Institute of Paper Science and Technology
Atlanta, Georgia

IPST Technical Paper Series Number 685

Biobleaching of Kraft Pulps with Laccase and Hydroxybenzotriazole

J.E. Sealey, T.M. Runge, and A.J. Ragauskas

October 1997

Submitted to
1997 TAPPI Pulping Conference
San Francisco, California
October 19–23, 1997

INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY
PURPOSE AND MISSIONS

The Institute of Paper Science and Technology is a unique organization whose charitable, educational, and scientific purpose evolves from the singular relationship between the Institute and the pulp and paper industry which has existed since 1929. The purpose of the Institute is fulfilled through three missions, which are:

- to provide high quality students with a multidisciplinary graduate educational experience which is of the highest standard of excellence recognized by the national academic community and which enables them to perform to their maximum potential in a society with a technological base; and
- to sustain an international position of leadership in dynamic scientific research which is participated in by both students and faculty and which is focused on areas of significance to the pulp and paper industry; and
- to contribute to the economic and technical well-being of the nation through innovative educational, informational, and technical services.

ACCREDITATION

The Institute of Paper Science and Technology is accredited by the Commission on Colleges of the Southern Association of Colleges and Schools to award the Master of Science and Doctor of Philosophy degrees.

NOTICE AND DISCLAIMER

The Institute of Paper Science and Technology (IPST) has provided a high standard of professional service and has put forth its best efforts within the time and funds available for this project. The information and conclusions are advisory and are intended only for internal use by any company who may receive this report. Each company must decide for itself the best approach to solving any problems it may have and how, or whether, this reported information should be considered in its approach.

IPST does not recommend particular products, procedures, materials, or service. These are included only in the interest of completeness within a laboratory context and budgetary constraint. Actual products, procedures, materials, and services used may differ and are peculiar to the operations of each company.

In no event shall IPST or its employees and agents have any obligation or liability for damages including, but not limited to, consequential damages arising out of or in connection with any company's use of or inability to use the reported information. IPST provides no warranty or guaranty of results.

BIOBLEACHING OF KRAFT PULPS WITH LACCASE AND HYDROXYBENZOTRIAZOLE

J. E. Sealey T. M. Runge
A. J. Ragauskas

Institute of Paper Science and Technology
500 10th Street, NW
Atlanta, GA 30318

ABSTRACT

Laccase was thought to be ineffective at delignifing kraft pulp until a small molecular weight compound called a mediator was introduced a few years ago. Mediator developments have introduced many new active mediators, but the most effective mediators contain an N-OH functional group. In this group of mediators, N-hydroxybenzotriazole has been shown to be as effective, if not more effective, than any other mediators at delignifing kraft pulp. When incorporating oxygen reinforced alkaline extraction, laccase HBT biobleaching can obtain more than 70% delignification in one stage. Selective radical quenching experiments have shown that the active delignification mechanism is radical based, and delignification can be completely stopped if a high dose of a radical quenching catalysis is used.

INTRODUCTION

In recent years, biobleaching kraft pulps with isolated extra-cellular enzymes has been shown to be effective (1-4). Because several oxidative enzymes are used in nature to degrade lignin, the exact mechanism of degradation is unknown. The two main classes of enzymes that are studied today, manganese peroxidase and laccase, are both ineffective at oxidizing the residual lignin in kraft pulp unless a small molecular weight compound called a mediator or co-factor is present (5). The mechanism that is generally accepted proposes that the mediator is oxidized by the enzyme. This activated mediator then diffuses into the pulps and oxidizes the lignin causing it to fragment and be removed from the pulp in the alkaline extraction (6).

Laccase biobleaching can be performed with oxygen as the original oxidant, which activates the enzyme and enables the enzyme to oxidize the mediator (Figure 1). This can be a major advantage over a peroxidase enzyme, which needs a very narrow range of peroxide present to activate the enzyme. Unfortunately, laccases have the disadvantage of a lower oxidations potential, which can vary depending on the origin of the enzyme and how it was isolated (7).

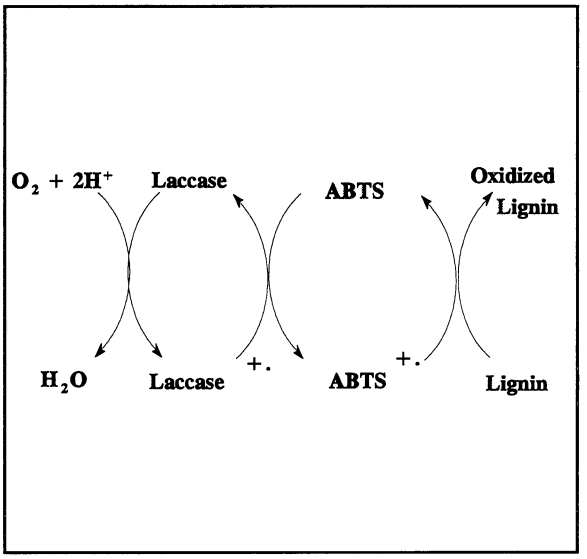

Figure 1. Proposed laccase mediator delignification mechanism.

