New strategies to bleach dissolving pulps using enzymatic treatments

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A biobleaching sequence was applied to sulphite pulp in order to explore new bleaching possibilities using enzymatic treatments. Therefore, the well-known laccase-mediator system was used with the aim to achieve dissolving pulp characteristics. The enzymatic sequence was compared with a conventional hydrogen peroxide treatment in order to elucidate the effect of a laccase stage (L) for a potential industrial application. The treated pulps showed satisfactory results: high cellulose reactivity, high brightness and low content of hemicelluloses.

Keywords: alkali resistance, brightness stability, carbohydrate composition, cellulose reactivity, dissolving pulp

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INTRODUCTION

Dissolving pulps are characterized by a high content of cellulose, low amount of hemicelluloses (<10%) and traces of residual lignin, extractives and minerals. These particular features can be obtained by a sulphite or a pre-hydrolysis kraft cooking process. Importantly, according to FAO (2012), demand for dissolving pulp has grown rapidly in latest years and the prospective consumer markets indicate that this trend will continue in the next decades. Dissolving pulps have traditionally been used in the production of viscose filament and viscose staple fibres for textile applications; however, new end-uses such as cellulose based casings and sponges, thickeners in food and paints, capsules for medicine, among others have been developed. In addition, agricultural restrictions on cotton cultivation and the fact that dissolving pulp can be an environmentally friendly alternative to synthetic fibers have also contributed to this market upturn.

The long-established procedures used to obtain dissolving pulps present some disadvantages in terms of economic cost, chemical consumption and production rate (Hillman, 2006). Hence, there are wide numbers of studies that have been done on the extraction of hemicellulose from wood under acidic conditions (Puls, Janzon, & Saake, 2006) or the use of xylanase and cellulase (Gehmayr, Schild, & Sixta, 2011; Ibarra, Köpcke, Larsson, Jääskeläinen, & Ek, 2010; Köpcke, Ibarra, Larsson, & Ek, 2010) to carry out this transformation. However, there are few works dealing with the bleaching ability of sulphite pulps. Therefore, taking into consideration the recent interest in this high-purity bleached pulp, the aim of this work was to explore new bleaching opportunities for unbleached sulphite cellulose using the well-known Laccase-Mediator System and provide an alternative solution to conventional bleaching processes.

In this paper, never-dried unbleached sulphite cellulose obtained from a mixture of spruce and pine was used as a raw material. The unbleached sulphite cellulose was subjected to an $L_{VA}QPo_4$ biobleaching sequence with the intention to elucidate the potential of LMS as an alternative to conventional bleaching processes. On the one hand, treated pulps were characterized via bleaching properties: kappa number, viscosity, ISO brightness, brightness

stability (aging treatments), hydrogen peroxide consumption and effluent properties (colour, toxicity and residual laccase activity). On the other hand, pulps were evaluated in terms of dissolving pulp characteristics: carbohydrate composition, cellulose reactivity, alpha cellulose and alkali solubility.

EXPERIMENTAL

Pulp and enzyme

Never-dried unbleached sulphite cellulose, cooked at Domsjö mill (Sweden), was used as a raw material. The initial pulp was a mixture of 60% spruce (*Picea abies*) and 40% pine (*Pinus sylvestris*). Commercial laccase from *Trametes villosa* was used in combination with the synthetic mediator violuric acid (VA). The enzyme was supplied by Novozymes® (Denmark) and the mediator was purchased from Sigma–Aldrich. Laccase activity was determined by monitoring the oxidation of ABTS 2,2'azino*bis*(3-ethylbenzthiazoline-6-sulphonate) in 0.1M sodium acetate buffer at pH 5 at 25ºC. One activity unit is defined as the amount of laccase required to convert 1 µmol/min of ABTS to its cation radical (ε_{436} = 29300 $M^{-1}cm^{-1}$).

