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The Path Forward to Practical Nascent Laccase Biobleaching Technologies

F.S. Chakar, L. Allison, D.H. Kim, A.J. Ragauskas, and T. Elder

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The path forward to practical nascent laccase biobleaching technologies

Fadi S. Chakar, Lenong Allison, Dong Ho Kim, and Art Ragauskas
Institute of Paper Science and Technology
Atlanta, GA 30318

Tom Elder
Auburn University
Auburn, Alabama 36849

ABSTRACT

The advantages of chemical bleaching agents are well established and include many factors such as ease of use, reliability, acceptable selectivity, and bleaching costs. Given these considerations, the successful implementation of a biobleaching stage in a modern kraft mill must meet several criteria including cost, dependability, and pulp performance properties. Research studies into the biobleaching of kraft pulps promise new bleaching technologies that could reduce capital expenditures and yield bleached products with superior physical pulp properties and negligible impact on the environment.

Over the past several years, the utilization of laccase-mediator systems (LMS) for delignifying kraft pulps has steadily improved in terms of delignification capabilities, enzyme and mediator dosages, and bleached pulp properties. Our studies have been primarily directed at understanding the fundamental chemical processes involved in this biodelignification process. A better understanding of the enzymatic and chemical mechanisms involved in the LMS system will improve the overall biobleaching effect and hasten its application in commercial bleaching operations. This paper reports on improving the LMS system using violuric acid (VA) or *N*-acetyl-*N*-phenyl-hydroxylamine (NHA) as mediators. The bleaching properties of LMS were investigated in terms of brightness, lignin content, and pulp viscosity.

INTRODUCTION

Bleached kraft pulp production is a dominant global industry that contributes 50% of the total world wood pulp production. Over the past decade, this part of the fiber line has been dramatically changed to improve the overall environmental performance of bleached kraft pulp operations. As a result, the demand for elemental chlorine has substantially decreased and the consumption of chlorine dioxide, hydrogen peroxide, oxygen, and ozone has increased. With the introduction of these new bleaching technologies, the research focus for future bleaching opportunities has begun to change. A research issue that is taking on added importance revolves around the need to reduce capital expenditures associated with bleaching kraft pulps. As discussed by several industry leaders in the recent TAPPI Journal Industry Leaders Interview Series (1,2,3), the production of wood pulp is not providing sufficient return on equity for the amounts of capital required to produce bleached kraft pulp. Furthermore, there is a growing belief

that this issue will not be addressed by the incremental research studies of the past. New pulping, bleaching, and papermaking technologies need to be developed that can substantially reduce the capital requirements of the pulp mill of the future. Certainly, under this vision, alternative technologies such as biopulping and biobleaching of kraft pulps appear attractive. The temperature, pH, and pressure requirements of many oxidoreductases, coupled with their catalytic properties, suggest that biopulping/bleaching systems with low-capital requirements and low operating costs could be developed for the pulp mill of the future.

Several research groups are actively investigating biobleaching systems that could be employed to remove lignin from kraft pulps. Hemicellulase-aided bleaching is the most well-established, economically feasible biotechnical application in the pulp and paper industry. The enzymatic treatments, based on xylanases (4) and mannanases (5), introduce modifications in the carbohydrate structures, leading to enhanced delignification in subsequent chemical bleaching operations. The mechanism is based on the partial depolymerization of hemicelluloses, which impede the chemical removal of residual lignin from pulp fibers. The benefits obtained by the enzymatic treatment depend on the type of raw material, pulping process, and bleaching sequence. The enzymatic treatment leads to reductions in chemical consumption, maintains product quality, and has been commercially implemented.

