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Report on Laccase modification of fibre joint strength.

Objective: *Increase delamination of CTMP middle layer for paperboard construction.*

INTRODUCTION

Enzymes are suitable tools for achieving new properties and reducing the amount of chemicals used within the paper industry. Some applications have been widely explored and were implemented at industrial level. The more recent developments in the field of molecular biology have prompt new opportunities from improved biocatalysers. In the last years the major focus of enzymatic applications in the pulp and paper field has shifted toward pulp fibre modification with the aim of producing improved and/or innovative materials.

Laccase is a multicopper oxidase that catalyses the oxidation of aminophenols, polyphenols and polyamines with the concomitant reduction of oxygen to water. Although the possibility of modifying pulp fibres with laccase is limited to lignin-containing pulp, it represents one of the most interesting enzymes with respect to fibre modification. Several papers show that laccase can enhance paper properties through direct lignin oxidation in the fibres or by grafting new molecules to lignin, thus creating new types of chemical bonds on the surface. In the first 18 months of the project we have explored the use of laccase for improving delamination properties of CTMP intended to be used in the middle layer of paperboard.

MATERIALS AND METHODS

Enzymes.

In this work a commercial laccase from Novozymes (Novozym 51003) and a partially purified laccase from *Trametes pubescens*, produced in our laboratory, have been tested. *T. pubescens* laccase was produced in a 12 litres LKB fermenter using a previously reported medium. The laccase production was induced by copper addition in the media the second day of growth, after the induction, laccase activity was measured daily and the fermenter was usually stopped after 15 days of fermentation. Commonly, the maximum laccase activity was in the range of 70-80 U/ml.

Pulp treatments.

The pulp has been usually treated for 1 hour at 50°C with 15-100 U of enzyme/g of oven dried pulp. The reaction with *T. pubescens* laccase has been performed at pH 5.0 in 50 mM citrate-phosphate buffer in a semi-closed reactor with 2% pulp consistency supplying a constant oxygenation (air) by a membrane pump. The same conditions were applied for the commercial laccase but the pH was set at 6.0. The laccase treatments were also conducted in the presence of nanofiltered low molecular weight lignin from black liquor (NFL) supplied from KTH.

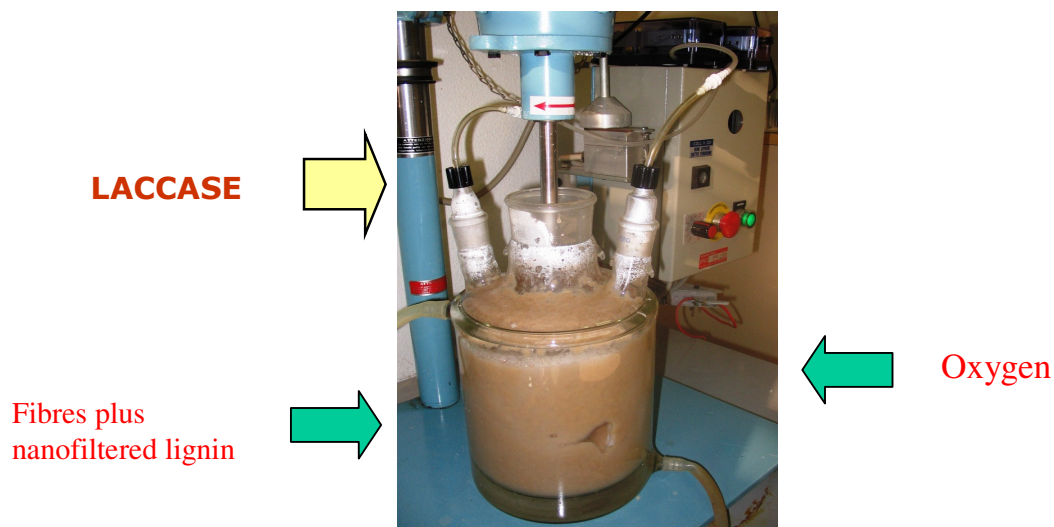


Fig. 1 Reactor used for laccase treatments

Handsheets preparation.

The handsheets were prepared according to a modification of ISO 5269-1:1998 method (Conventional sheet-former method). In the modified method the target grammage was 140 g/m² and the handsheets were pressed at 6 bars instead of 4 bars. In some experiments the different densities were obtained by pressing at 4, 6 and 8 bars in a cold press apparatus. After pressing, the handsheets were dried for 1 hour at 90°C and conditioned 24 hours at 23°C /50% RH before testing.

