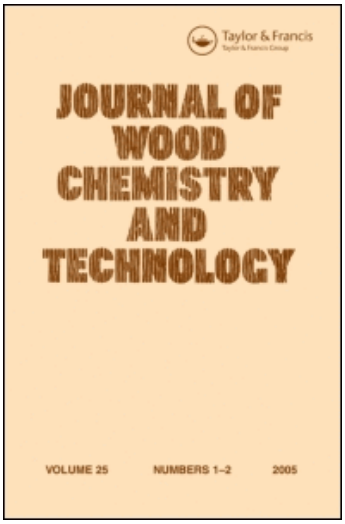

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

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To cite this Article Chakar, F. S. and Ragauskas, A. J.(2000) 'The Effects of Oxidative Alkaline Extraction Stages After Laccase$_{HBT}$ and Laccase$_{NHAA}$ Treatments-An NMR Study of Residual Lignins', Journal of Wood Chemistry and Technology, 20: 2, 169 — 184
To link to this Article: DOI: 10.1080/02773810009349631
URL: http://dx.doi.org/10.1080/02773810009349631

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 20(2), 169–184 (2000)

THE EFFECTS OF OXIDATIVE ALKALINE EXTRACTION STAGES AFTER LACCASE$_{HBT}$ AND LACCASE$_{NHAA}$ TREATMENTS-AN NMR STUDY OF RESIDUAL LIGNINS

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ABSTRACT

Two laccase-mediator systems (LMS) were performed with 1-hydroxybenzotriazole (HBT) and N-acetyl-N-phenylhydroxylamine (NHAA), as the mediators, on a southern softwood conventional kraft pulp with an initial kappa of 33.8. The LMS$_{HBT}$ and LMS$_{NHAA}$ treated pulps were then subjected to various reinforced alkaline extraction stages with oxygen (E+O), peroxide (E+P), and peroxide/oxygen (E+P+O). The kappa data suggested that both LMS$_{HBT}$ and LMS$_{NHAA}$ are effective at delignifying kraft pulps. However, under the conditions employed in this study, a greater level of delignification was obtained with an LMS$_{HBT}$ stage than with an LMS$_{NHAA}$ stage. The viscosity measurements confirmed the selectivity of LMS. Oxidative reinforcement of the alkaline treatments was beneficial for regaining the loss in brightness from LMS when the mediator was either NHAA or HBT. ^{31}P NMR spectral analysis of phosphitylated residual lignins revealed a greater enrichment of carboxylic acid groups after an LMS$_{HBT}$ than after an LMS$_{NHAA}$ stage. Depletion of free phenolic groups was evident after either an LMS$_{HBT}$ or an LMS$_{NHAA}$ treatment. However, it was greater after an LMS$_{NHAA}$ stage.

INTRODUCTION

In response to environmental regulations, research efforts in pulp and paper have been directed toward finding novel delignification and bleaching technologies with minimal impact on the environment. Currently, alternative bleaching technologies to chlorine or to hypochlorous acid include hydrogen peroxide, oxygen, chlorine dioxide, and ozone.[1] Although these chemical bleaching agents have been successfully implemented in mills around the world, the constant search for other bleaching methods is still very active. The potential for using enzymatic treatments was realized in the mid 80s, when it was discovered that xylanase pretreatments of pulps prior to their subsequent bleaching with chlorine, chlorine dioxide, and hydrogen peroxide could yield substantial savings in bleaching chemicals.[2] More recently, the use of lignin-degrading enzymes, such as laccase, has been shown to be more effective than xylanases.

