Chapter 7

Laccase Modification of the Physical Properties of Bark and Pulp of Loblolly Pine and Spruce Pulp

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Pine bark, pine pulp, and spruce pulp were reacted with laccase in the presence of phenolic laccase substrates to modify the fiber surface properties. The acid–base and dispersive characteristics of these modified steam-treated thermomechanical loblolly pine pulps were determined by inverse gas chromatography. Different combinations of substrates with laccase modified the Lewis acid–base characteristics of the pulp. The binding of methylene blue was found unsuitable as an indicator of negative charges on pulps modified by laccase with phenolic substrates; however, its binding did indicate undefined changes in the pulp. Thermomechanical spruce pulp treated with manganese peroxidase or laccase with 4-hydroxyphenylacetic acid increased handsheet strength. Manganese peroxidase treatment also decreased the amount of refining needed to obtain the same freeness, which would indicate potential energy savings. Incremental addition of resorcinol in the presence of laccase altered spruce pulp to a greater extent than did bulk addition. Laccase with substrates also altered the binding of phosphate by modified pine bark.

Introduction

Many species of woody plants are used for particular purposes, with their end use being directed by the properties of the wood and economic concerns. Generally, the functional use of wood products is determined by the hydrophilic, hydrophobic, dimensional, and ionic properties of the cellulosic fiber. Small diameter and mixed species of wood have limited uses and are thus low in value. If we could understand how to change the cellulose fiber properties of such materials, alternative sources of fiber could be better exploited and more valuable products made from mixed woody species or recycled fiber.

The structure of wood and other lignocellulosic materials is on first inspection quite simple. It consists of very few polymeric materials: cellulose, hemicellulose, and lignin. Upon further consideration, three-dimensional aspects and species-specific variability make the structure of lignocellulose very complicated. Understanding and optimizing the surface properties of lignocellulosic materials for structural or paper applications will result in better use of these resources.

Our knowledge of lignocellulose structure and function is increasing, but we cannot as yet convert a source of lignocellulose to a desired product at will. Much of our knowledge is based on empirically separating the components of the material to create a product with the desired strength and flexibility. Processes following this approach, which are still being developed, will reduce costs and provide enhanced products. Thus to use lower value resources, the best approach might be to regard them as a mixture of polymeric materials and empirically derive ways of altering their properties with a target product in mind. The next stage in our understanding will come from developing ways to actively build strength and other properties into the materials.

The goal of this research is to investigate the feasibility of grafting compounds or polymers onto softwood materials to alter the surface characteristics. Laccase and peroxidases have been used to modify the properties of lignocellulosic materials (*1-7*). We have used laccase with substrates and manganese peroxidase to alter lignocellulose materials. Methylene blue binding (to determine carboxylic acid content), inverse gas chromatography (to determine Lewis acid–base characteristics), and pulp handsheet properties were used to explore the effectiveness of the treatments in altering surface properties.

Materials and Methods

Pulp Sources

Dried thermomechanical pulp (TMP) (mature loblolly pine, *Pinus taeda*) was obtained from the U.S. Department of Agriculture at Pineville, Mississippi. Steam pressures used during the TMP process were 4, 8, and 12 bar. Spruce (*Picea glauca*) chips were refined at 103 kPa, followed by atmospheric refining to ~600 mL Canadian standard freeness (CSF). Spruce pulps were further refined to 70 mL CSF after enzyme treatments using a PFI mill to reduce the freeness.

Laccase Treatment

Dry loblolly pine pulp samples (2 g) were weighed into Erlenmeyer flasks, 75 mL deionized water was added, and the mixture was stirred for at least 3 h at room temperature to ensure complete wetting. Sodium tartarate buffer (5 mL, 200 mM, pH 5) and one unit laccase (NovoSample 51002, 1 unit/5µL, unit = 1 µMole syringaldazine oxidized/min at 30°C, pH 5.5) were added and stirred for 20–25 min before 1 mL substrate (0.3 M) was added. After approximately 25 min of stirring, another 20 µL laccase was added. The samples were stirred for 1 to 2 h at room temperature, then loosely covered with foil and refrigerated for 15 to 17 h. After warming to room temperature, the Supernatant solution was filtered off and the pulps were washed three times with 1.5 L distilled water. Finally the pulps were strained through a sieve, freeze-dried for at least 48 h, and stored in a desiccator until analyzed.