RESULTS

Chemical characterisation of materials and treated samples.

The CTMP pulp supplied by KCL has been characterised with respect to Kappa number, Klason lignin and the amount of phenolic groups in lignin.

Table 1. CTMP pulp characterisation.

Parameter	
Kappa number	112
Klason lignin	24
Average molecular weight, Daltons (GPC in THF)	22.000
Phenols/aromatic ring by NMR, %	14

The CTMP pulp contains a significant amount of lignin which represents the laccase substrate, however, taking into account the percentage of phenols per unit of aromatic ring it appears clear that laccase can react with a limited number of “reactive sites” along the chain. Laccase oxidises phenols in lignin through the formation of phenoxy radicals. However, the enzyme is too large to access the fibre wall and therefore the oxidation is most likely restricted to the surface of the fibres. Besides direct lignin oxidation, laccase can perform an indirect reaction on lignin, through the oxidation of low molecular weight aromatic compounds (mediators), which in turn oxidise lignin present in the fibres. The advantage of such compounds are generally a higher redox potential and a greater diffusion within the fibre cell wall, that allows to reach the lignin not located on the surface. In our work we have

selected a low molecular weight nanofiltered lignin (NFL) supplied by KTH, to replace the commonly used aromatic mediators.

The nanofiltered lignin was characterised in our laboratory with respect to its molecular weight and phenolic content. The average molecular weight corresponds to an oligomer formed by approximately 13-15 phenylpropanoid units. Although the distribution of molecular weights is quite narrow, units as small as tetramers can also be present in the mixture. The relevance of this compound for laccase is mainly related to its size and high phenolic content that makes it a suitable reactive substrate for obtaining fibres cross-linking.

Table 2. Nanofiltered lignin characterisation.

Parameter	NFL
Average molecular weight, Daltons (GPC in THF)	2500
Phenols/aromatic ring by NMR, %	36

Preliminarily, laccase was tested for its ability to polymerise lignin present into fibres. Lignin extracted by dioxane/water from CTMP pulp samples treated with laccase were analysed by gel permeation chromatography to verify whether or not lignin polymerisation occurred. The limited number of phenol active sites of CTMP lignin (14%) did not prevent laccase polymerisation of lignin as shown in figure 2, the average molecular weight was increased from 22.000 Daltons of reference pulp to 35.000 Daltons after the treatment with laccase.

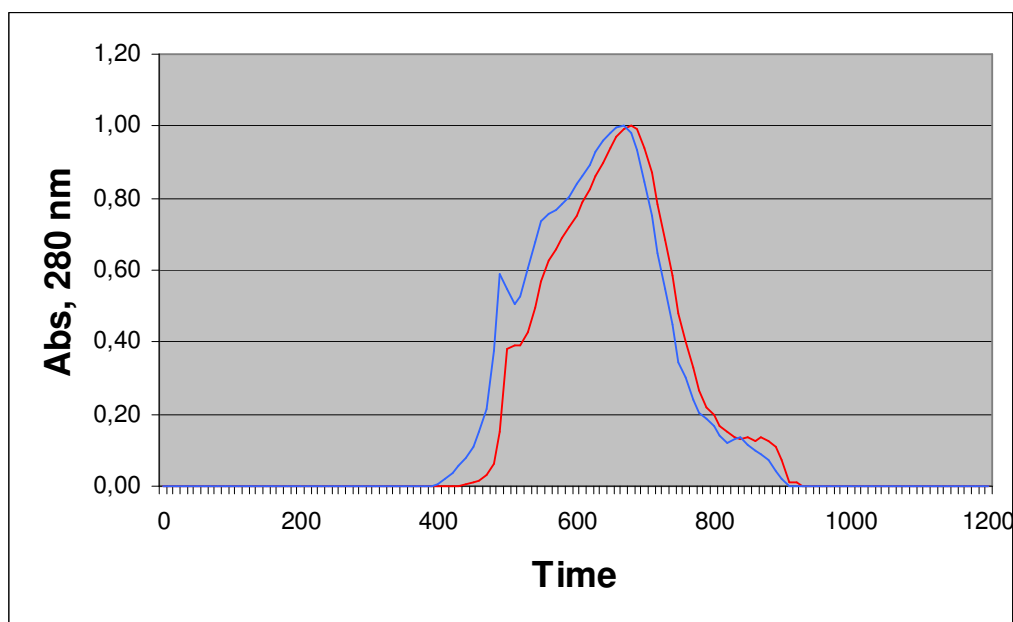


Fig.2. Effect of laccase on molecular weight distribution of dioxane isolated lignin from CTMP pulp. Reference (red line). Laccase treated sample (Blue line).