Historically, the use of laccase for bleaching kraft pulps had limited acceptance due to the minimal delignification that could be achieved. This inefficiency was attributed to the size of the enzyme and, therefore, to its inability to diffuse into the pulp fibers to catalyze the oxidation of lignin.[3] Fortunately, this problem was circumvented when Bourbonnais and Paice discovered that laccase in the presence of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) could delignify kraft pulps.[4,5] The introduction of HBT by Call,[6,7] which followed that of ABTS, demonstrated that much higher levels of delignification could be achieved with HBT than with ABTS. Since then, various research groups have been focusing their attention on laccase-mediator systems.[8-12] More recently, mediators such as violuric acid, NHAA, and others have been discovered.[13,14]

Despite these advancements in biobleaching systems, much remains to be discovered and learned. Further understanding of the fundamental chemistry of LMS delignification is, therefore, of paramount importance. This paper examines the effects of LMS_{NHAA}, LMS_{HBT} and of subsequent oxidatively reinforced alkali extraction stages on the structure of residual lignin.

EXPERIMENTAL

Materials

All materials were purchased from Aldrich Chemical Co., Milwaukee, WI, and used as received, except for *p*-dioxane, NHAA, and laccase. *p*-Dioxane was freshly distilled over $NaBH_4$ prior to using it for the lignin isolation experiments. NHAA was synthesized in accordance with Oxley's method.[15] Laccase, from *Trametes villosa*, was donated by Novo Nordisk Biochem. The conventional southern softwood kraft pulp was prepared at Potlatch Corp. facilities in Cloquet, MN. The wood source originated from *Pinus taeda* and was acquired from Union Camp. The wood was approximately 25 years of age, void of visual disease and of compression wood. The chips were cooked to an H-factor of 1390 using 19.5 % active alkali and a 4:1 liquor:wood ratio. The pulp was thoroughly washed, screened, centrifuged, fluffed, and stored at 4 °C prior to LMS bleaching treatments.

Enzyme 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 min per mL of enzyme solution, in a 100 mM phosphate buffer (2.2 mL) and 0.216 mM syringaldazine in methanol (0.3 mL). The procedure was carried out at 23°C. The activity of the laccase used in this study was 1.87E+06 (U/mL of enzyme solution).

Laccase-Mediator Delignification Procedure

A 1000-mL capacity Parr reactor equipped with a stirrer, a pressure gauge,

a heating mantle, and connected to a temperature controller was charged with 15 g of never-dried fibers (solid basis). The pulp consistency was adjusted to 9% by adding distilled water. The slurry was then heated to a temperature of 45°C and was maintained at this temperature throughout the incubation period. HBT (2 x 10^{-3} moles) was then added (or 2.2 x 10^{-3} moles of NHAA when NHAA was used) to the heated slurry. Subsequent to mixing the slurry (ca. 5 min), the pH was adjusted to 4.5 with glacial acetic acid. Laccase was then added (372,000 U per gram of o.d. pulp) and the reactor was sealed and pressurized with oxygen to 145 psig. Subsequent to the four-hour treatment, the pulp was thoroughly washed and subjected to various reinforced alkaline stages (E*). All E* stages were performed for one hour at 80°C. These stages are summarized in Table 1. Kappa, brightness, and viscosity measurements were performed on the extracted pulps in accordance with TAPPI methods T236, T452, and T230, respectively.[16]

Control experiments were also conducted on the brownstock in the absence of laccase and mediator to evaluate the effect of E* treatments. The conditions for the E, E+O, E+P, and E+P+O stages are summarized in Table 1.

Laccase-Mediator Procedure for Lignin Isolation Purposes

In order to isolate the residual lignin, larger batches of LMS treated pulps were needed. In order to accomplish this task, a 2000-mL instead of the 1000-mL capacity Parr reactor was employed. In this case, the reactor was charged with 60 g of never-dried fibers (solid basis). The experimental protocol for the larger batches was identical to the one described above, except 8.9 x 10^{-3} moles of HBT and NHAA were added instead of 2.2 x 10^{-3} moles.