Several other promising enzymatic delignification technologies are under active investigation including, the use of laccase-mediator systems (6), manganese peroxidase (7), and EPOC (i.e., mixture of lipase/ketone/fatty acid/H_2O_2) (8). Mn-peroxidase is secreted by white-rot fungi and participates in the degradation of lignin (9). Ehara et al. have shown that this treatment reduced the total effective chlorine charge required to obtain a pulp of 85% brightness in a (CD)ED sequence by 51, 66, and 69% for normal- and low-lignin-content softwood pulps and hardwood pulps (10). Call (5) has presented data indicating that the EPOC system can delignify commercial kraft pulps by 40% under mild conditions and short retention times. Laccase-mediator bleaching technology has advanced significantly over the past decade. Since the initial reports highlighting the need of employing a mediator with laccase to achieve efficient delignification, significant improvements in this technology have come forth. Advances in mediator design (11,12), fundamental LMS chemistry (13,14), and pulp bleachability have demonstrated that (LMS)E treatment of conventional HW and SW kraft pulps can achieve 20-55% delignification (15).

The application of LMS technology toward high kappa kraft pulps is another promising opportunity. Studies by Parthasarathy (16), Bokstrom et al.(17), and others have shown that by halting the kraft cook prior to reaching the residual phase, pulp yield can be improved. For example, Magnotta, et al.(18) reported that halting a SW kraft cook at kappa 44 and then performing a OO-stage followed by D(E+O+P)D provides a 3.8% yield increase over OD(E+O+P)D for a 30-kappa SW kraft pulp. By extension, an LMS-stage could potentially replace an O or OO-stage for high kappa kraft pulps providing improved delignification properties over a typical O-stage. Our initial studies in this field have certainly demonstrated that LMS treatments can remove 16-38% of the lignin from

SW kraft pulps ranging in kappa numbers from 70 to 90 (19,20). Interestingly, we have also demonstrated that a double LMS treatment can extend the biodelignification effect, removing 53% of the lignin from a 73-kappa SW kraft pulp via (LMS)(LMS)(E). In addition, the overall performance of an LMS(E) can be significantly improved by oxidatively reinforcing the E-stage with hydrogen peroxide and/or oxygen (21,22). This effect is due, in part, to the in-situ formation of quinones (23) during the LMS stage, which can then be oxidatively removed with alkaline peroxide. This manuscript further explores the biodelignification effects using a new generation (12) of N-hydroxy-based LMS mediators with high-lignin content SW and HW kraft pulps. The effects of biodelignification are explored in terms of % delignification, yield, and fundamental LMS bleaching chemistry.

EXPERIMENTAL

Materials

All materials employed in this study were purchased from Aldrich Chemicals, Milwaukee, WI, and used as received except for *N*-acetyl-*N*-phenylhydroxylamine (NHA) and laccase. NHA was synthesized in accordance with Oxley's method (24) and laccase from *Trametes villosa* was donated by Novo Nordisk Biochem.

Furnish

Table 1 provides a brief description of the pulps employed in this study. In brief, the LMS_{NHA} and LMS_{VA} treatments were performed on a series of high-lignin-content laboratory-prepared conventional batch southern softwood and hardwood kraft pulps. In addition, two commercial hardwood and softwood kraft pulps were employed with the LMS_{VA} system.

Table 1. Description of Kraft Pulps Employed

Pulp	Kappa #	Viscosity/cP
SW-lab prepared	73.4	-
SW-lab prepared	50.0	45.0
SW-commercial	27.5	24.2
HW-lab prepared	26.9	67.6
HW-commercial	10.8	21.6

Laccase Assay

Laccase activity was measured by monitoring the rate of oxidation of syringaldazine. One unit of activity (U) was defined as the change in absorbance at 530 nm of 0.001 per minute, per ml of enzyme solution, in a 100 mM phosphate buffer (2.2ml) and 0.216 mM syringaldazine in methanol (0.3ml). The procedure was carried out at 23°C. The activity of the enzyme was 1.87 E+06 (U/ml of enzyme solution).

Laccase-mediator treatment procedure

All LMS experiments were carried out using a 1000-ml-capacity Parr reactor equipped with a pressure gauge, a stirrer, and a temperature controller. The pulp (20g o.d. basis) was placed in the reactor and the consistency was adjusted to 5% using distilled water.