Operating Conditions and Standards

An extended biobleaching sequence using violuric acid (VA) as mediator was studied in order to more accurately assess the performance of LMS in combination with a pressurised hydrogen peroxide treatment. The used of VA was based on the results obtained from a preliminary bleaching treatments, where natural mediators such as *p*-coumaric acid (PCA) and syringaldehyde (SA) and the synthetic mediator 1-hydroxybenzotriazole (HBT) were also tested (Quintana, Valls, Vidal, & Roncero, 2013). The biobleaching sequence was named LQPo, where L denotes an enzymatic stage, Q a chelating stage and Po a pressurized hydrogen peroxide bleaching treatment. The enzymatic stage (L) was carried out with the presence of *Trametes villosa* laccase and violuric acid (VA). The enzymatic treatment was performed in an oxygen pressurized reactor (0.6 MPa) at stirring rate of 30 rpm, using 50 mM sodium tartrate buffer (pH 4) to adjust 5% (w/w) pulp consistency. The enzyme dose was 20 U/g odp (oven-dry weigh of pulp) of laccase and 1.5% odp of mediator at 50 ºC for 4 h. After treatment, pulp sample was filtered and extensively washed for further processing. The enzymatic treatment was followed by a Q stage which was performed with 1% odp DTPA at 5% consistency at pH 5–6 (adjusted with H_2SO_4 1N) in polyethylene bags at 85ºC for 1h. The biobleaching sequence was completed with a chemical bleaching stage involving alkaline hydrogen peroxide bleaching procedure (Po) which consisted of a multiple sequential step. Thus, Po was carried out at 5% consistency in oxygen pressurized (0.6 MPa) reactor, using a stirring rate of 30 rpm under the following conditions: 1.5% odp NaOH, 0.3% odp DTPA and 0.2% odp $MgSO_4$ at 90ºC for 4h. This stage was performed in three consecutive steps (Po_1 = 1h reaction, Po_2 = 1h reaction, Po_4 = 2h reaction) each involving the addition of 10% odp H_2O_2 and no interstep washing. A small amount of pulp was removed after each Po step, filtered, residual liquors collected and then extensively washed with de-ionized water in a filter funnel for subsequent analysis (Quintana et al., 2013). The results were compared with those for a control sequence (KQPo) without laccase and mediator in order to confirm that neither the buffer suspension nor the incubation time had any effect on the process. In addition, the effect of an enzymatic stage was assessed via a conventional

treatment; thus, the enzymatic stage was omitted and a chemical hydrogen peroxide (Po) treatment was directly applied to the initial pulp under the same conditions as in the enzymatic sequence.

Treated pulps were characterized via kappa number, brightness stability, viscosity, cellulose reactivity, carbohydrate composition, alkali solubility and α-cellulose.

RESULTS AND DISCUSSION

The variation in the residual lignin content during the bleaching process was assessed in terms of kappa number. As can be seen from **Table 1**, in all bleaching sequences, the greatest delignification (i.e. kappa number reduction) was detected after a hydrogen peroxide stage. However, the marked decrease obtained after Po_1 step of the enzymatic sequence (LQPo) was directly due to the Laccase–VA action since the control sequence (KQPo) only provided 56% delignification after $KQPo_1$ and with respect to the previous stage. The conventional hydrogen peroxide process (Po) caused 61% delignification at Po_1 relative to the initial pulp, but failed to reach the same level as the $LQPo_1$ sequence. This result further confirms the boosting effect of L stage in the bleaching process and as a consequence, the alkaline pH used in the Po stage facilitated the dissolution of this degraded lignin. In terms of brightness (**Table 1**), a drop in brightness was observed after the enzymatic stage (L-0h) but this sequence ($LQPo_4$-0h) was more efficient in raising pulp brightness (~90 % ISO) at the end of the whole sequence than was the control sequence ($KQPo_4$-0h). The particular characteristics of starting pulp (i.e. no presence of hexenuronic acids and low content of lignin) provided high brightness stability when samples were thermally aged (Initial-144h). In particular, the control treatment (KQ-144h) only suffered 0.3% brightness loss while, the L_{VA} treatment underwent a 10% brightness loss after 144h of aging treatment. These results suggested that the enzymatic treatment generated amounts of chromophores or oxidizable structures, which in turn resulted in increased reversion of optical properties. However, the final brightness loss detected for $L_{VA}QPo_4$ and $KQPo_4$ after an aging treatment of 144h, was very similar in both cases (13% and 12%, respectively), suggesting that the chromophore groups created during the laccase treatment were removed or modified along the hydrogen peroxide stage (Po).

