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Laccase-Mediator Systems and Oxygen Delignification—A Comparative Study

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LACCASE-MEDIATOR SYSTEMS AND OXYGEN DELIGNIFICATION- A COMPARATIVE STUDY

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ABSTRACT

A conventional SW kraft pulp with an initial kappa number of 73.4 was subjected to a series of treatments using laccase-violuric acid (LMS_{VA}=L), oxygen, and a combination of both systems. The treated pulps were characterized for kappa, brightness, and viscosity. Based on the experimental conditions employed in this study, an L, LE, and LLE exhibited superior retention in pulp viscosity than an O or a double O. The delignification response obtained with an LLE and an OLE treatment was comparable to a OO stage. Relative to the brownstock, the laccase treated pulps were significantly lower in brightness. However, an E stage subsequent to an L treatment alleviated this effect. Overall, the application of laccase-mediator systems on high-kappa pulps may be a viable technology that can yield substantial delignification without detrimental ramifications on viscosity.

INTRODUCTION

The bleaching of kraft pulps is a pivotal operation in the production of high-value paper products. Over the last two decades, research activities in pulp and paper have largely focused on environmental issues. As these issues continue to be addressed, new opportunities are developing. There has been a renewed interest in pulping and bleaching yields research [1-7]. Improvements in yields will have positive ramifications on wood utilization practices, as well as on operating and capital cost associated with the production of kraft pulps. One promising method for improving pulp yield consists of halting the kraft cook at a relatively high kappa (> 40). The pulp is then treated with a single or double oxygen stage before it is bleached.

Recently, we have begun exploring competing enzymatic systems, more specifically laccase-mediator systems (LMS), as potential cost-effective alternatives to oxygen delignification [8,9]. Several research groups [10-15] have established the high selectivity of LMS and the ability to achieve substantial levels of delignification (> 45% delignification) on low-lignin content SW and HW kraft pulps (kappa # < 30). Based on these findings, we hypothesized that an LMS could delignify high-lignin content kraft pulps. Our initial results [8] shown in Figure 1 supported this claim.

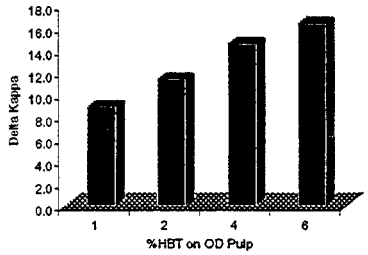

Figure 1. Effect of 1-hydroxybenzotriazole (HBT) charge on delignification of a 97.5 kappa commercial SW kraft pulp with constant laccase charge.

Further LMS studies on a conventional SW kraft pulp (kappa # 73.4) demonstrated that violuric acid (VA) was a superior mediator with respect to both 1-hydroxybenzotriazole (HBT) and *N*-acetyl-*N*-phenylhydroxylamine (NHAA) [16]. These results are summarized in Figure 2.

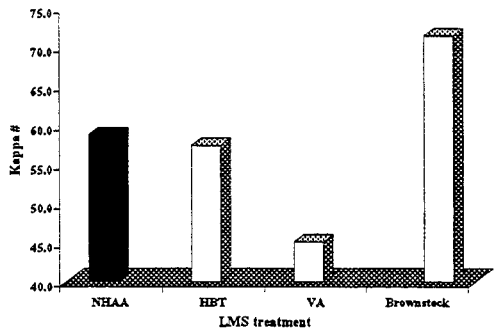

Figure 2. Kappa number of pulps subjected to LMS followed by an alkaline extraction, using equal molar doses of NHAA, HBT and VA.

This report summarizes our continued efforts in examining LMS systems on high-kappa kraft pulps. The objective of this study was to compare the delignification response of an LMS_{VA}, and a two stage LMS_{VA} to an oxygen and a double oxygen treatment. In addition, the delignification response of sequence treatments with both systems was also investigated.

MATERIALS AND METHODS

Materials

All materials were purchased from Aldrich Chemical Co., Milwaukee, WI, and used as received, except for NHAA. NHAA was synthesized in accordance with Oxley's [17] method. Laccase from *Trametes villosa*, was donated by Novo Nordisk Biochem, Franklinton, NC.

Furnish

A conventional southern USA softwood kraft pulp (kappa # 73.8) was prepared from *Pinus taeda* chips at Potlatch Corp. facilities in Cloquet, MN. The chips were cooked to an H-factor

of 573 using 18.5% active alkali. The pulp was thoroughly washed, screened, centrifuged, fluffed, and stored at 4°C prior to carrying out experiments.

Methods

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 530nm of 0.001 per minute per ml of enzyme solution, in a 100 mM potassium phosphate buffer (2.2 ml) and 0.216 mM syringaldazine in methanol (0.3 ml, pH 6.7). The procedure was carried out at 23°C. The activity of the laccase was 1.87 E + 06 U/ ml of enzyme solution.

