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LACCASE-LIGNIN REACTIONS

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ABSTRACT

Residual lignin samples isolated from a SW kraft pulp (kappa no. 71.4) were subjected to laccase and laccase-mediator treatments using HBT, NHA, and VA as mediators. Structural changes relative to the brownstock lignin were measured via ^{13}C NMR and ^{31}P NMR. ^{31}P NMR spectral analysis of residual lignins treated with laccase and with laccase in the presence of mediators revealed a depletion in phenolic lignin groups, non-condensed and condensed at C-5, as well as in aliphatic hydroxyl groups. ^{13}C NMR spectral data revealed a decrease in methoxy groups and a substantial increase in carboxylic acid groups.

INTRODUCTION

The pulp and paper industry continues steadily and successfully to displace traditional chlorine and hypochlorous acid bleaching methods with more environmentally compatible technologies such as ECF and TCF. Despite these remarkable advances, new challenges and opportunities are emerging. There is a renewed interest in improving pulping and bleaching yields of kraft pulps, since the accessibility to inexpensive fibers is expected to decrease in the long run. One promising method for improving overall pulp yields consists of halting a kraft cook at a relatively high kappa and then treating the pulp with a single or a double oxygen stage before it is bleached. This type of approach has been reported to improve the overall yield of bleached kraft pulps by 2-4% (1,2).

For the last few years, our research activities have been directed at examining laccase-mediator systems as alternatives to oxygen delignification for improving pulp yields (3,4). In the past, the use of laccase for delignifying kraft pulps was restricted due to the size of the enzyme and thus to its inefficacy in diffusing into pulp fibers to react with the lignin (5). This limitation was first overcome with the aid of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), a mediator (6). Since this initial discovery, research efforts into laccase-mediator systems (LMS) have intensified and remain very active on lignin model compounds and low-lignin-content kraft pulps (7-13). The application of LMS for delignifying high-kappa kraft pulps remains to a large extent understudied. Our initial studies on high-kappa kraft pulps demonstrated that LMS treatments using 1-hydroxybenzotriazole (HBT) as the mediator could efficiently delignify such furnishes (3). Further LMS studies using *N*-acetyl-*N*-phenylhydroxylamine (LMS_{NHA}), violuric acid (LMS_{VA}), and HBT (LMS_{HBT}) as mediators revealed that VA outperforms both HBT and NHA vis-à-vis delignification (14). Nonetheless, all three LMS systems lead to pulp darkening. Our studies have established that this effect is attributed, in part, to the formation of quinonoid-type structures during LMS (14). Structural analysis of phosphorylated residual lignins isolated subsequent to LMS treatments (with VA, NHA, or HBT) using a high-kappa SW kraft pulp revealed that all three mediators preferentially attack noncondensed at C-5 phenolic lignin structures. C-5-condensed phenolic lignin structures exhibited resistance towards an LMS_{HBT} and an LMS_{NHA} treatment, but less towards LMS_{VA} . A decrease in aliphatic hydroxyl groups and an increase in carboxylic acid groups relative to the brownstock lignin was also evident with all three mediators. Nonetheless, VA was the most potent mediator when considering the magnitude of change in lignin functional groups.

This report summarizes our continued LMS research efforts. The purpose of the present study was to examine the structural changes of residual lignins isolated from a SW kraft pulp (kappa no. 71.4) and then treated with LMS_{VA} , LMS_{HBT} , and LMS_{NHA} .

EXPERIMENTAL

Materials

All materials used in this study were purchased from Aldrich Chemical Co., Milwaukee, WI, and used as received except for *p*-dioxane, *N*-acetyl-*N*-phenylhydroxylamine (NHAA), and laccase. *p*-Dioxane was distilled over NaBH_4 , and NHAA was synthesized in accordance with Oxley's method (15). Laccase from *Trametes villosa* was donated by Novo Nordisk Biochem, Franklinton, NC.

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 minute per ml of enzyme solution, in a 100 mM potassium phosphate buffer (2.20 ml) and 0.216 mM syringaldazine in methanol (0.30 ml, pH 6.7). The

procedure was carried out at 23°C. The activity of the laccase was 3.8E+07 U/ml of enzyme solution. The activity of the laccase was also measured in several solutions of water:dioxane as shown in Figure 1.