The slurry was then heated to 45°C while mixing and was maintained at that temperature throughout the incubation time. The appropriate dose of mediator was added and further mixing (approx. 5 min) was allowed. The pH was then adjusted to 4.5 and the appropriate dose of laccase was added. The reactor was sealed and pressurized with oxygen (10 bar). Subsequent to the treatment, the pulp was removed from the reactor and thoroughly washed with distilled water. The treated pulp was subjected to an alkaline extraction stage and then characterized.

Pulp Characterization

The delignification response of the LMS_{NHA} (E) treatments was expressed as kappa number. Kappa numbers were determined in accordance with TAPPI Standard Method T-236. Each reported kappa number represents the average of two individual measurements. Typically, the kappa number of duplicates varied by +/- 0.3 kappa units.

Experimental Design

A two-phase central composite design (CCD) was carried out in this study. A total of 33 LMS_{NHA}(E) treatments were performed at various times, doses of NHA, and laccase. The data obtained from Phases I and II were then subjected to conventional multiple linear regression analysis. Regression models from each phase were constructed from a set of variables consisting of the original variables, together with their squares and pairwise cross products. The regression models contained only those terms that were justified by a significance test at a 95% confidence level (t-test) and that yielded the highest value of the multiple correlation coefficient (R^2). In addition, the lack of fit (LOF) for the two regression models was calculated and was determined to be insignificant at the 95% confidence level.

RESULTS AND DISCUSSION

The delignification response of LMS treatments is critically dependent on the properties of the mediator. Although a host of mediators have been proposed for LMS delignification, some of the most effective mediators are N-hydroxy-based structures (see Figure 1).

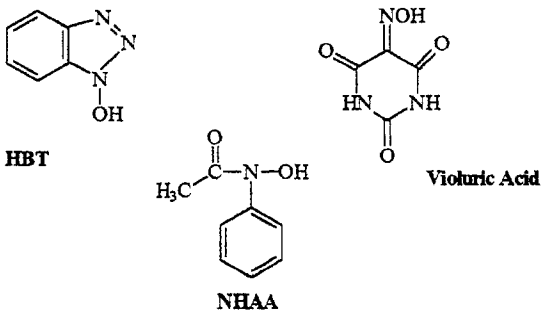

Figure 1. N-hydroxy-based laccase mediators.

The first part of this investigation dealt with improving the LMS conditions using NHA as the mediator. This task was accomplished by carrying out a two-phase central composite design. In Phase I, 20 enzymatic treatments were performed at various times doses of NHA, and laccase. In Phase II, 13 treatments were carried out at various doses of NHA and laccase and at a constant time of 2 hours. Phases I and II, along with the kappa results, are summarized in Tables 2 and 4 respectively. In order to simplify the regression analysis, the original variables (i.e. time, % NHA, and dose of laccase) were nondimensionalized and coded. Tables 3 and 5 define the relationships for the coded variables for Phases I and II respectively.

Table 2. LMS(E)[1] experimental conditions and data for Phase I

Experiment #	**Time, h**	**% NHA, o.d. pulp**	**Laccase solution, ml/10 g o.d. pulp**	**Kappa number**
Starting pulp	-	-	-	73.4
1	2.0	1.0	1.0	67.2
2	2.0	1.0	9.0	68.1
3	2.0	4.0	9.0	67.2
4	2.0	4.0	1.0	63.7
5	6.0	4.0	9.0	67.5
6	6.0	1.0	1.0	67.1
7	6.0	4.0	1.0	63.0
8	6.0	1.0	9.0	68.4
9	4.0	2.0	3.0	66.7
10	4.0	2.0	3.0	66.0
11	4.0	2.0	3.0	66.4
12	4.0	2.0	3.0	66.9
13	4.0	2.0	3.0	66.6
14	4.0	2.0	3.0	66.0
15	7.4	2.0	3.0	66.0
16	4.0	6.4	3.0	63.8
17	4.0	2.0	19.0	69.2
18	0.6	2.0	3.0	67.1
19	4.0	0.6	3.0	68.5
20	4.0	2.0	0.5	65.3

[1]E stages were performed with 2.5% NaOH, for 1 h, at 80° C and 10% csc.