Laccase-mediator delignification procedure

A 300-ml capacity Parr reactor equipped with a stirrer, a pressure gauge, a heating mantle, and connected to a Parr 4842 temperature controller was charged with 10 g of o.d. fibers. The pulp consistency was adjusted to 10% with distilled water. The slurry was then heated to 45°C and was maintained at this temperature throughout the incubation period. VA (4.4 mmol/ 10 g of o.d. pulp) was then added to the heated slurry. Subsequent to mixing the slurry (approx. 5 minutes), the pH was adjusted to 4.5 with glacial acetic acid or saturated sodium bicarbonate solution. Laccase (93,500 U, or 0.05 ml of enzyme solution/ g of o.d. fiber) was added, and the reactor was sealed and pressurized with oxygen to 145 psig. After a mixing period of 1 hour, the pulp was removed from the reactor and thoroughly washed with distilled water (12L per 10 g of o.d. pulp). The treated and washed pulp was either followed by subsequent treatments (oxygen delignification or LMS) or simply subjected to an alkaline extraction stage (E).

Alkaline extraction stage

Alkaline extractions (E) were carried out for 1 hour at 80°C, 10% consistency in 4mm thick heat-sealable Kapak pouches. All E treatments employed 2.5% charge (o.d. basis) of NaOH.

Oxygen delignification

Oxygen delignification was carried out in a 300-ml capacity Parr reactor. All treatments were conducted at 95 °C, 10 % consistency, for 1 hour. A charge of 2.5% NaOH (o.d. basis) was employed. Subsequent to a treatment, the pulp was thoroughly washed with distilled water.

Hexenuronic acid content in brownstock

The content of hexenuronic acids in the brownstock was indirectly measured in accordance with a modified procedure reported by Vuorinen *et al.* [18]. In brief, a 1000-ml round bottom flask was charged with 25 g of pulp (o.d. basis). The pulp consistency was adjusted to 3% by adding distilled water. The pH was then lowered to 3 using concentrated sulfuric acid. The slurry was refluxed for three hours at 100°C. The change in kappa number before and after the treatment was then determined and served as indirect measurement of hexenuronic acids (see Table I). Clearly, the hexenuronic acid content of this pulp was negligible.

TABLE I. CHANGES IN KAPPA # AFTER ACID TREATMENT OF BROWNSTOCK

Replicate #	Initial kappa	Final kappa	% change
1	73.4	71.5	2.6
2	73.4	71.9	2.0

Pulp characterization

The kappa, brightness, and viscosity before and after treatments were measured in accordance with TAPPI Test Methods T236 and UM246, T452, and T230, respectively [19-22]. Only the terminal viscosity of the treated pulps was measured. The initial viscosity of the starting material could not be determined using TAPPI Test Method T230 due to the high-lignin content in the pulp. Kappa and viscosity measurements were duplicated. Each brightness value was based on the average of five readings.

Experimental design

A conventional SW kraft pulp with an initial kappa of 73.8 was subjected to a series of L (L= LMS_{VA}) and oxygen delignification treatments. The list of experiments is summarized in Table II.

TABLE II. SUMMARY OF TREATMENTS

Treatment #	Description
1	L
2	LE
3	LLE
4	O
5	OO
6	OLE
7	LO
8	OLO

RESULTS

Based on our previous studies [9,16], we have demonstrated that an LMS_{NHAA}, an LMS_{HBT}, and an LMS_{VA} can yield substantial delignification when applied on high-kappa pulps. However, under the experimental conditions used in our studies, we noted that VA outperformed both NHAA and HBT [16]. This study summarizes our continued research efforts in this field. A conventional SW kraft pulp (kappa # 73.4) was subjected to LMS_{VA}, oxygen, double oxygen, and a combination of LMS and oxygen treatments. The pulps were then characterized for viscosity, kappa, and brightness.

Kappa

The delignification results shown in Figure 2 clearly demonstrate than an LMS system can yield substantial delignification on a high-kappa pulp. As expected, the delignification effect was further enhanced after an E stage, as the NaOH solubilizes the oxidized lignin. The data also suggest that under the experimental conditions employed in this study, an oxygen stage subsequent to an LMS treatment did not yield substantial delignification. This inefficiency could be linked to the oxidized nature of the residual lignin after an LMS. We have previously shown that the nature of the residual lignin of an LMS treated pulp is enriched with carboxylic acid groups and with quinone type structures, and is depleted of phenolic hydroxyl groups [9,16]. Hence, if an LMS treated pulp is further

subjected to an oxygen delignification stage, then we might expect that some of the oxygen chemistry will be impeded. Presumably, the alkalinity of the oxygen system is directed towards the ionization of acid groups, the solubilization of already oxidized lignin material, and the destruction of quinone type structures. Hence, less oxygen delignification chemistry occurs. The same rationale could also be applied to the observed delignification response of the O stage subsequent to the OL treatment (i.e., OLO).