Spruce pulps were treated at 37°C for 24 h with manganese peroxidase at 12% consistency with 50 mM malonate buffer (pH 5), 0.1 mM $MnSO_4$, and 0.8 mM H_2O_2 (added slowly with time). Treatment of spruce pulp with 4-hydroxyphenylacetic acid was performed under the conditions described by Chandra and Ragauskas (*8*). For treatments with resorcinol, samples of 243 g spruce pulp (37% solids) were weighed into plastic bags and mixed with 64 mL 200-mM tartarate buffer pH 4.5 and 250 mL distilled water. Laccase (0.6 mL) was added in 43 mL distilled water. Resorcinol (2 g/50 mL water) was either added with the 250 mL distilled water or added in increments (1/3 added at 0, 60, and 105 min of incubation). The bags were sealed and incubated at 37°C for 2 h with the contents mixed by hand every 30 min, followed by storage overnight at 5°C. Pulps were rinsed with distilled water until the filtrate was colorless; the

filter cake was then dewatered by pressing on adsorbent sheets and frozen until used. Where oxygen was used, the plastic bags were flushed with a stream of oxygen for 1 min, then sealed.

Methylene Blue Binding

Known weights of dry pulp (up to 150 mg) were placed in 50-mL disposable centrifuge tubes with 5 mL 1M borate buffer (pH 8.5) and five clean steel balls and mixed to break clumps. Aqueous methylene blue (35 mL of 1 mM) was added, and the tubes were tumbled for 1 h. Samples (1.5 mL) were removed and centrifuged at 20,000× g for 5 min. Duplicate dilutions were made of 0.5 ml supernate into 5 mL H_2O + 0.125 mL 1 N HCl. Two samples (0.2-mL) from each dilution were analyzed at 610 nm on a Molecular Devices Spectra Max Plus microtiter plate reader. Micromoles of dye adsorbed by pulp equals $35(1-A_{sample}/A_{blank})$. Methylene blue binding was determined by plotting amount of dye bound as a function of weight of pulp and determining the slope of the line (with at least three points). All methylene blue results in which less than half the methylene blue was adsorbed from the assay were combined for one calculation for each sample.

Inverse Gas Chromatography

Approximately 1 g pulp was packed into stainless steel chromatographic columns of 0.5 m length and 0.63 mm diameter. A Hewlett Packard 5790A gas chromatograph was used with helium as carrier gas at a flow rate of 15 mL/min. The flame ionization detector was set to 200°C and the injector at 150°C. Measurements were performed at five different oven temperatures, from 40°C to 80°C in 10°C increments. The reference line used five n-alkanes (n-pentane to n-nonane), and tetrahydrofuran, acetone, and chloroform were selected as the basic, amphoteric, and acidic probes, respectively. Results for the dispersive energy component were obtained according to Felix and Gatenholm (9) by plotting $RT\ln V_n$ versus $2Na(\gamma_l^d)^{1/2}$, with V_n the net specific retention volume, a the surface area occupied by the probe, γ_l^d the dispersive energy of the probe, R the gas constant, and T the temperature. The plot of $RT\ln V_n$ versus $2Na(\gamma_l^d)^{1/2}$ gives a straight line. From the slope of this line, the surface energy of the substrate can be determined at each temperature. Polar probes deviate from the straight relationship due to specific interactions, including Lewis acid–base interactions. If the vertical distance of each polar probe is measured to the straight line, the free energy of sorption can be determined and plotted as a function of temperature (*10*). The slope of that line is related to the free enthalpy

of adsorption/desorption. Donor (DN) and acceptor (AN) numbers of the polar probes are available from the literature. A plot of the free enthalpy of sorption versus DN/AN allows the determination of acidity (K_A, slope) and basicity (K_D, intercept) of the substrate (*10*). Determinations were made for two independent samples and the results averaged.

Paper Testing

Handsheets (60-g/m^2) were made according to TAPPI standard T 205 sp-95 (*11*). Burst, tear, and tensile strengths were determined according to TAPPI standards T 403 om-97, T 414 om-98, and T 494 om-96, respectively (21). Results represent the average of 10 handsheets.