Table 3. Definition of the relationships for the coded variables for Phase I

Original Variable	Coded Variable	Coding Relationship	Sample Values
Time, h	T	T= (time-4)/2	-1 when Time = 2.00; 0 when Time= 4.00; +1 when Time = 6.00; -1.682 when Time = 0.64; +1.682 when Time = 7.40
NHA, %	M	LogM= 0.301NHA+0.301	-1 when NHA = 1.00, 0 when NHA= 2.00; +1 when NHA = 4.00; -1.682 when NHA = 0.62 +1.682 when NHA = 6.40
Laccase, ml	L	Log L= 0.477Laccase+0.477	-1 when Laccase = 1.00; 0 when Laccase = 3.00 +1 when Laccase = 9.00; -1.682 when Laccase = 0.47; +1.682 when Laccase =19.00

The final regression model obtained for Phase I is as follows:

$$\text{Kappa} = 66.33 - 1.26\,M + 1.23\,L + 0.74\,M*L + 0.28\,L^2$$

The R^2 value obtained is 0.960 and the standard deviation is 0.37. The variable time was not included in the regression model since this term was not statistically significant (at the 95% confidence level). In turn, this highlights the efficiency of an LMS in that most of the delignification takes place in a relatively short time. Figure 2 demonstrates that there is good agreement between the observed and predicted values.

The best delignification response was observed at a 4% dose of NHA and at 1 ml of laccase/10g of o.d pulp, resulting in 9.8 kappa units with respect to the brownstock kappa. Although the results were promising, we believed that we could further improve the delignification, and thus we proceeded to carry out Phase II of the experimental design. Table 4 summarizes our results from Phase II.

The final regression model for Phase II is as follows:

$$\text{Kappa} = 63.55 - 1.86\,M + 0.91\,L + 0.37\,M*L + 0.69\,M^2$$

The R^2 value obtained is 0.984 and the standard deviation is 0.28. As in Phase I, there was a good agreement between the predicted and observed kappa numbers (see Figure 3)

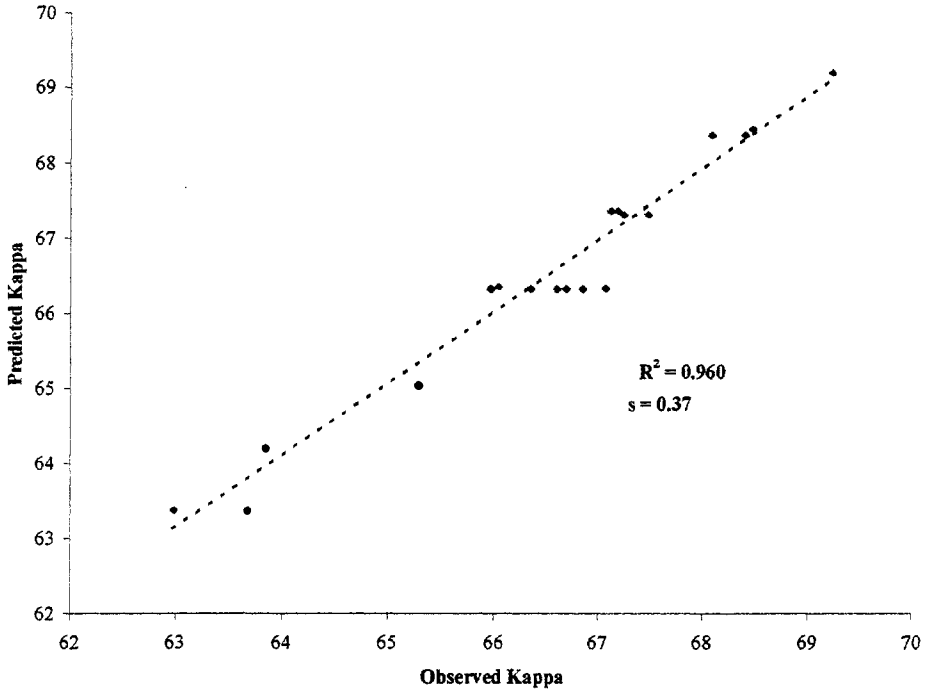

Figure 2. Predicted kappa by the regression mode vs. the observed kappa for Phase I

