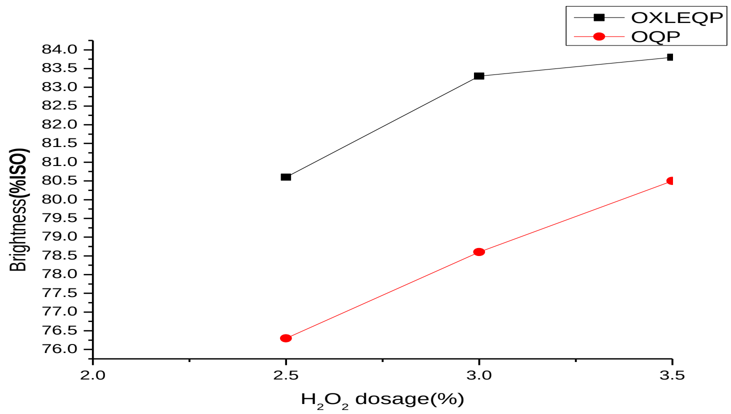


Fig. 1. Pulp brightness of OXLEQP and OQP bleached pulp at the same H_2O_2 dosage

In a comparison of the OXLEQP sequence with OQP bleaching (Fig. 1), it was found that the OQP bleaching sequence only achieved a brightness of 80.5%ISO when the H_2O_2 consumption was 3.5%. But OXLEQP bleaching reached the same brightness when the H_2O_2 consumption was 2.5%. So for the same brightness target level over 80%ISO, the XL treatment could save the dosage of 28.6% bleaching chemicals. Meanwhile, the pulp viscosity increased 6.7% from 804 mL/g to 858 mL/g. When the H_2O_2 dosage was kept the same, the brightness of OXLEQP bleached pulps was on average 3%ISO higher than that of OQP bleached pulp. This indicated that the synergetic biobleaching of laccase and xylanase is feasible for the improvement of delignification and bleachablity. In conclusion, synergetic biobleaching of laccase and xylanse not only decreases the bleaching chemicals consumption but also improves selectivity and bleachability.

Beating Characteristics of Pulps

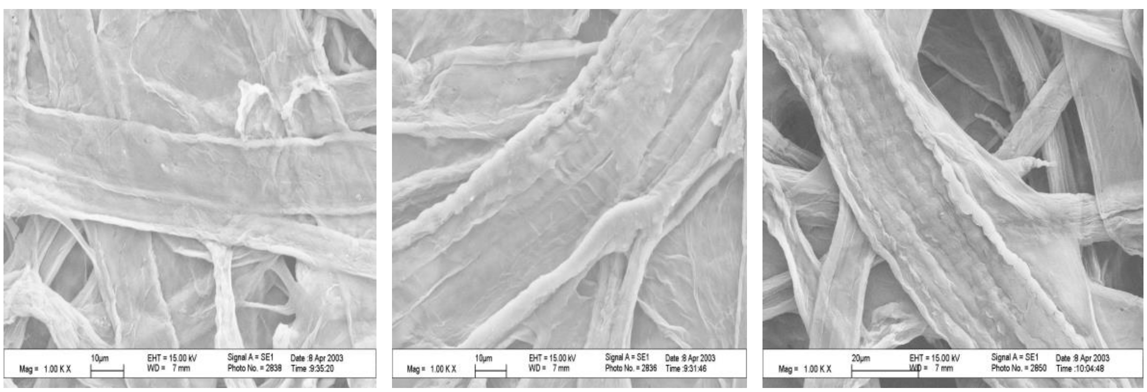
The strength properties of the beaten pulps are listed in Table 3. The results show that TCF bleached pulps including XL treatment were easier to beat to a specified beating degree than those of OQP and CEH bleached pulps. At about 1800 to 2000 revolutions with a PFI mill, the beating degree of OXLEQP could reach 40°SR. To get the same beating degree, OQP and CEH bleached pulps required more energy (2800 to 3200 revolutions). These results indicated that the xylanase and laccase synergetic treatment has the potential to save energy consumption in pulp beating. The breaking length, tear index, and burst index of enzymatic TCF bleached pulps and OQP bleached pulp were better than those of CEH bleached pulps. The breaking length of CEH bleached pulp was only 5.3 km. But the breaking length of OXLEQP3 bleached pulp reached as high as 6.4 km.

Table 3. Strength Properties of Beaten Pulps

Bleaching sequences	Beating Degree (ºSR)	Revolutions	Breaking Length (Km)	Tear Index ($mN \cdot m^2/g$)	Burst Index ($kPa \cdot m^2/g$)
OXLEQP1	40	2000	5.8	4.6.	5.0
OXLEQP2	41	2000	6.1	4.7	5.1
OXLEQP3	41	1800	6.4	4.5	5.4
OQP1	40	3000	6.1	4.4	5.2
OQP2	41	3000	6.2	4.5	5.1
OQP3	40	2800	6.0	4.5	5.2
CEH	40	3200	5.3	4.3	4.6

Morphology of Fiber

In order to observe the morphology of fiber, SEM observations were carried out. The results in Fig. 2 show that the surface of OXLEQP bleached pulp was more three-dimensional than those of CEH and OQP bleached pulp.



A B C

Fig. 2. Observation of fiber morphology (A) CEH bleached pulp (B) OQP bleached pulp (C) OXLEQP bleached pulp

Bleaching Effluent Load

The properties of bleaching effluent of TCF and CEH bleaching sequences are listed in Table 4.

Table 4. Properties of Bleaching Effluents

Bleaching sequences	AOX(mg/L)	SS(mg/L)	BOD5(mg/L)	CODCr(mg/L)
OQP_3	---	65	50	167.4
$OXLQP_3$	---	81	57	175.5
CEH	545.3	243	143	2041.4

AOX was not found in effluent from the TCF bleaching sequences OQP and OXLEQP. But in the CEH bleaching effluent AOX was detected at a level of 545.3 mg/L. AOX is very toxic towards the environment. In addition, the SS, BOD_5, and COD_{Cr} of TCF bleaching sequences were much lower than those of CEH bleaching sequences (243 mg/L, 143 mg/L, and 2041.4 mg/L). These properties of effluent showed that the biobleaching sequences can be more friendly to the environment.

CONCLUSIONS

The XLE biobleaching sequence was found to be the most suitable for wheat straw pulp enzyme pretreatment. The results indicated that xylanase pretreatment could increase the accessibility of laccase to fibers. In comparison of OXLEQP bleaching sequence and OQP bleaching sequence, for a specific target brightness level of over 80% ISO, XL pretreatment can save 28.6% H_2O_2 and increase the viscosity by 6.7%. It was also found that synergetic biobleaching not only decreases beating energy consumption but also is environmentally friendly.

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Article submitted: March 12, 2013; Peer review completed: April 16, 2013; Revised version received and accepted: April 18, 2013; Published: May 2, 2013.