Bark Reactions

Bark of *P. taeda* obtained from a pulp mill in Mississippi was milled, extracted, activated, and reacted with bisaminopropylethylenediamine (BAPED). The modification was made to increase the capacity of the bark to adsorb anions. Modified bark (0.5 to 0.7 g) in 5 mL of 40 mM tartarate buffer pH 5.0 and 10 µL laccase was mixed for 30 min at room temperature. Phloroglucinol (1 mL of 0.3 M in ethanol) or catechol (1 mL of 0.3 M) were added and incubated for 3 h at room temperature, followed by 18 h at 5°C. The particles were rinsed and recovered by filtration and freeze dried. Controls included laccase addition without substrate and untreated modified bark. The bark was tested for the ability to remove phosphate from solution.

Results and Discussion

In initial testing, loblolly pine fiberboard furnishes produced at 4, 8, and 12 bar steam pressure were evaluated for laccase reactivity. Laccase was incubated with a sample of each furnish at 37°C, and the rate and extent of oxygen consumption were measured. Oxygen consumption by laccase was highest and fastest for the 12 bar furnish, with 12.0 nmoles oxygen consumed per milligram furnish. This would correspond to 48 moles of one-electron oxidative events per milligram of knish. Assuming the reaction was with lignin and 28% lignin in loblolly pine, this corresponds to the reaction of approximately 1 syringaldehyde-like unit in 30. The 4 bar and 8 bar knishes had 4.0 and 8.4

moles oxygen consumed per milligram furnish. The majority of subsequent screening work was performed with the 12 bar pulp.

Oxygen was consumed during laccase treatment, but the actual structures in lignocellulose that are substrates could not be determined. The affinity of laccase for its substrates depends upon the structure and its accessibility. The affinity of enzymes for substrates and concentrations of substrates will alter the rate of reaction. Although it is difficult to assess the enzyme affinity for substrates already present in the lignocellulose, the affinity of the enzyme for phenolic substrates was measured. The approximate K_m values for several substrates were determined by measuring the oxygen consumption rate at different substrate concentrations (data shown in Table I). The laccase substrates tested ranged from quite insensitive with phenol (18 mM) to sensitive with 2,5-dihydroxy benzoic acid (0.04 mM). Though there are some differences with various laccases and their affinity for the substrates, the values presented here are similar to those reported for *Polysporous pinsitus* laccase (*12*).

Table I. Approximate K_m for Laccase Substrates Tested

Substrate[a]	K_m (mM)
Phenol	18
4-hydroxyphenylacetic acid	7
Guaiacol	3
4-methylcatechol	2
Resorcinol	2
o-coumaric acid	2
Isovanillic acid	2
Vanillic acid	1
p-coumaric acid	1
Hydroquinone	<1
3,4-dihydroxybenzoic acid	<1
Catechol	<1
3,4-dihydroxyphenylacetic acid	<1
2,5-dihydroxybenzoic acid	<1

[a]Substrates were tested in 20 mM pH 5.0 tartarate buffer and 0.2 units laccase using a YSI oxygen meter with a temperature-controlled stir cell (2 mL). Substrates are listed in order of increasing reactivity with laccase.

Laccase creates free radicals that can further react with other compounds. Ideally, conditions for each substrate would be optimized for the grafting reaction, which would require investigation of concentration, pH, affinity of the enzyme, and other reactants in the pulp. Because optimizing the conditions for each substrate is beyond the scope of a screening test, the concentration of substrate (3.75 mM), enzyme (5 units), and fiber were held constant. We incubated 12 bar loblolly pine pulp in the presence of laccase with and without various laccase substrates, as described in Methods. These pulps were treated with laccase, washed, and freeze dried. High affinity laccase substrates were oxidized once before 1 h had elapsed. The enzyme reaction was continued for several hours to allow for lower affinity substrates to react.

Carboxylic acid groups are beneficial in the bonding of pulp fibers in paper and can increase the strength of the paper (13). Phenolic substrates containing carboxylic acid groups could be attached by the grafting of the substrate. Carboxylic acids could also be created by extensive oxidation of substrates or the fiber. Anionic groups from dissociated carboxylic acids can be estimated by conductometric titrations or by adsorption of cations such as metal atoms or dyes (e.g., methylene blue) (*14*, *15*). Conductometric titrations are very time consuming, and methylene blue adsorption correlated well with the conductometric results on pulp (*14*). Thus, we modified a method of methylene blue adsorption (*15*) to the fibers to determine the amount of anionic groups bound to the fiber.