Table 4. Experimental conditions and data for Phase II

Experiment #	**Time, h**	**% NHA, o.d. pulp**	**Enzyme Solution, ml/10 g o.d. pulp**	**Kappa number**
Starting pulp	-	-	-	73.4
1	2.0	2.00	0.50	65.3
2	2.0	2.00	1.50	66.5
3	2.0	6.82	1.00	62.2
4	2.0	4.00	1.00	63.8
5	2.0	4.00	1.0	63.7
6	2.0	1.17	1.00	67.8
7	2.0	4.00	1.70	64.9
8	2.0	4.00	0.29	62.5
9	2.0	6.00	0.50	61.1
10	2.0	4.00	1.00	63.6
11	2.0	6.00	1.50	63.8
12	2.0	4.00	1.00	63.4
13	2.0	4.00	1.00	63.0

Table 5. Definition of the relationships for the coded variables for Phase II

Original Variable	Coded Variable	Coding Relationship	Sample Values
NHA, %	M	M= (NHA-4)/2	-1 when NHA = 2.00, 0 when NHA= 4.00; +1 when NHA = 6.00; -1.414 when NHA = 1.17 +1.414 when NHA = 6.83
Laccase, ml	L	L= (Laccase-1)/0.5	-1 when Laccase = 0.50; 0 when Laccase = 1.00 +1 when Laccase = 1.50; -1.414 when Laccase = 0.29; +1.414 when Laccase = 1.71

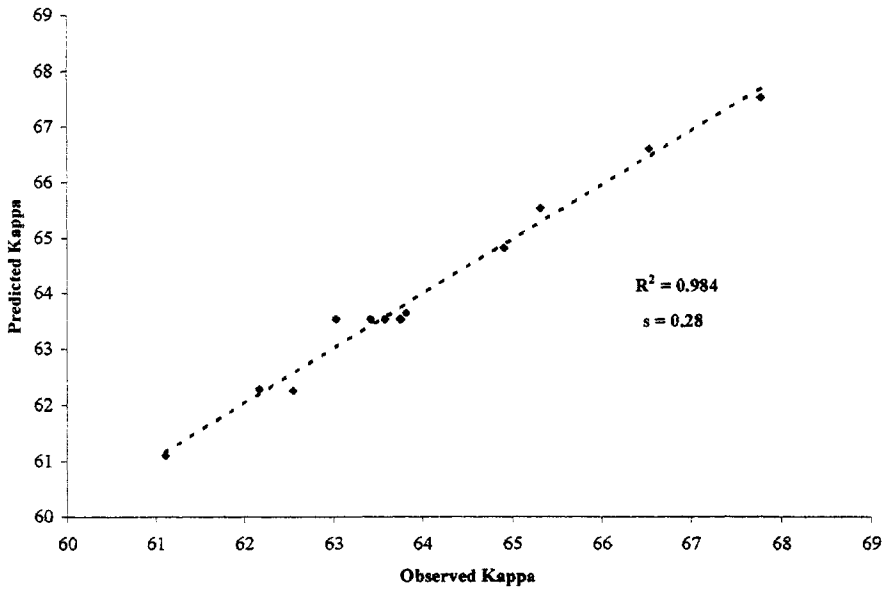

Figure 3. Predicted kappa by the model vs. observed kappa for Phase II.

Based on our experimental conditions in Phase II, the best delignification response was observed at a dose of 6% of NHA and 0.50 ml of enzyme solution. These conditions enabled us to reduce the kappa number by additional 2.50 units using 50% less of enzyme solution. The dose of mediator had to be increased from 4% to 6%. In turn, this suggests that an LMS_{NHA} treatment is not truly catalytic. Freudenreich et al.(25) have recently shown that NHA can be reduced to a nondelignifying agent, and that may explain in part, the need for higher doses of NHA.