Isolation of Residual Lignins

The isolation of residual lignins was carried out in accordance with standard literature methods.[17] A 5000-mL three-necked round bottom flask

TABLE 1

Summary of Extraction Stage Conditions[1,2]

Extraction Stage (E*)	%NaOH (o.d. basis)	$\%H_2O_2$ (o.d. basis)	O_2 (psig)
E	2.5	-	-
E+O	2.5	-	60
E+P	2.5	0.5	-
E+P+O	2.5	0.5	60

[1] All E* were applied to LMS_{HBT} and LMS_{NHAA} treated pulps.

[2] Similarly, All E* conditions were applied on the kraft softwood brownstock (initial kappa # = 33.8). These experiments served as controls.

equipped with a Friedrichs condenser was charged with 50 g of o.d. pulp (air-dried). The consistency of the pulp was adjusted to 4% by adding a 0.10N (HCl) 9:1 *p*-dioxane:water solution. The slurry was then refluxed for 2 hr under an argon atmosphere. Subsequent to the treatment, the pulp was filtered and the filtrate was passed through celite to remove any fines. The filtrate was then neutralized and concentrated under reduced pressure to approximately 10% of the original volume. Water (ca. 400 mL) was added and the mixture was concentrated again under reduced pressure to remove the last traces of *p*-dioxane. The solution's pH was then adjusted to 2.5 with 1.00 N HCl. The precipitate (i.e., the lignin) was collected, washed several times, and freeze-dried. Lignin yields ranged from 45.4 to 48.3%.

Characterization of Residual Lignins

The residual lignins isolated from the brownstock (kappa # 33.8) and from LMS_{HBT} (E), LMS_{HBT} (E+P+O), LMS_{NHAA} (E), and LMS_{NHAA} (E+P+O) treated

pulps were phosphitylated and characterized by ^{31}P NMR in accordance with established literature methods.[18, 19] NMR data was acquired with a DMX400 MHz Bruker spectrometer.

NMR Error Analysis

The NMR error analysis was performed by repeating the isolation of the brownstock residual lignin three times under identical conditions and comparing the results. The isolated lignin samples were then phosphitylated and analyzed by ^{31}P NMR, as described above. A least significant difference (LSD) value at a 95% confidence interval was calculated by using the standard deviations along with the Student-t value. The LSD values for the functional groups acquired by ^{31}P NMR are illustrated in Table 2.

RESULTS AND DISCUSSION

The purpose of this study was to compare the delignification efficiency of an LMS_{HBT} and LMS_{NHAA} treatment. This was accomplished by using the same molar equivalence of mediators for all LMS_{HBT} and LMS_{NHAA} treatments (note: all other experimental conditions such as dose of enzyme, temperature, time, O_2 pressure, and pH were held constant). These experimental conditions were selected so that the difference in biobleaching could be attributed to the mediators. In addition, the effects of reinforcing the subsequent alkaline extraction stages to the laccase treatments with oxygen and peroxide were examined. These changes in biobleaching were studied by measuring the physical and optical properties of the pulps, and the structural changes of the residual lignins. HBT was chosen as the reference mediator since it is probably one of the most studied mediators currently available for kraft pulps.

TABLE 2

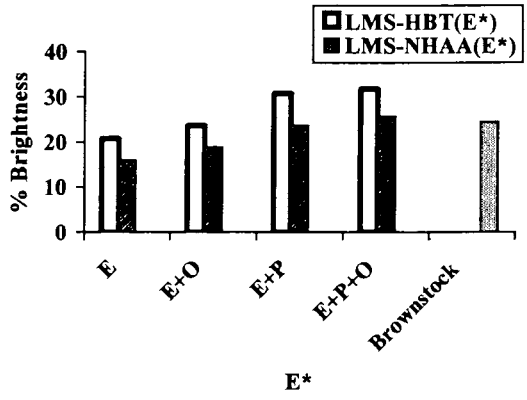
^{31}P NMR Least Significant Difference Values

Functional group	Average (mmol/g lignin)	St. dev.	LSD
Carboxyl OH	0.23	0.003	0.013
Guaiacyl OH	1.02	0.025	0.095
Condensed OH	0.82	0.016	0.060
Aliphatic OH	1.40	0.019	0.069