Although lignin polymerisation is an indirect evidence of cross-linking, this last phenomena cannot be taken for granted for at least three reasons:

- The molecular weight distribution was obtained on dioxane isolated lignin, which represents approximately 20-30% of total Klason lignin.
- The polymerisation could occur mainly on colloidal lignin giving raise to the formation of a matrix around the fibres instead of cross-linking in the fibre wall.
- The bi-dimensional ^{13}C -NMR analysis failed to show us the formation of new type of bonds in the isolated lignin.

Physic-mechanical characterisation

A preliminary screening of laccase and laccase NFL system effects on mechanical and optical properties was performed using *Trametes pubescens* laccase (Table 3).

Table 3. Mechanical properties of CTMP pulp after laccase and laccase-NFL treatments.

Property	Reference	Tpl 15 U/g	Tpl 30 U/g	Tpl 15 U +NFL(1%)
Scott Bond (J/m ²)	58.9	65.2	70.4	64.1
Tensile Strength Index (Nm/g)	26.8	28.6	26.5	28.5
Wet Tensile Strength index (Nm/g)	0.47	0.68	0.64	0.80
Tensile Energy Absorption index (mJ/g)	351	416	348	359
Tensile Stiffness Index (kNm/g)	2.51	2.78	2.53	2.58
Modulus of elasticity (MPa)	938	1074	961	977
Tear resistance (mNm ² /g)	8.75	10.49	9.16	8.64

Tpl = *Trametes pubescens* laccase.

NFL = Low molecular weight nanofiltered lignin from black liquor.

It can be observed that the whole mechanical properties were increased by laccase treatment (15 U/g); however, a dramatic effect was seen only for wet tensile strength index, which showed the greatest improvement, 45%. The use of 1% NF lignin in combination with laccase led to an increase only of the wet tensile strength index, which under these conditions was 70% higher than the reference CTMP and 25% with respect to laccase alone treatment.

Besides wet tensile strength, tear resistance and tensile energy absorption were also increased by approximately 20%, while Scott bond enhancement was only 10%.

The increase of *Trametes pubescens* laccase dosage from 15 U/g to 30 U/g affected only the Scott bond value, which was 10 % higher than the reference using the low laccase dosage and 20% when twice the amount of laccase was applied. In contrast all the other parameters were unaffected and for most properties the results with the higher laccase dosage were even worse than the lower dosage. Since the enzyme was only partially purified the presence of interfering substances that might affect the results at high dosage could not be ruled out.

Wet tensile strength enhancement was function of density as shown in fig. 3. When the handsheets were made at higher density, obtained pressing at 6 bars instead of 4 bars, the effect of the laccase/NF lignin system on the wet tensile index was much greater reaching 140% increase of the value versus the reference pulp.

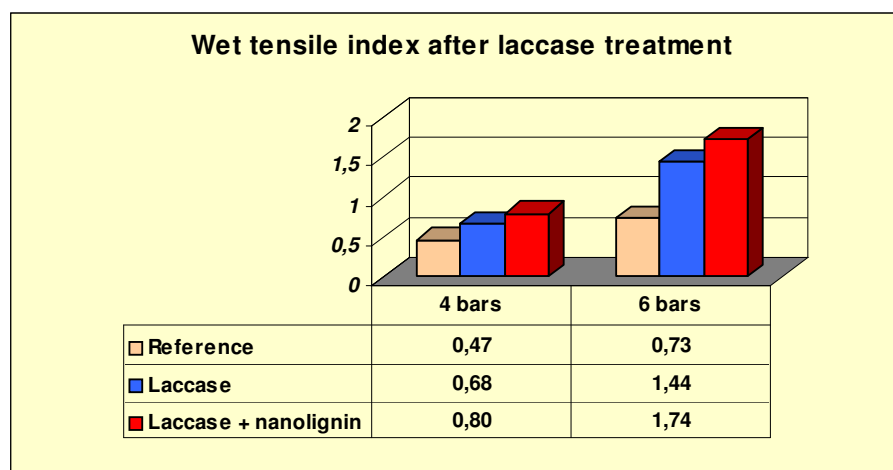


Fig. 3. Effect of laccase nanofiltered lignin system on wet tensile strength index at two different densities.