Although we anticipated that the delignification properties of LMS_{NHA} could be further improved, preliminary concurrent investigative studies employing violuric acid (VA) as a LMS mediator indicated that this agent exhibited superior biodelignification properties. Employing identical LMS conditions and the same pulp as in Phase II (i.e. 0.5ml/10-g o.d. pulp; 6% molar equivalence of NHA), an $LMS_{VA}(E)$ treatment was found to substantially outperform the LMS_{NHA} system. Indeed, we were able to reduce the pulp kappa number by 34.0 kappa units. In addition, we found this trend holds true regardless of the LMS conditions and the starting kappa number. For example, an $LMS_{VA}(E)$ treatment (4% molar equivalence of NHA on o.d pulp; 5% consistency; 3ml of laccase solution/10 g of o.d. pulp; 45°C; 2 hr. Extraction: 2.5% NaOH on o.d. pulp; 80°C; 2 hr.) on a 50-kappa SW kraft pulp led to a drop of 17 kappa units. When the identical treatment was carried out using NHA, we observed a total drop of 7.2 kappa units.

A notable observation of these studies is the dramatic differences in delignification efficiency despite the fact that both VA and NHA are N-hydroxy-based mediators. Following these investigations, we further explored the response of the $LMS_{VA}(E)$ system to laccase charge. These results are shown in Figure 4. In general, the behavior of an LMS_{VA} treatment was consistent with the trends observed with the LMS_{NHA} system.

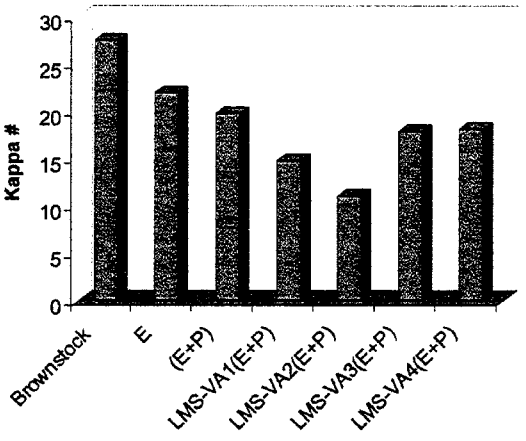

Figure 4. Delignification of 27.5 kappa SW kraft pulp with $LMS_{VA}(E^*)$[1]

[1]: VA1 used 1.87 E+04 U of laccase, VA2 used 5.61 E+06 U laccase, VA3 used 11.22 E+06 U of laccase, VA4 used 22.4 E+06 U of laccase. Charge of VA was 4%, E-stage was at 80°C, 10% csc, 2.5% NaOH, for 1.5 h. (E+P) stages were performed in a similar manner except 0.5% H_2O_2 was added.

Our interest in LMS treatments is based not solely on its delignification response but also on its low reactivity with cellulose. Indeed, treatment of an LMS system with a fully bleached pulp was shown by Haynes (26) to have no impact on pulp viscosity, suggesting that the LMS system is highly specific for lignin. This high bleaching specificity should provide very high pulp bleaching yields. To test this hypothesis, a series of HW and SW kraft pulps were treated to an $LMS_{VA}(E+P)$ sequence and gravimetric yields were determined. The results shown in Table 6 demonstrate the improved selectivity and yield benefits that can be achieved from an $LMS_{VA}(E+P)$ stage for both high- and low-kappa kraft pulps.

Hexenuronic acid analysis of the two HW kraft pulps was performed in accordance with a modified procedure established by Vuorinen et al. (27). The analysis indicated that the contribution of these groups to the initial kappa number was 18.2% (initial kappa no. 26.9) and 24.0% (initial kappa no. 10.8), respectively. It is known that an LMS treatment does not oxidatively remove hexenuronic acid groups (28). Hence, further substantial delignification could be accomplished after an LMS_{VA}(E+P) treatment if the two HW kraft pulps had been treated with a mild acid stage.