All LMS treatments were performed on a laboratory-prepared southern softwood conventional kraft pulp with an initial kappa of 33.8. The LMS_{HBT} and LMS_{NHAA} pulps were then subjected to various reinforced alkaline extraction stages (see Table 1), and the extent of delignification, brightness, and viscosity were measured after LMS (E*) treatments. The residual lignins were then isolated from the brownstock and from LMS_{HBT} (E), LMS_{HBT} (E+P+O), LMS_{NHAA} (E) and LMS_{NHAA} (E+P+O) treated pulps and characterized via ^{31}P NMR.

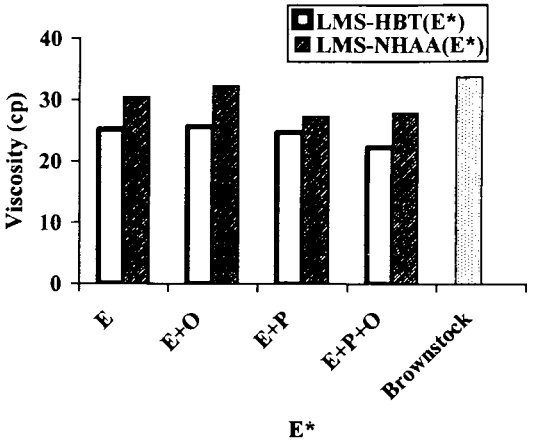
Physical and Optical Properties of LMS Treated Pulps

The changes in TAPPI brightness and viscosity results are depicted in Figures 1 and 2, respectively. Oxidative reinforcement of the alkaline extraction stage was beneficial after either an LMS_{HBT} or an LMS_{NHAA} treatment. These results are in agreement with the trends seen by Paice *et al.* when they incubated a softwood kraft pulp with LMS_{ABTS} and subsequently treated it with a QP stage.[20] The largest increases in brightness were observed after an (E+P) and an (E+P+O) stage. Interestingly, relative to the brownstock (BS), an LMS_{NHAA} (E) treatment suffered a greater loss in brightness than an LMS_{HBT} (E) treatment. This greater loss in brightness may be attributed to a higher content of quinone-type structures.



N.B: Each data point represents the average of 5 brightness readings

FIGURE 1. TAPPI brightness after $LMS_{HBT}(E^*)$ and $LMS_{NHAA}(E^*)$ treatments on a softwood kraft pulp with starting kappa number of 33.8.



N.B.: Viscosity testing was performed in duplicates. Each data point represents the average of two measurements.

FIGURE 2. Viscosity after $LMS_{HBT}(E^*)$ and $LMS_{NHAA}(E^*)$ treatments on a softwood kraft pulp with starting kappa number of 33.8.

Viscosity measurements are in agreement with previously reported data [21, 22] and once again confirm the selectivity of a laccase-mediator system. Although both treatments were selective, the LMS_{NHAA} system exhibited a higher degree of selectivity than the LMS_{HBT} system. This trend was evident despite the type of reinforcement used in the extraction stage.

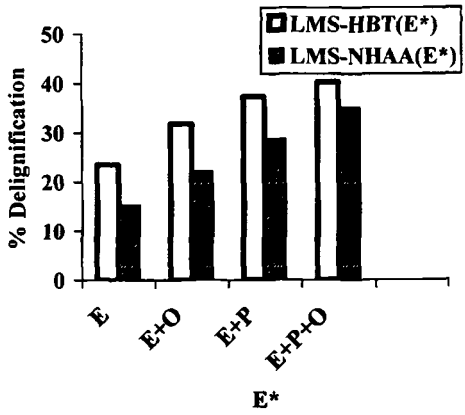
The delignification results shown in Figure 3 suggest that both an LMS_{HBT} and LMS_{NHAA} treatment yielded substantial levels of delignification. However, under the conditions used for this study, HBT was more effective than NHAA. The reinforcement of the alkaline stage further extended the level of delignification. Interestingly, reinforcement of the alkaline extraction stage with both peroxide and oxygen seemed to have narrowed the lignin content difference between the two LMS systems.