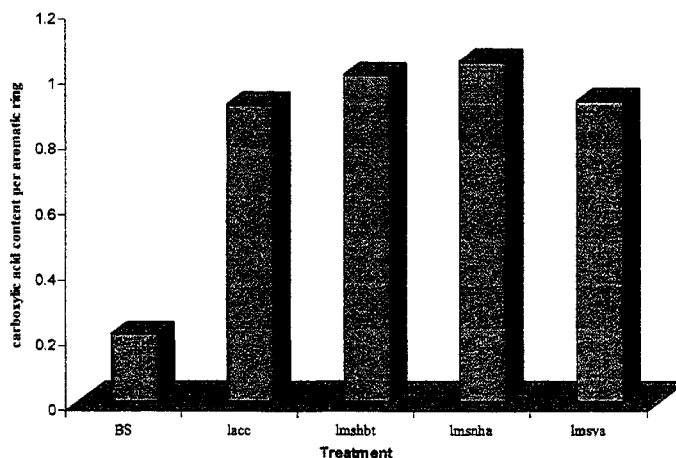


Figure 1. Percent decrease in laccase activity at different water:dioxane ratios

Isolation of residual lignins

Residual lignins were isolated from an acetone-extracted, never-dried, laboratory-prepared SW kraft pulp with a starting kappa number of 71.4 in accordance with established literature method (16,17). In brief, a 5000-mL three-necked-round bottom flask was charged with 120 g of pulp (o.d. basis). The consistency was adjusted to 4% by adding a 0.10 N HCl 9:1 *p*-dioxane:water solution. The slurry was then refluxed for two hours under an argon atmosphere. The pulp was filtered and the filtrate was filtered through celite, 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 precipitated lignin was collected, washed three times, and freeze-dried. A total of four isolations were carried out. The residual lignins were combined and then used to carry out the enzymatic treatments.

LMS procedure on residual lignins

Residual lignin (500 mg) was dissolved in 150 ml of a 1:1 solution of *p*-dioxane:distilled water. The solution was first transferred to a teflon beaker which was then placed into a 300-mL-capacity Parr reactor. The lignin solution was heated to 45°C and was maintained at this temperature throughout the incubation period (2 hours). NHAA (1.85×10^{-3} moles) was then added (or 1.85×10^{-3} moles of HBT or VA) and the pH was adjusted to 4.5 using glacial acetic acid. After mixing the solution (approx. 5 minutes), laccase (2 ml of enzyme solution) was added and the reactor was sealed and pressurized with oxygen to 145 psi. Subsequent to the treatment, the dioxane was removed under reduced pressure. The lignin-water solution was then diluted to approximately 250 ml using fresh distilled water, and the pH was adjusted to 2.2 with 1.00 N HCl. The precipitated lignin was washed three times using acidified water. The treated lignin was then subjected to an alkaline extraction stage.

Extraction stage of LMS treated residual lignins.

Subsequent to the LMS treatments, the residual lignins were subjected to an alkaline extraction stage. The lignins were diluted to approximately 180 ml using distilled water. The initial pH was adjusted to 11 using a 1.00 N NaOH, and the lignin solutions were treated for 1.5 hours at 80°C. Following the treatment, the lignin solution was acidified to pH 2.2 using 1.00 N HCl, centrifuged, and decanted. The precipitated lignin was washed three times using fresh acidified water (pH 2.2), freeze-dried, and then characterized.

Characterization of residual lignins

The treated residual lignins as well as the starting residual lignin were characterized by ^{31}P NMR and ^{13}C NMR in accordance with established literature methods (18-20). NMR data were acquired with a DMX 400 MHz Bruker spectrometer.

RESULTS AND DISCUSSION

The bleaching of kraft pulps with LMS continues to be extensively studied. Knowledge on residual lignins isolated and characterized subsequent to LMS treatments of kraft pulps has substantially expanded over the past few years. Furthermore, our studies have demonstrated that LMS treatments deplete high-kappa pulps primarily of noncondensed C-5 phenolic lignin groups, as well as of side chain aliphatic hydroxyl groups leading

to the formation of carboxylic acids and *o,p* quinones. This study further explores the reactions of laccase and LMS with residual lignins in the absence of kraft pulp fibers.