With reference to the optical properties reported in Table 4 it can be observed that laccase treatment did not substantially decreased the ISO brightness and L^*/b^* values while the change of a^* value was more pronounced. On the contrary, the addition of low molecular weight nanofiltered lignin from black liquor caused a relevant change of all optical parameters but the b^* value.

Table 4. Optical properties after laccase treatments.

Treatment	ISO Brightness	L^*	a^*	b^*
Reference	66.1	92.6	-1.6	14.0
<i>T. pubescens</i> laccase (TpL)	63.1	91.2	-0.8	14.0
Tpl + NF lignin	54.4	86.6	2.9	14.1

Delamination properties.

The improvement of the internal bond strength was the major objective of our work and therefore further experiments were carried out with the commercial laccase at higher enzyme dosage. CTMP pulp was treated with Novozym 51003 at low dosage (15 U/g of oven dried pulp) and high dosage (100 U/g oven dried pulp). A comparison between TpL and commercial laccase was also carried out. As can be observed in the figure 4, TpL was slightly more effective than commercial laccase and showed a little synergistic effect in combination with nanofiltered lignin. On the contrary, in the presence of nanofiltered lignin Novozyme laccase showed even lower values than the reference pulp. TpL posses a higher redox potential in comparison to Novozyme laccase that may lead to a greater oxidation of nanofiltered lignin, however, our conclusion is that nanofiltered lignin does not significantly improve the laccase action for the modification of delamination properties. The difference

observed in the pulp reference Scott bond values is probably due to the different pH of the process that could influence handsheet formation.

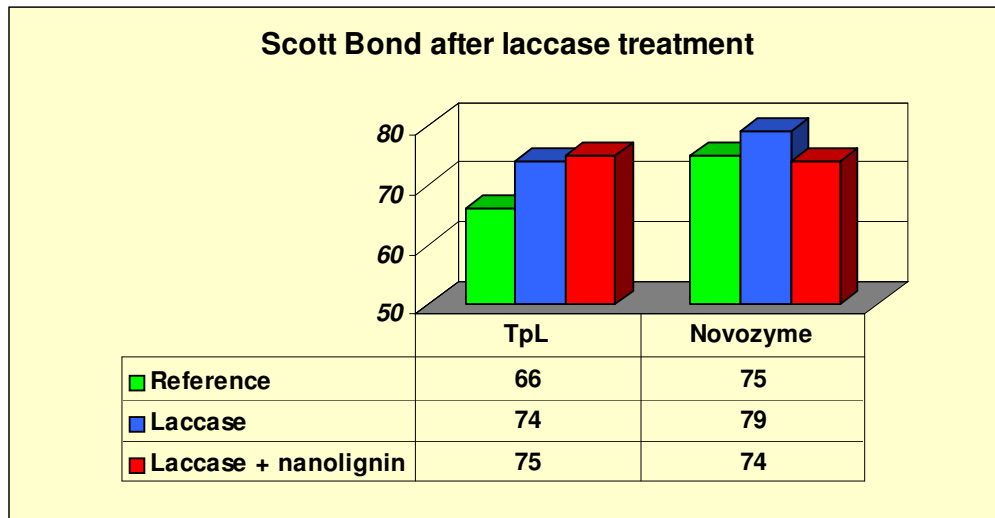


Fig. 4. Effect of laccase and laccase nanofiltered lignin system on Scott Bond values.

In the figure 5 the behaviour of Scott bond values versus handsheets density is reported for Novozyme laccase and laccase/nanofiltered lignin treatments. Apparently, increasing the handsheet density the laccase treatment seems more effective whereas the use of nanofiltered lignin did not lead to higher values at any density.

The laccase treatments with commercial laccase increased the Scott bond values in the range of 10% depending on laccase and handsheet density. The increase of enzyme dosage from 15 U/g of pulp to 100 U/g did not further increase the Scott bond values (data not shown).