Several mediators have been shown to be effective with laccase (5). The first mediator that was shown to be effective in delignifying kraft pulp was 2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonate) (ABTS), but N-hydroxybenzotriazole (HBT) was soon introduced. The first studies of HBT showed it to be highly effective with 50+% delignification. This discovery introduced a new class of mediators with the N-OH functional group. The proposed active delignification mechanism is a NO· radical, which is partially stabilized by the mediator structure and is selective for lignin oxidation. Currently, little data have been reported to prove or disprove this theory.

A review of the mediator structures that have been introduced reveals that the N-OH mediators are the most effective. While several problems exist with HBT for commercial-scale bleaching, it is as or more

effective than any other mediator reported. As work continues in mediator development, drawbacks to the laccase biobleaching system may be solved. Biodegradability, stability, effectiveness, and cost of the mediator are just a few of the hurdles that need to be crossed before a laccase biobleaching system can be implemented. Recently, Lignozym has introduced a new mediator, N-hydroxyacetanilide (NHAA), which is biodegradable and has been claimed to be cost-effective (8). Biobleaching with NHAA also allows the activity of the enzyme to maintain about 80% of its original activity after an hour treatment where biobleaching with HBT causes severe loss of enzyme activity. The exact mechanism that causes the loss of activity in the presence of HBT is not known, but the effectiveness of HBT and NHAA are virtually identical. Mediator instability may limit NHAA effectiveness because most mediators, that have been studied extensively, have been shown to be unstable during biobleaching conditions (9).

The purpose of this study is to examine the effectiveness of biobleaching with the laccase mediator system and also to determine the active delignification mechanism. While the commercial viability of HBT is low, HBT was chosen as the mediator in this study to create the most active delignification environment available with laccase today. Because HBT severely lowers the activity of laccase over time, a large dose of enzyme was used to optimize delignification.

EXPERIMENTAL PROCEDURES

Laccase Assay:
The activity of the laccase was measured by monitoring the rate of oxidation of syringaldazine. The change in A_{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) was set to one unit (U) of activity. This test was performed at 23°C.

Biobleaching Conditions:
All laccase treatments were performed with 200 mg of HBT per 10 g of OD pulp. The reactions were performed at 4.5 pH for 4 hrs at 10% consistency under 145 psi O_2. The amount of laccase used was 1.6 million U per 10 g of OD pulp.

Chlorine Dioxide and Hydrogen Peroxide Stages:
D stages were performed at 10% consistency for 3 hrs in a sealed bag at 70°C, after the initial pH was adjusted to 10. P stages were performed at 10% consistency at 90°C in a sealed bag for 4 hrs. E stages were performed at 70°C for 1 hr at 10% consistency with a 2% NaOH charge. EOP stages were performed at the same conditions as E except 0.5% P was added, and O_2 was maintained at 60 psi for the first 15 minutes, and the pressure was decreased by 20 psi every 5 minutes until atmospheric pressure was reached.

RESULTS AND DISCUSSION

The first experiments were carried out to determine the effect of reinforced alkaline extraction on laccase HBT treated pulps. For a standard laccase HBT treatment, about 50% delignification can be achieved with a typical industrial softwood kraft post-O_2 pulp with an initial kappa number of 13. If oxygen reinforced alkaline extraction (EO) was used, delignification was close to 60%, but if an industrial EOP was used more than 70% delignification can be obtained with one laccase HBT treatment (Figure 2).