Control Experiments

Past literature results indicate that in order for an LMS system to yield significant levels of delignification, both the mediator and the laccase must be present.[20,23] In this study, we carried out a series of control experiments, which enabled us to discriminate between the effects of LMS and the oxidatively reinforced alkaline extraction stages on the brownstock. The alkaline extraction conditions for these control experiments are summarized in Table 1. The results, shown in Table 3, demonstrate that in all cases, an LMS stage before an E* improves delignification. The improved delignification effects were unfortunately accompanied by a decrease in pulp brightness and a slight decrease in viscosity.

^{31}P NMR

Based on the physical and optical properties, an LMS_{NHAA} and an LMS_{HBT} treatment followed by an (E+P+O) extraction stage yielded the highest brightness



N.B.: Kappa testing was performed in duplicates. The average of the two measurements was used to calculate the % delignification. % delignification was calculated in accordance with the following formula: % delig =[(kappa # of brownstock – kappa # after LMS (E*))/ kappa # of brownstock]*100

FIGURE 3. % Delignification after $LMS_{HBT}(E^*)$ and $LMS_{NHAA}(E^*)$ treatments on a softwood kraft pulp with starting kappa number of 33.8.

TABLE 3

Kappa, Tappi Brightness and Viscosity of Control Experiments, $LMS_{NHAA}(E^*)$ and $LMS_{HBT}(E^*)$ Treatments

Pulp	Kappa[1]	Brightness[2] (%)	Viscosity[3](cp)
Brownstock (BS)	33.8	24.5	33.7
BS(E)	31.7	25.6	33.5
BS(E+O)	28.9	26.4	28.8
BS(E+P)	27.4	30.2	24.6
BS(E+P+O)	25.1	31.9	22.5
$LMS_{HBT}(E)$	25.9	20.8	25.2
$LMS_{HBT}(E+O)$	23.1	23.6	25.6
$LMS_{HBT}(E+P)$	21.2	30.8	24.8
$LMS_{HBT}(E+O+P)$	19.9	31.7	22.3
$LMS_{NHAA}(E)$	28.6	15.9	30.4
$LMS_{NHAA}(E+O)$	26.3	18.9	32.2
$LMS_{NHAA}(E+P)$	24.1	23.6	27.3
$LMS_{NHAA}(E+P+O)$	22.0	25.6	27.9

[1] The pooled standard deviation of all kappa measurements was 0.1.

[2] The pooled standard deviation of all brightness measurements was 0.41.

[3] The pooled standard deviation of all viscosity measurements was 0.58.

and level of delignification, whereas enzymatic treatments followed by a simple E stage yielded the opposite. The next step was for us to further our understanding of LMS_{NHAA} and LMS_{HBT} by studying structural changes of the residual lignins.

^{31}P NMR was used to evaluate the structural changes in phosphitylated residual lignins isolated from the brownstock and after an LMS_{HBT} (E), LMS_{HBT} (E+P+O), LMS_{NHAA} (E) and LMS_{NHAA} (E+P+O) treatment. ^{31}P NMR is a facile and effective method for evaluating various types of hydroxyl groups such as those present in carboxyl, free phenolic, condensed phenolic and aliphatic lignin moieties.

It is clearly evident from the data shown in Figure 4 that relative to the brownstock lignin, the LMS_{HBT} (E) and LMS_{NHAA} (E) lignins were both enriched in carboxyl groups. However, this enrichment was more pronounced with HBT than with NHAA. As expected, reinforcement with peroxide and oxygen further increased the content of carboxylic acid of both LMS_{NHAA} and LMS_{HBT} residual lignins. However, this increase was more substantial when NHAA was used. We had earlier suggested that the greater loss in brightness after an LMS_{NHAA} treatment than after an LMS_{HBT} could be attributed to a greater content in quinone- type structures. If our speculation in regard to this matter is correct, then the greater increase in carboxylic acid content after the LMS_{NHAA} (E+P+O) treatment stage could be attributed to the well-known ring opening reactions of quinones to generate muconic acid-type structures.