Single measurements of methylene blue adsorption on pulp fibers gave different results, probably as a result of non-specific binding. The conditions of the assay (volume, sample amount, and use of new glassware and plastic ware) were adjusted so that .the adsorption of methylene blue to a pulp sample could be determined reproducibly. The final form of the assay is described in Methods and uses at least three different samples of pulp, all of which adsorb less than half the methylene blue in the assay. The adsorption of methylene blue was determined from the slope of methylene blue adsorbed as a function of weight of the pulp (Figure 1).

Methylene blue is positively charged at pH 8.5, and adsorption should be correlated to negative charges on the pulp. We expected that the laccase substrates containing carboxylic acid groups would interact more than those without acid groups. However, methylene blue adsorption did not directly correlate with acid content of the substrate. The highest values of methylene blue adsorption were observed for pulp treated with resorcinol, which by itself contains no carboxylic acid groups. The nature of the attachment of resorcinol

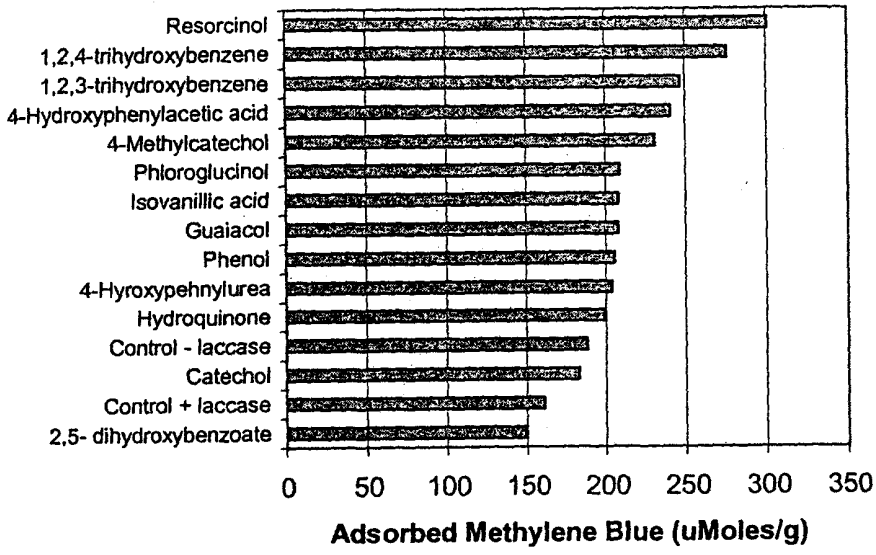

Figure 1. Methylene blue adsorption to 12 bar loblolly pine pulp after treatment with laccase and indicated substrates,

(or other substrates) to the pulp is unknown. A resorcinol free radical could also have reacted with oxygen, possibly increasing the acidity of the fiber.

We analyzed many of these pulps by IGC to determine if there was an increase in donor or acceptor character for the pulp or if the dispersive energy characteristics of the pulp were changed in a manner that correlates to the methylene blue binding results. Table II shows the surface acid–base characteristics and dispersive component for these pulps. The ability to act as a Lewis acid was the only statistically significant correlation when compared with methylene blue binding, with $r_s = -0.56$ (Spearman rank-order correlation coefficient) and $P = 0.03$. The ability to act as a Lewis base, the dispersive component, and the ratio of acid to base had no relationship with methylene blue binding.

Because methylene blue is a cationic dye, it would be expected to have activity as a Lewis acid. The correlation with methylene blue was weak but showed the expected trend, with increased methylene blue binding being negatively correlated to increased K_A. Phloroglucinol and isovanillic acid were the only laccase substrates that increased the K_D of the fiber. All substrates other than resorcinol, 4-hydroxyphenylacetic acid, 1,2,3-trihydroxybenzene, and 1,2,4-

trihydroxybenzene increased the K_A of the fiber. The ratio of K_A/K_D was increased for many samples after these treatments. We expected that the methylene blue adsorption would decrease for those treated fibers with K_A/K_D ratios greater than the control, but for many substrates the opposite effect was observed. This indicates that methylene blue does not interact with the fibers solely by Lewis acid–base interactions.