Table 1. Kappa number (± confidence intervals) of treated pulps obtained from each stage of the sequences: LQPo (enzymatic treatment), KQPo (control treatment) and Po (conventional treatment). Brightness values before (0h) and after an accelerated ageing by moist heat treatment (144h) at the different bleaching stages.

Kappa number			
Initial	5.3 ± 0.12		
	LQPo	KQPo	Po
L/K	2.3 ± 0.06	4.7 ± 0.18	-
Q	2.5 ± 0.07	4.2 ± 0.19	-
Po_1	0.5 ± 0.33	1.9 ± 0.24	2.1 ± 0.35
Po_2	0.6 ± 0.37	1.1 ± 0.0	1.9 ± 0.04
Po_4	0.2 ± 0.00	1.0 ± 0.21	1.5 ± 0.0

ISO Brightness (%)		
	0h	144h
Initial	58.74	57.15
L	55.43	49.81
LQ	56.86	54.94
$LQPo_4$	89.18	71.83
KQ	57.85	57.65
$KQPo_4$	81.83	77.61

In **Figure 1** is shown reactivity (according to Fock's method) and viscosity values for the different bleaching sequences. In general, it can be said that in terms of reactivity there were no differences between treatments. The introduction of enzymatic stage (L) did not

improve reactivity; however obtained values were similar to those reported elsewhere (Ibarra, Köpcke, & Ek, 2010; Köpcke, Ibarra, & Ek, 2008). With regard to cellulose integrity, the highest viscosity loss was detected by the enzymatic sequence (LQPo) after applying a Po stage; nevertheless this sequence gained the highest brightness value (~90%). Importantly, for a target brightness of ~84% ISO, similar yields of viscosity were obtained for the different bleaching sequences.

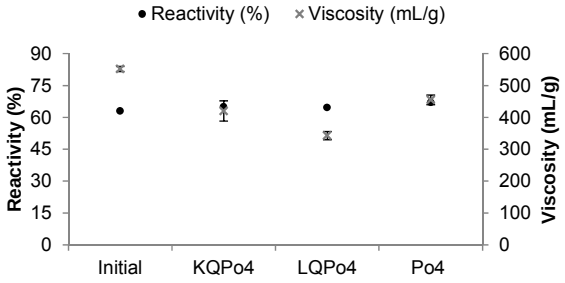

Fig. 1. Viscosity and reactivity values for the different bleaching treatments. Initial (unbleached sulphite pulp), $KQPo_4$ (control treatment with no presence of laccase neither mediator), $LQPo_4$ (enzymatic stage and pressurized hydrogen peroxide stage) and Po_4 (conventional bleaching treatment).

In order to evaluate the effectiveness of the bleaching process, it is important to monitor the residual amount of chemical products used in the process. **Table 2** shows the hydrogen peroxide consumption (%) at each addition step of H_2O_2 for the different bleaching sequences. Interestingly, during the first hour of treatment (i.e first addition of H_2O_2), Po sequence consumed 19% and 24% less of H_2O_2 than LQPo and KQPo, respectively. Moldes & Vidal (2008) reported that in the case of laccase treatment in combination with HBT or VA, during the P stage, the hydrogen peroxide is consumed mainly to oxidize chromophoric groups and as a result a brightness enhance is detected. Unlike LQPo, the conventional sequence (Po) included no enzymatic treatment, so no chromophores groups resulted from the laccase treatment were there; this accounts for the response of the pulp to the Po_1 step. Overall, the evaluation of residual H_2O_2 in the bleaching effluents confirmed that an excess of H_2O_2 dose was employed and therefore this stage needs to be optimized for any industrial application.

Table 2. Hydrogen peroxide consumption (%) for each bleaching sequence: Po (conventional treatment), LQPo (enzymatic treatment) and KQPo (control treatment).