Inspection of Figures 5 and 6 suggests a depletion of guaiacyl and condensed phenolic hydroxyl groups, and is consistent with trends seen by other researchers when HBT was employed.[23,24] This decrease was greater with NHAA than with HBT. In turn, this may suggest that the oxidative selectivity of LMS_{NHAA} toward phenolic lignin structures may be different than that of LMS_{HBT}. Reinforcement of the alkaline extraction stages seems to narrow the gap between the two systems.

The aliphatic lignin hydroxyl groups content, shown in Figure 7, also decreased relative to the brownstock. This decrease is consistent with recent

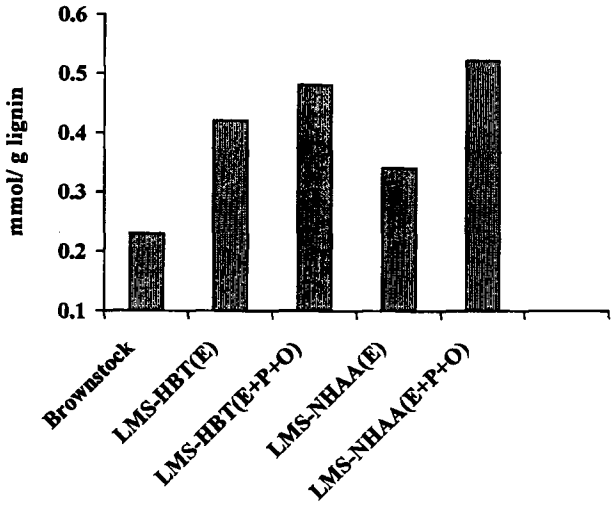


FIGURE 4. Carboxyl OH groups in residual lignins isolated after $LMS_{HBT}(E)$, (E+P+O), and $LMS_{NHAA}(E)$,(E+P+O) treatments.

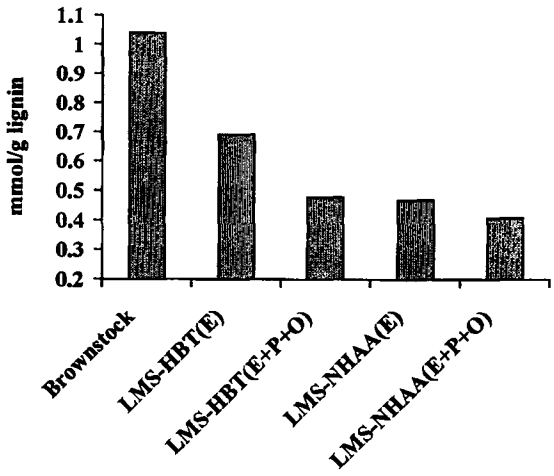


FIGURE 5. Guaiacyl OH groups in residual lignins isolated after $LMS_{HBT}(E)$, (E+O+P), and $LMS_{NHAA}(E)$,(E+P+O) treatments.