Table II. Analysis of Laccase Treated Pulps by IGC

Substrate for 22 bar pulp or pulp controls[a]	K_A	K_D	K_A/K_D	*Dispersive component*
2,5- Dihydroxybenzoate	0.261	0.019	13	29
12 bar control + laccase	0.223	0.037	6	28
Catechol	0.276	0.010	28	31
12 bar control - laccase	0.199	0.052	4	30
Hydroquinone	0.245	0.049	5	27
4-Hyroxyphenylurea	0.224	0.011	20	30
Phenol	0.268	0.017	16	27
Guaiacol	0.271	0.010	26	27
Isovanillic acid	0.277	0.074	4	28
Phloroglucinol	0.244	0.119	2	27
4-Methylcatechol	0.207	0.023	9	28
4-Hydroxyphenylacetic acid	0.180	0.035	5	29
1,2,3-Trihydroxybenzene	0.182	0.043	4	29
1,2,4-Trihydroxybenzene	0.186	0.011	16	26
Resorcinol	0.172	0.040	4	30
4 bar control - laccase	0.208	0.065	3	36
8 bar control - laccase	0.214	0.041	5	32

[a]The substrates are listed for the I2 bar pulp reacted with the substrate and laccase. The 4 bar, 8 bar, and 12 bar controls were also included.

Methylene blue binding does not predict the Lewis acid–base characteristics of the pulp but might be useful as an indicator of other properties in a product derived from the pulp. It does indicate that some change was made in the binding properties of the pulp.

Several conditions were selected to test the effects of oxidative enzyme treatment on spruce pulps and determine if handsheet properties were altered by the treatment. Spruce pulp of high freeness (CSF 600 mL) was treated with

manganese peroxidase or laccase in the presence of 4-hydroxyphenylacetic acid, followed by refining in a PFI mill. Handsheets were made from the treated pulps. The results of methylene blue binding by the pulps and strength testing on handsheets made from these pulps are shown in Table III.

Table III. Properties of Treated Spruce TMP After Refining by PFI Mill and Handsheet Preparation

Pulp treatment	*Methylene blue adsorp.* (μMol/g)	*Tear index* (mN m²/g)	*Burst index* (kN/g)	*Tensile index* (Nm/g)	*CSF* (mL)
Control	ND	3.52	1.54	33.5	65
MnP complete	54	3.80	1.64	34.1	69
H_2O_2 + Mn	50	3.51	1.52	33.5	70
Buffer	42	3.56	1.50	33.4	71
Laccase + 4-HPA	101	3.61	1.59	32.3	69

ND= not determined; 4-HPA is 4-hydroxyphenylacetic acid; and the complete reaction mix for MnP contained enzyme, buffer, H_2O_2, and Mn.

Handsheets from manganese peroxidase (MnP) treated pulp showed increases in burst, tear, and tensile indexes. The manganese peroxidase treated pulp also required less refining in the PFI mill to obtain the same degree of freeness (requiring only 8,500 revolutions compared with 10,000 for the controls and laccase treated material). This may indicate a savings in refining energy (*16*). The buffer, Mn, and H_2O_2 had no effect on the strength of the handsheets. The laccase treatment with 4-hydroxyphenylacetic acid brought about small increases in the burst and tear indexes and a decrease in the tensile index. Chandra and Ragauskas (*8*) reported an 18% increase in the burst index, an 11% increase in the tear index, and about an 8% increase in the tensile index for softwood linerboard pulp treated with laccase and 4-hydroxyphenylacetic acid.

The MnP treatment did not increase the binding of methylene blue above that of the controls. The laccase together with 4-hydroxyphenylacetic acid did increase the adsorption of methylene blue. Both the MnP treatment and laccase treatment together with 4-hydroxyphenylacetic acid increased handsheet strength. Methylene blue adsorption does not appear to correlate with handsheet strength.

The conditions under which the pulp is treated can influence the extent of the modification. Using resorcinol as the modifying substrate on spruce TMP,

we investigated the method of substrate addition using the adsorption of methylene blue to assay the treated pulp. Table IV shows the results of the methylene blue adsorption on pulps treated with the same amount of resorcinol but under different conditions. Wen the entire amount of resorcinol was added at the start of the experiment, there was an increase in methylene blue adsorption above that of the laccase treated control. If the atmosphere of the incubation bag was replaced with oxygen, then no discernable changes in methylene blue adsorption were observed. Methylene blue adsorption was highest when resorcinol was added in three aliquots during incubation. The color of all the resorcinol treated pulps changed from brown to deep red and could not be removed by washing with distilled water. Samples reacted in an oxygen atmosphere turned red much more rapidly those reacted in air.