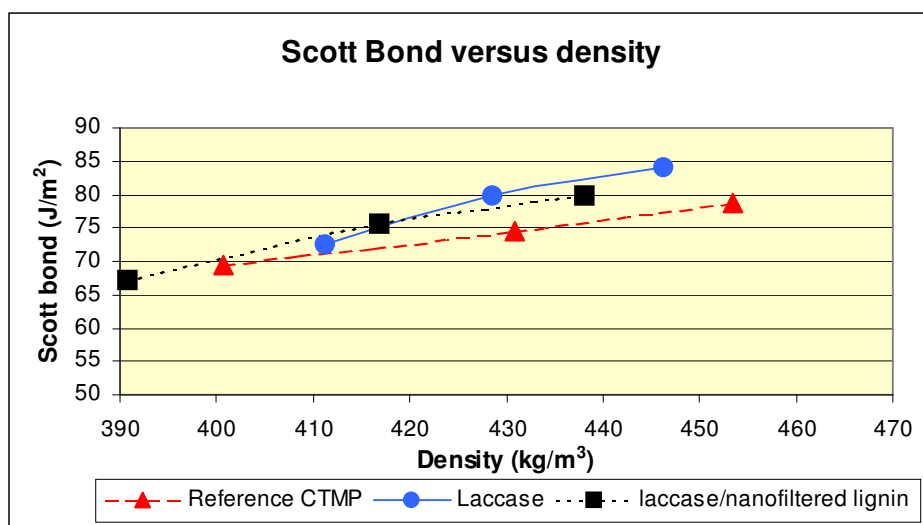


Fig. 5. Effect of Novozym 51003 laccase and laccase nanofiltered lignin system on Scott Bond values for different handsheet densities.

The Scott bond value is considered a fair method for measuring the bonding strength of a single ply, however, a comparison between Scott bond and z-directional strength methods was

performed. The same handsheets, reference and laccase treated samples, made at SSCCP were used to determine the Scott bond value (SSCCP) and z-directional strength (STFI). As evidenced in Fig.6, the trend of the results was similar, in both cases the laccase treatment increased the value with respect to the reference CTMP pulp and the addition of nanofiltered lignin did not result in any significant gain. However, the percentage of increase was greater when measured with the z-strength method.

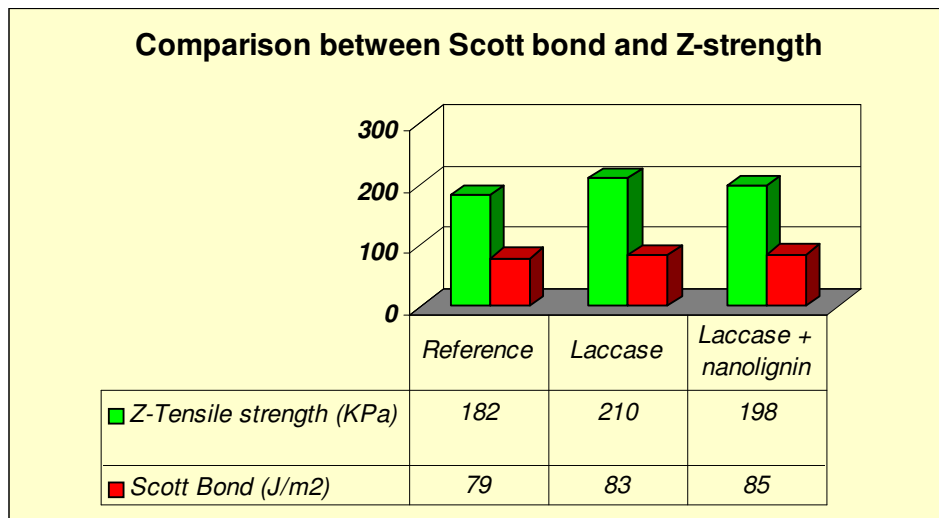


Fig. 6. Effect of Novozym 51003 laccase and laccase nanofiltered lignin system, on Scott Bond and Z-strength values.

Taking into account the z-directional tensile index the bonding strength of CTMP middle layer can be increase up to 15% by laccase treatments.

Conclusions

Laccase nanofiltered lignin system showed a remarkable improvement of wet tensile strength of CTMP pulp that was function of handsheet density. Tear resistance, tensile energy absorption and Scott bond were also affected, although to a lower extent, by the laccase treatments. The addition of nanofiltered lignin did not show any gain with respect to laccase alone but for wet tensile strength.

With reference to the more detailed experiments concerning the joint strength in the z-direction it has been observed that Scott bond values were 10% higher after laccase treatments moreover, increasing handsheet density a slight improvement of Scott bond values after laccase treatment versus reference pulp was observed. On the contrary, no significant differences were obtained using different laccases.

Measuring the effect of laccase treatment by z-directional strength method a 15% enhancement was detected instead of 10% measured by Scott bond.

In the next months besides the optimisation of laccase treatment with respect to time, temperature, pH, oxygen and fibre concentration the following approaches will be undertaken:

- laccase activation of fractionated lignin-rich fines to be added in the middle layer,
- fibre functionalisation with low molecular weight monomers (i.e. isoeugenol/gallic acid) for improving z- directional strength.