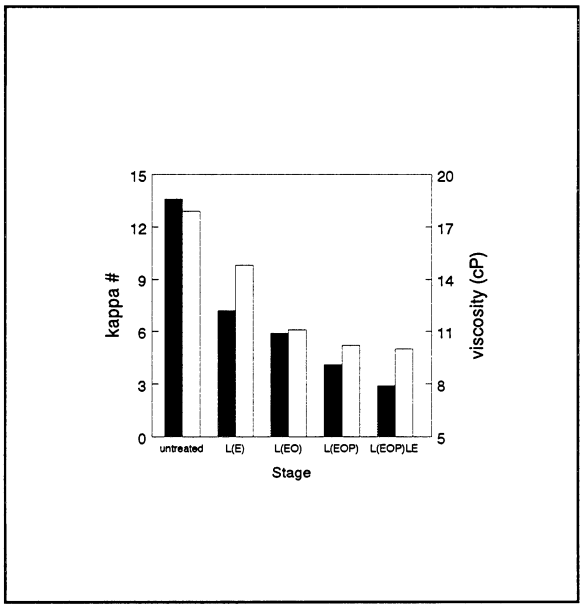

Figure 2. The effect of reinforced alkaline extraction and two stages of laccase HBT treated softwood kraft Post-O_2 industrial pulp. Solid bar is kappa number, and open bar is viscosity.

If the L(EOP) pulp is treated with another laccase HBT stage, the kappa number decreases significantly

again. This observation suggests that laccase HBT biobleaching is capable of reacting with the last vestiges of residual lignin, which are typically very unreactive. The selectivity of this system also appears to be equal to, if not significantly better than, most TCF sequences.

The L(EOP)LE treated pulp was then brightened by hydrogen peroxide and chlorine dioxide (Figures 3 and 4).

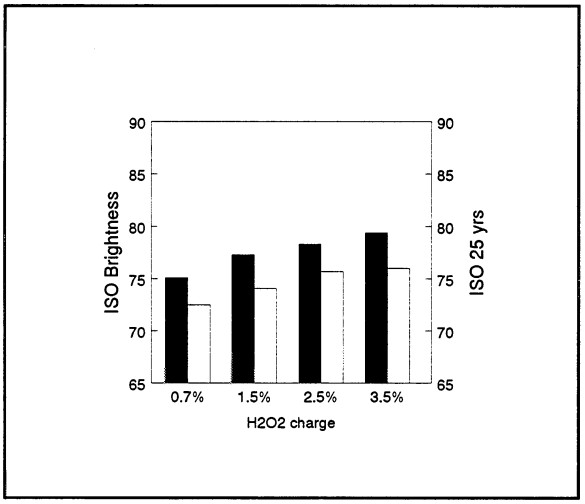

Figure 3. Hydrogen peroxide brightening of L(EOP)LE pulp with different H_2O_2 charges. Solid bar is ISO brightness, and open bar is ISO brightness after thermal reversion equal to 25 years.

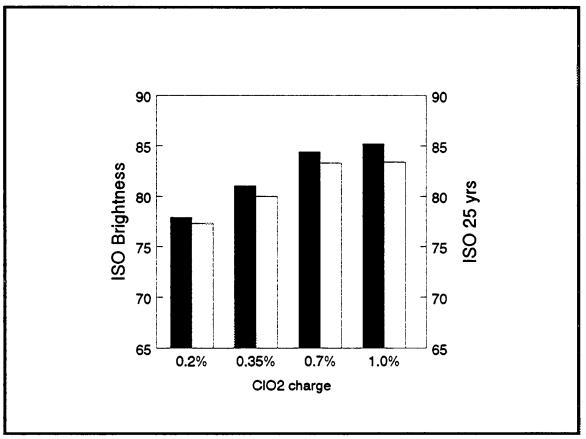

Figure 4. Chlorine dioxide brightening of L(EOP)LE pulp with different ClO_2 charges. Solid bar is ISO brightness, and open bar is ISO brightness after thermal reversion equal to 25 years.

The hydrogen peroxide treated pulp approached an 80 ISO brightness with the higher peroxide charge, but the pulp showed minimal reversion over a 25 years thermal reversion test.

The pulp treated with chlorine dioxide exceeded 80 ISO brightness with a relatively low charge, and the higher charge brightened the pulp to more than 85 ISO brightness with low reversion detected. A bleaching sequence of OL(EOP)D was also performed, which produced an pulp with a 83 ISO brightness.

Because the effectiveness of laccase HBT was clearly seen in these experiments, the mechanism of delignification was studied. The proposed mechanism is a radical mechanism where the radical is partially stabilized and made selective for lignin by the mediator structure. To test this mechanism, a selective radical quenching agent was needed that would not react with laccase directly. The radical quenching agent that was chosen was superoxide dismutase (SOD).

SOD has been used with laccase to inhibit polymerization of soluble lignins (10). This effect was believed to be due to the quenching of radicals and not the quenching of superoxide. SOD has also been used to quench radicals in many systems, and it is very active at pH 4.5, which proved beneficial for our studies (11).