Residual lignin samples isolated from a SW kraft pulp (kappa no. 71.4) were treated with laccase or LMS using VA, NHA, and HBT as the mediators. The treatments involved placing the lignin, laccase, and/or the mediator in a 1:1 water:dioxane solution for 2 hours at 45°C in a pressurized Parr reactor (145 psi of oxygen). The treated lignins were recovered and treated to an alkaline extraction stage at 80°C for 1.5 hours. Lignin structural changes were evaluated via ¹³C and ³¹P NMR. Figures 2-5 summarize the most important changes observed in lignin structures. As illustrated in Figure 2, the carboxylic acid content of the lignins treated with laccase and LMS substantially increased under the conditions employed. This increase in acid groups was accompanied by depletion in phenoxy groups as shown in Figure 3. It is interesting to note that both condensed and noncondensed C-5 phenolic groups were reactive towards LMS. Although we have previously observed this reactivity towards lignin from low-kappa pulps, the same did not hold true on high-lignin-content kraft pulps. Indeed, LMS treatments of high-kappa pulps yielded residual lignins predominantly depleted of noncondensed C-5 phenolic groups. Several factors may be contributing to this difference in selectivity, including lignin content, mass-transfer effects, and reaction parameters. Accompanying the loss in phenolic hydroxyl groups was a substantial decrease in aliphatic hydroxyl group content, as seen in Figure 4. This trend can be attributed to side chain oxidation of the lignin and corroborates LMS lignin model compound studies. The LMS treatments in this study were found to slightly decrease the methoxy group content of the recovered lignins, suggesting that demethoxylation took place (see Figure 5).

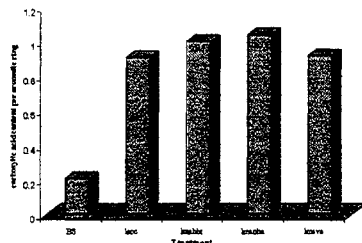


Figure 2. Carboxylic acid content per aromatic ring

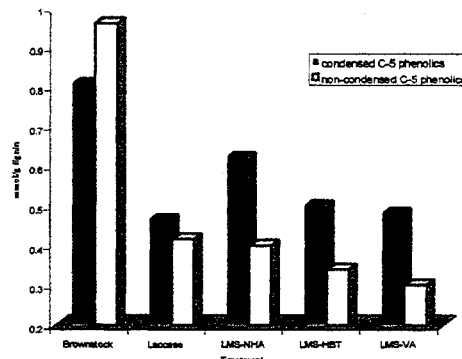


Figure 3. Condensed and noncondensed at C-5 phenolics (mmol/g lignin)

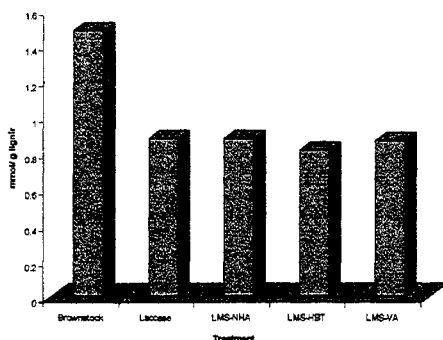


Figure 4. Aliphatic hydroxyl group content (mmol/g lignin)

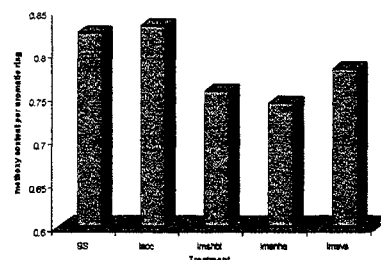


Figure 5. Methoxy content per aromatic ring

CONCLUSIONS

This study demonstrates that, overall, LMS treatments on residual lignins follow the same general oxidative trends as have been observed with kraft pulp fibers. In addition, these results suggest that laccase or an LMS treatment could be employed to modify the structure of lignin. This technology may have future applications to tailor the properties of lignin for commercial applications.

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