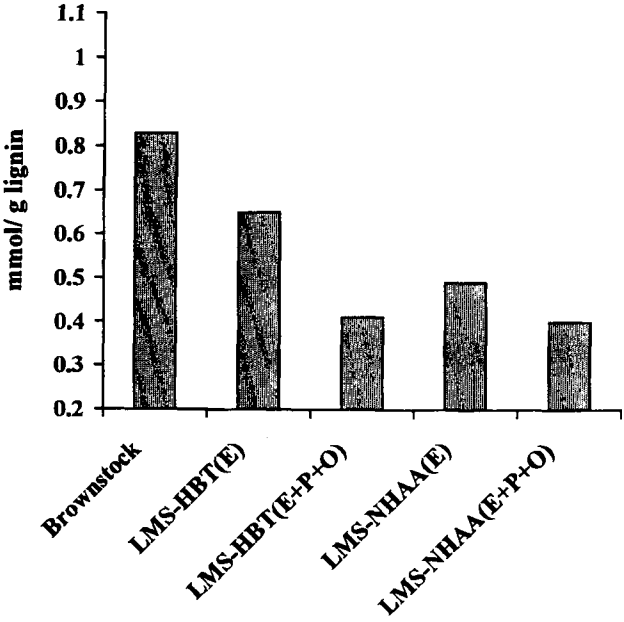


FIGURE 6. Condensed OH groups in residual lignins isolated after $LMS_{HBT}(E)$, (E+P+O), and $LMS_{NHAA}(E)$,(E+P+O) treatments.

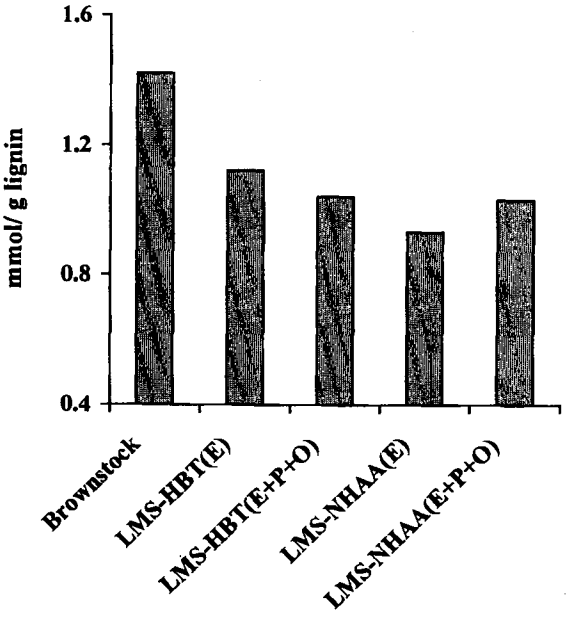


FIGURE 7. Aliphatic OH groups in residual lignins isolated after $LMS_{HBT}(E)$, (E+P+O), and $LMS_{NHAA}(E)$,(E+P+O) treatments.

observations of side chain oxidation and fragmentation of model compounds during LMS (E) treatments reported by Freudenreich *et al.*[25] and Li *et al.*[26] Reinforcement of the alkaline extraction stages with peroxide and oxygen did not further deplete these types of groups, as expected.

Overall, the structural analysis of the residual lignins suggests that the oxidative chemistry of an LMS_{NHAA} and LMS_{HBT} system is different. This is supported by the observed differences in the structure of the isolated residual lignins after the alkaline extractions. If the LMS bio-delignification chemistry was proceeding via the same pathway, when either mediator was used, then the residual lignins after an LMS_{NHAA}(E) and an LMS_{HBT}(E) would have had identical ^{31}PNMR spectra, and this was not the case. Obviously, the same rationale applies to the residual lignins isolated after an LMS_{NHAA}(E+P+O) and LMS_{HBT}(E+P+O) treatments.

CONCLUSIONS

In summary, these results confirmed the reported effectiveness of HBT and NHAA as mediators in LMS systems. Based on the conditions used in this study, we observed that HBT yielded higher levels of delignification than NHAA. The oxidatively reinforced alkaline extraction stages were shown to be very beneficial and seem to narrow the gaps between the two LMS systems.

Overall, the structural analysis of the residual lignins was consistent with the delignification properties of the pulp. The improvements in the LMS_{HBT} vs. LMS_{NHAA} systems were reflected in the NMR analysis of the lignin samples. For example, the residual lignin isolated after an LMS_{HBT}(E) treatment was enriched in lignin carboxylic acid moieties more so than after an LMS_{NHAA} (E) treatment. The increased delignification observed when using an E+P+O stage after the LMS treatment was accompanied with increased amounts of carboxylic acid groups in the residual lignin.