The conditions used for these experiments indicate that a greater effect can be created by metering in the substrate. Oxygen is required in these incubations to reoxidize the laccase. It might also react with laccase generated radicals and propagate oxygen free radicals that could be additional reactants.

Table IV. Effect of substrate addition on methylene blue binding to treated spruce TMP

Pulp treatment	Methylene blue adsorption (µMol/g)
No substrate + Laccase	66
Resorcinol–all at start	99
Resorcinol–all at start–Oxygen	60
Resorcinol-3 aliquots	126

Southern pine bark treated with BAPED is effective at removing phosphorous from aqueous solutions. The bark was treated with laccase and also with phloroglucinol and catechol to generate additional sites where more BAPED could be bound. The laccase treated material decreased the capacity of the bark to remove phosphorous by 31%. The catechol and phloroglucinol treatments decreased this capacity by 38% and 45%. respectively. It appears the reaction with laccase and substrate competes for sites already occupied by BAPED, diminishing the capacity of the bark. It is also possible the phenolic substrates attached to the nitrogen of BAPED and reduced the phosphorous binding capacity. These reactions will be explored further.

Conclusions

Laccase and manganese peroxidase are able to generate free radicals in pulps and substrates. In this paper, we showed that oxygen was consumed but we did not directly demonstrate free radical formation. The results of fiber treatment are influenced by treatment of the pulp prior to enzyme treatment, the conditions under which the pulp is treated with enzymes, and the types of substrates used for the treatment. Laccase reactions changed the surface characteristics of the fiber, and the changes were different with different substrates. Results obtained with IGC indicated that both acidic and basic modifications could be made. If these changes can be made economically, there may be some use in increasing paper strength. Methylene blue adsorption did correlate with the K_A results from IGC but is not suitable as a primary indicator of carboxyl group attachment on these treated pulps. Incremental additions of laccase substrate appeared to alter the fiber to a greater extent than did bulk addition. Some oxidative modifications by enzymes can result in increased strength properties or lower energy needs during refining. These enzyme studies need to be explored further to determine if there is a possibility of applications in industry.

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References

1. Lund, M.; Ragauskas, A. J. *Applied Microbiology and Biotechnology* **2001,** 55,699-703.
2. Huttermann, A.; Mai, C.; Kharazipour, A. *Applied Microbiology and Biotechnology* **2001,** 55,387-394.
3. Huttermann, A.; Majcherczyk, A.; Braun, L. A; Mai, C.; Fastenrath, M.; Kharazipour, A.; Huttermann, J.; Huttermann, A. H. *Naturwissenschaften* 2000,87,539-541.
4. Mai, C.; Milstein, O.; Huttermann, A. *Applied Microbiology and Biotechnology* **1999,** 51,527-53 1.
5. Kharazipour, A.; Schindel, K.; Huttermann, A. In *Enzyme applications in fiber processing,* ed.; Paulo, A. C., American Chemical Society: Washington, 1998; pp 99-115.

6. Felby, C.; Olesen, P. O.; Hansen, T. T., In *Enzyme applications in fiber processing,* ed.; Paulo, A. C., American Chemical Society: Washington, 1998; pp 88-98.
7. Felby, C.; Hassingboe, J.; Lund, M. *Enzyme and Microbial Technology* **2002,** 31,736-741.
8. Chandra, R. P.; Ragauskas, A. J. In *Laccase: The renegade of fiber modification,* Tappi Pulping Conference, 2001. Tappi Press, Atlanta: 2001.
9. Felix, J. M.; Gatenholm, P. *Nordic Pulp and Paper Research Journal* **1993,** 1,200.
10. Tshabalala, M. A. *Journal of Applied Polymer Science* **1997,** 65, 1013-1020.
11. *Tappi test methods.* ed.; TAPPI Press: Atlanta, GA, 2000..
12. Xu, F., *Biochemistry* **1996,** 35,7608-7614.
13. Waleka, J. A., *TAPPI* **1956,** 39, (7), 458-463.
14. Fardim, P.; Holmbom, B.; Ivaska, A.; Karhu, J. *Nordic Pulp and Paper Research Journal* **2002,** 17,346-350.
15. Philipp, B.; Rehder, W.; Lang, H., *Papier* **1965,** 19, 1-10.
16. Leatham, G.; Myers, G. C. *Tappi Journal* **1990,** (April), 192-197.

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