The SOD tests were performed with active biobleaching conditions. Dry SOD powder was added to the pulp before the addition of laccase (Figure 5). A strong decrease in the ability of the laccase HBT biobleaching system to reduce the kappa number of the pulp was seen as the dose of SOD was increased from 29,000 U to 116,000 U. Surprisingly, the high dose of SOD completely stopped delignification. Numerous controls were performed to determine if the effect was due to the addition of active SOD. The most definitive control used was to add thermally denatured SOD at the highest dose of SOD used. This addition detected no inhibition of delignification (D-116 in Figure 5).

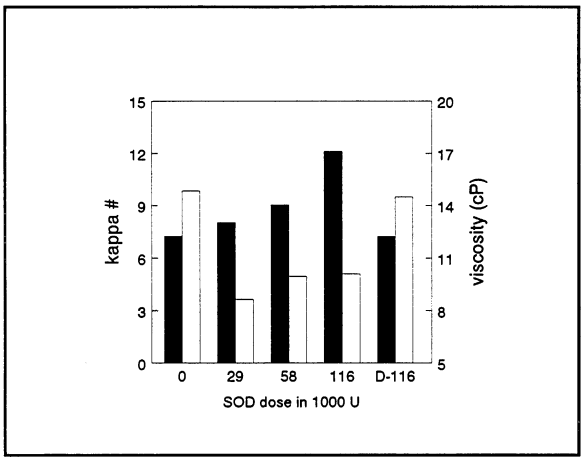

Figure 5. Superoxide dismutase treatments of active biobleaching conditions of laccase HBT. D-116 is thermally denatured SOD. Solid bar is kappa number, and open bar is viscosity.

CONCLUSIONS

Laccase HBT biobleaching can be an effective stage in TCF and ECF bleaching. When an oxidant reinforced alkaline extraction stage is used, more than 70% delignification of a softwood kraft pulp can be achieved with one stage of biobleaching. The active delignification mechanism of laccase HBT appears to be radical based. While several factors limit delignification of laccase and HBT, the radicals created appear to be selective for lignin and very reactive because laccase HBT can react effectively with very low kappa pulps.

ACKNOWLEDGMENTS

The authors thank the Institute of Paper Science and Technology and its Member Companies for supporting this research. The gift of the laccase by Novo Laboratories Inc. is gratefully appreciated. Portions of this work were used by J. S. and T. R. as partial fulfillment of the requirements for the Ph.D. degree at the Institute of Paper Science and Technology.

REFERENCES

1. Call, H.P., "Multicomponent Bleaching System", WO #94/29425.
2. Call, H.P., "State of the Art of Enzyme Bleaching and Disclosure of a Breakthrough Process", *Tappi/ Emerging Pulping and Bleaching Technology Workshop*, Durham, NC, 15-17, (1995).
3. Bourbonnais, R. and Paice, G.M., "Enzymatic Delignification of Kraft Pulp using Laccase and a Mediator", *Tappi J.* 79(June):199-204 (1996). 148 (1996).
4. Bourbonnais, R., Paice, M.G., Reid, I.D., Lanthier, P., Yaguchi, M., "Lignin Oxidation by Laccase Isozymes from *Trametes versicolor* and Role of the Mediator 2,2'-Azinobis(3-ethylbenzthiazoline-6-sulfonate) in Kraft Lignin Depolymerization" *Applied and Environmental Microbiology* 61(5): 1876-1880 (1995).
5. Paice, M.G. et al., "Kraft Pulp Bleaching by Redox Enzymes", *ISWPC* Montreal, PL1-1-PL1-4 (1997)
6. Call, H.P. and Mucke, I., "The Laccase-Mediator System (LMS)- a New Concept", *6th International Conference on Biotechnology in the Pulp and Paper Industry* Vienna, Austria, 27-32 (1996).
7. Yaropolov, A.L. et al., "Laccase: Properties, Catalytic Mechanism, and Applicability" *Applied Biochemistry and Biotechnology* 49, 257-280 (1994).
8. Amann, A., "The Lignozym Process Coming Closer to the Mill" *ISWPC* Montreal F4-1-F4-5 (1997).
9. Sealey, J., Ragauskas, A.,"Fundamental Investigation into the Chemical Mechanisms Involved in Laccase Mediator Biobleaching" *ISWPC* Montreal F1-1-F1-4 (1997).
10. Milstein, O. et. al.;"Enzymatic Transformation of Organosolv Lignin" 6th ISWPC p481-488.
11. Ellerby, L.M. et al.,"Copper-Zinc Superoxide Dismutase: Why Not pH-Dependant?" *J. Am. Chem. Soc.* 118, 6556-6561 (1996).