The spectral analysis of the residual lignin samples after $LMS_{NHAA}(E^*)$ and $LMS_{HBT}(E^*)$ treatments indicated that NHAA, as a mediator, has different selectivity than HBT. Studies into the effect of quinone-type structures on LMS systems are currently underway.

ACKNOWLEDGEMENTS

The authors would like to thank the Institute of Paper Science and Technology and its member companies for their support for these ongoing studies. The authors would also like to express their gratitude to Union Camp for supplying the *Pinus taeda* tree, Potlatch Corp. for pulping the chips, and Novo Nordisk Biochem for furnishing the laccase needed for these studies. Portions of this work were used by FSC as partial fulfillment of the requirements for the Ph.D. degree at the Institute of Paper Science and Technology.

REFERENCES

1. T. McDonough, Tappi J., 78, 55 (1995).
2. L. Viikari, M. Ranua, A. Kantelinen, et al., Proc. of the 3rd Intl. Conf. on the Biotech. Pulp and Paper Industry, 67 (1986).
3. L. Jurasek, J. Pulp and Paper Sci, 21, J274 (1995).
4. R. Bourbonnais, M. Paice, FEBBS Lett, 267 (1), 99 (1990).
5. R. Bourbonnais, M. Paice, Tappi Journal, 79(6), (1996).
6. H. Call, Multicomponent Bleaching System, Patent WO 94/29425 (1994).
7. H. Call, Tappi/Emerging Pulping and Bleaching Tech. Workshop, 15 (1995).

8. F.S. Archibald, R. Bourbonnais, L. Jurasek, M.G. Paice, I.D. Reid, J. of Biotech., 53, 215 (1997).

9. J. Sealey, A.J. Ragauskas, ISWPC, F1-1 (1997).

10. A. Potthast, T. Rosenau, H. Koch, K. Fischer, Holzforschung, 53, 175 (1999).

11. M. Balakshin, E. Capanema, C.-L. Chen, J. Gratzl, A. Kirkman, H. Gracz, ISWPC, I572 (1999).

12. T. Tamminen, B. Hortling, M. Ranua, E. Luonteri, A. Suurnakki, M. Tenkanen, J. Buchert, ISWPC, I 590 (1999).

13. M. Amman, ISWPC, F4-1, (1997).

14. P. Schneider, Patent WO 95/01426, Novo Nordisk, (1995).

15. P.W. Oxley, B.M. Adger, M.J. Sasse, M.A. Forth, Org. Synth., 67, 187 (1989).

16. TAPPI Test Methods. Tappi Press, Atlanta, GA, 1998-99.

17. P.M. Froass, A.J. Ragauskas, J. of Wood Chem. 16(4), 347 (1996).

18. A. Granata, D. Argyropoulos, J. of Agric. and Food Chem., 43, 1538 (1995).

19. P. Froass, A.J. Ragauskas, J. Jiang, Ind. Eng. and Chem. Research, 37, 3388 (1998).

20. M. Paice, R. Bourbonnais, I. D. Reid, Tappi J., 78 (9), 161 (1995).

21. J. Sealey, T.M. Runge, A.J. Ragauskas, Bio. Sci. Symposium, 339 (1997).

22. K. Poppius-Levlin, W. Wang, M. Ranua, M. Niku-Paavola, L. Viikari, Bio. Sci. Symposium, 329 (1997).

23. K. Poppius-Levlin, W. Wang, T. Tamminen, B. Hortling, L. Viikari, M. Niku-Paavola, ISWPC, F3-1 (1997).

24. J. Sealey, A.J. Ragauskas, Enzyme Microb. Technol., 23,422 (1998).

25. J. Freudenreich, M. Amman, E. Fritz-Langhals, J. Stohrer, IPC, 71 (1998).

26. K. Li, H. Bermeck, K.-E.L. Eriksson, Bio. Sci. Symposium, 349 (1997).