Based on the experimental protocol used in this study, the delignification response of the LLE and OLE sequence was comparable to that of a OO stage and better than that of a single O stage. In addition, the LE treatment responded more favorably than a single O.

Overall, the LMS delignification system exhibits an additive effect that is reflected in the percent delignification of the LE and LLE treatments. Clearly, these results highlight the efficiency of the laccase-mediator system.

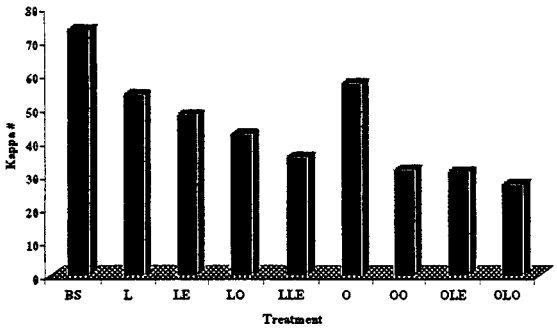

Figure 2. Kappa numbers of brownstock (BS) and pulps treated with O, L, and O/L.

Viscosity

Accompanying the substantial delignification response of a laccase-mediator system was a high retention in pulp viscosity. The viscosity results shown in Figure 3 indicate that L, LE, and LLE treatments are much more selective than an O or a OO stage. An L treatment applied after an oxygen delignification stage was more beneficial to the viscosity than when applied before, which reemphasizes the uniqueness of the enzymatic system.

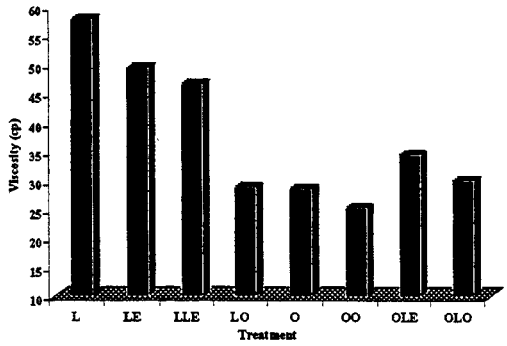

Figure 3. Terminal viscosity of pulps treated with O, L, and O/L.

The incorporation of an L treatment between a double oxygen stage was also beneficial, since the terminal viscosity was higher than that of a double oxygen treatment. Furthermore, the viscosity of an OLO treatment was comparable to a single O stage. In turn, this may prove to be an attractive approach in the future for obtaining the typical enhanced delignification benefits from a double oxygen stage without further loss in pulp viscosity. Nonetheless, further experimental work will be needed to support this claim.

Brightness

The brightness data shown in Figure 5 demonstrate that an LMS treatment leads to pulp darkening. This effect is attributed to the formation of quinone structures during an L treatment [16]. The alkaline extraction stage subsequent to an L treatment was beneficial in regaining some of the brightness loss, obviously because of the well-known ability of NaOH to destroy quinone type structures. The combination of LMS with oxygen had less of a detrimental effect on brightness than LMS alone. Overall, the brightness results suggest that if an LMS system is to be a viable technology then the darkening effect will need to be addressed. We believe that further bleaching with peroxide could be a viable approach in dealing with this problem. However, further studies will be needed to validate this claim.

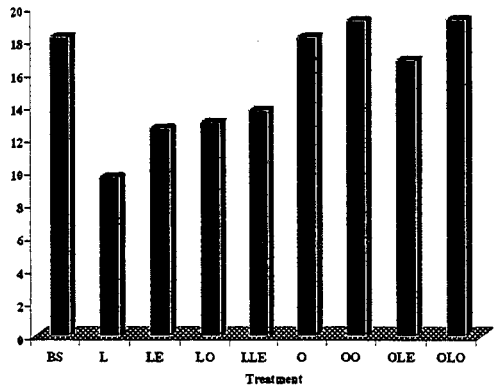

Figure 4. Brightness of brownstock (BS) and pulps treated with O, L, and O/L.

CONCLUSIONS

In summary, our results suggest that substantial delignification with high retention in pulp viscosity can be achieved *via* a laccase-violuric acid system. This study examined the delignification capabilities of an LMS system operating under extreme conditions (i.e., kappa # 73). The observed selectivity of the LMS system should provide a technology in the future to delignify kraft pulps with high yields.

It is now well established that the yield benefits of stopping a kraft cook prior to reaching the residual phase can be achieved with SW kraft pulps of kappa # 40-50. Based on our studies using a kappa 75 SW kraft pulp, we believe that a 50% delignification level using the lower kappa pulps (i.e. kappa # 40-50) is readily achievable with retention of pulp viscosity. Nonetheless, several research issues remain to be addressed. The loss of brightness during an LMS treatment of high-kappa pulps is a high-priority issue that will need to be examined.

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