Table 6. Yield and delignification response after treating high- and low-kappa SW and HW kraft pulps with LMS_{VA}(E+P)[1].

Pulp	SW 50.0 kappa 45.0 cP	SW 27.5 kappa 24.2 cP	HW 26.9 kappa 67.6 cP	HW 10.8 kappa 21.6 cP
% Delign.	42.6	61.1	65.4	70.4
% Viscosity Loss	25.8	20.4	45.9	22.6
Yield[2]	99.9	100.0	99.4	99.1

[1](E+P) stage was for 1.5 h, 80°C, 10% csc, 0.5% H_2O_2, 2.5% NaOH; [2]gravemetric yield corrected for loss of lignin (i.e., mass lignin lost = 0.15% (initial – final kappa #).

The pulp bleachability of the LMS_{VA}-treated pulps was examined using a DED sequence. These results are summarized in Figure 5 and Table 7.

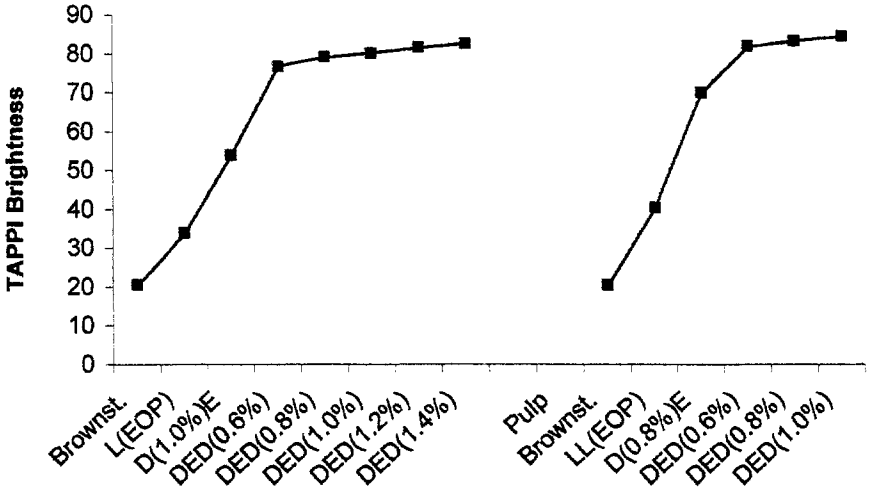

Figure 5. Changes in TAPPI brightness bleaching SW kraft pulp (kappa 30) with LMS_{VA}(E+P+O)DED and $LMS_{VA}LMS_{VA}$(E+P+O)DED.

Table 7. Bleaching conditions employed for LMS_{VA}(E+P+O)DED and $LMS_{VA}LMS_{VA}$(E+P+O)DED.

LMS_{VA}	(E+P+O)	D
2% charge VA. See experimental section for remaining details.	70°C, 10% csc, 1 h, 0.05% $MgSO_4$, 2.5% NaOH, 15 min 60 psi, 12 psi vented every 5 min.	D_0, 10% csc, 50°C, 45 min. D_1 70°C, 3 h, 10% csc See Fig 6 for ClO_2 charges in brackets

These results indicated that the LMS-treated pulps can achieve +80 TAPPI brightness values employing ECF bleaching technologies.

CONCLUSIONS

The results of this study yield a series of conclusions. First, high-kappa pulps respond favorably to both LMS_{NHA} and LMS_{VA}, although the latter mediator exhibits a superior biodelignification response. For the LMS_{NHA} system studied, the reaction is complete within 36 minutes, and high doses of laccase do not provide improved delignification. In contrast, higher doses of mediator provide improved lignin removal. These experimental trends appear to also hold true with the LMS_{VA} system. The high selectivity of the LMS_{VA} system provides exceptionally high pulp yields after alkaline extraction. Finally, the LMS-treated pulps are amendable to ECF bleaching conditions providing +80 brightness pulps. Despite the significant delignification that is achievable with NHA and VA on high- and low-kappa pulps, these mediators cannot be considered ideal. Hence, this creates once again an opportunity for finding other mediators compatible with laccase.

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