Hydrogen peroxide consumption (%)			
H_2O_2 addition step	**Po**	**LQPo**	**KQPo**
Po_1 (1h)	67	86	91
Po_2 (2h)	80	76	86
Po_4 (4h)	88	86	78

As dissolving pulp definition said, a low content of hemicellulose is desired since hemicelluloses can affect the cellulose processability, e.g. the filterability and the xanthanation in the viscose process, and properties of the cellulose-end products such as the viscose strength (Christov & Prior, 1993; Ibarra, Köpcke, Larsson, et al., 2010). As can be seen

from **Table 3**, the carbohydrate composition of the bleached pulp samples was similar in all cases and essentially the same composition as in the initial pulp. This result was to be expected since, unlike cellulases or xylanases, a laccase–mediator system acts on the bleaching process (i.e. diminution of lignin content) rather than on the hemicellulose content of pulp (Gübitz, Lischnig, Stebbing, Saddler, & Gÿbitz, 1997; Ibarra, Köpcke, & Ek, 2010; Köpcke et al., 2008). However, it is important to emphasize that the hemicellulose content fell in the acceptable range for commercial dissolving pulp (< 10%) in all studied cases (Christov, Akhtar, & Prior, 1998; Köpcke et al., 2008).

Table 3. Mean values (± standard deviation) of carbohydrate composition for the different bleaching treatments.

	Initial	L	$KQPo_4$	$LQPo_4$	Po_4
Glucan	91.1±0.53	91.9±0.44	91.9±1.48	92.6±0.15	92.7±0.14
Xylan	6.5±0.09	6.9±0.35	7.03±1.11	6.5±0.12	6.5±0.17
Arabinan	-	0.13±0.02	0.33±0.28	0.06±0.00	0.08±0.01
Glucuronic acid	0.76±0.01	0.66±0.17	0.60±0.05	0.03±0.26	0.48±0.15

Some other important characteristics for dissolving pulps are shown in **Table 4**. On the one hand, determining the α-cellulose proportion is a way to know the cellulose purity since corresponds to cellulose with high molecular weight. As can be seen in **Table 4**, the α-cellulose contents were similar with all bleaching treatments and consistent with the carbohydrate composition, particularly with glucan content. However, the β–cellulose fraction of enzymatic sequence was slightly diminished with respect to initial and control sequence. On the other hand, alkali solubility is a measure of cellulose degradation, and also of loss or retention of hemicellulose during pulping and bleaching processes. A 10% sodium hydroxide solution (S10) possesses maximum dissolving power and dissolves both degraded cellulose and hemicellulose; while in 18% NaOH (S18) the hemicellulose is soluble (Tappi T-235 cm-00). Slight differences in terms of 10% alkali resistance were observed between bleaching treatments. The conventional bleaching treatment suffered the lowest alkali resistance indicating higher degradation. The small differences in R18 (or S18), during the bleaching process, can be ascribed to differences in carboxylic acid content.

Table 4. Characteristic parameters used to describe market-like dissolving pulp. Initial (unbleached) sulphite pulp, $LQPo_4$ (enzymatic stage plus pressurized hydrogen peroxide stage), $KQPo_4$ (control treatment without laccase and mediator) and Po_4 (pressurized hydrogen peroxide stage or conventional treatment). The standard deviations were below ± 0.7 in all cases.

	Initial	$KQPo_4$	$LQPo_4$
α-cellulose	88.3	88.1	87.6
β–cellulose	7.5	7.0	5.8
λ-cellulose	4.2	5.4	6.1

Alkali Resistance		Initial	$LQPo_4$	Po_4
R10	%	88.7	86.1	85.2
R18	%	90.3	86.6	90.2

CONCLUSIONS

The outstanding results obtained with the introduction of L_{VA} system fulfil the characteristics of commercial dissolving pulps: high ISO brightness, high cellulose reactivity, preserved cellulose integrity and low content of hemicellulose. Additionally, from a bleaching point of view, the enzymatic treatment saved 2h of reaction time and about 70% of hydrogen peroxide consumption, with respect to a conventional hydrogen peroxide sequence (Po).

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