

# Lignin-Laccase-Mediator-Systems (LLMS) for the Production of Binderless Medium Density Fiberboards (MDF)

Markus Euring<sup>1</sup>, Alexander Kirsch<sup>1</sup>, Patricia Schneider<sup>1</sup> & Alireza Kharazipour<sup>1</sup>

<sup>1</sup> Faculty of Forest Science and Forest Ecology, Büsingen-Institute, Department of Molecular Wood Biotechnology and Technical Mycology, Georg-August-University of Goettingen, Germany

Correspondence: Markus Euring, Faculty of Forest Science and Forest Ecology, Büsingen-Institute, Department of Molecular Wood Biotechnology and Technical Mycology, Georg-August-University of Goettingen, Germany.  
E-mail: meuring@gwdg.de

Received: November 30, 2015 Accepted: December 14, 2015 Online Published: January 4, 2016

doi:10.5539/jmsr.v5n2p7

URL: <http://dx.doi.org/10.5539/jmsr.v5n2p7>

## Abstract

Medium density fiberboards (MDF) are produced mainly by urea-formaldehyde resins (UF) as binding agent, which are synthesized from finite fossil resources. Those boards may emit critical amounts of formaldehyde, which can influence the health of humans and animals. In recent times the wood panel board industry is looking for alternative glues which contain less or no formaldehyde. In order to avoid potential formaldehyde emissions altogether it would be preferable not to use binders which are formaldehyde based at all. One possibility is to use natural binders or to activate the wood fibers' own binding forces by applying Laccase-Mediator-Systems (LMS). As a support of the LMS interactions with wood fibers technical lignin can be added. In this study it was found out that the addition of technical lignin intensified the fiber to fiber bindings. Two Lignin-Laccase-Mediator-Systems (LLMS) were analysed by Gel-Permeation-Chromatography and Cyclic voltammetry. Later the LLMSs were tested in the pilot scale production of MDF. The determination of the physical technological properties revealed that the LLMS treated MDF have higher dimension stabilities than only LMS treated MDF and approximately the same thickness swelling after 24 h. The results indicate that the application of LLMSs have a high potential for natural bonded MDF.

**Keywords:** technical lignin, laccase, mediator, Laccase-Mediator-System, Lignin-Laccase-Mediator-System, Medium density fiberboard, Gel-Permeation-Chromatography, Cyclic voltammetry

## 1. Introduction

Approximately ten million square meters of medium density fiberboards (MDF) are produced yearly in Europe (EPF, 2013). About 20% of the production costs can be attributed to the use of mainly petrochemical binders (Kharazipour, 2004). Here we refer to mainly the use of urea-formaldehyde resins (UF) and melamine-urea-formaldehyde resins (MUF) which are synthesized from finite fossil resources. Due to fluctuating raw oil prices, as well as the dependence of the wood-based materials industry on these petrochemical binding agents, there has been a noticeable price increase in wood-based products in recent years (Kharazipour, 2004). In addition the formaldehyde emissions from wood-based building materials are regarded critically and can be attributed to the use of formaldehyde based binders. At certain concentrations formaldehyde can induce irritation of the eyes, mucus membranes and skin (Yang et. al., 2001; IARC, 2004). In 2004 formaldehyde was classified as a carcinogen by the International Agency for Research on Cancer (IARC, 2004). Accordingly, increased research attention has focused on appropriate measures of reducing formaldehyde emissions in products in which it is contained (Oberdorfer, 2008).

At the beginning of 2009 in California, the first stage in reducing the formaldehyde emissions in the field of wood based products came into force. The "CARB" standards (CARB = California Air Resources Board) as an example, required for medium density fiberboards (MDF) a reduction of the formaldehyde emissions from the local norm of 0.3 ppm to 0.21 ppm (CARB, 2008). In 2011 the second stage in the reduction of emissions to an upper limit of 0.11 ppm came into force. For economic reasons, the European manufacturers of wood-based products oriented themselves on CARB and EN13986 with the emissions class E1 and E2 and also followed the recommendations of IKEA IOS MAT 003 as well as EPF-S (European Panel Federation-standard) in which the actual permitted formaldehyde emissions are lower than the top limit recommended by the CARB standards (SUBSPORT, 2013). It

remains to be seen what the future will bring in terms of regulations and measures to be taken with regard to formaldehyde emissions, considering that in 2014 formaldehyde was upgraded in terms of carcinogenicity from category 2 (suspected of carcinogenic properties) to category 1B, and in terms of mutagenicity from category 3 (possibly causing genetic mutations) to category 2 according to EU regulation 605/2014 (2014). Category 1B means that formaldehyde now is considered to be a probable cause of cancer in humans and that the cancer causing effect has been proven in animals (EG 1272/2008, 2008). Category 2 in terms of mutagenicity, classifies formaldehyde as a material potentially harmful for humans in that it could possibly cause genetic mutations in germ cells (EG 1272/2008, 2008). Many manufacturers of petrochemical products containing formaldehyde are exploring new ways for handling formaldehyde, while taking into consideration all current regulations (Formacare, 2015). On the one hand discussions are taking place regarding a reduction of formaldehyde in these products and on the other a reduction in the use of formaldehyde in the actual production process (Wiechmann & Kohla, 2014). In local industry, no harmful emission levels for employees in the manufacturing process of wood-based materials with binders containing formaldehyde have been measured. However various possibilities to reduce emissions are being evaluated (Wiechmann & Kohla, 2014). In order to avoid potential formaldehyde emissions altogether it would be preferable not to use binders which are formaldehyde based at all.

In this respect several wood processing factories, in particular those for wood-based products, are currently occupied with testing low-emission or emission free binders. A possible solution can be found in the development of natural binding agents (Müller et al., 2007, Euring et al., 2011, 2013, and 2015). The Lignin-Laccase-Mediator-System is a naturally based binding agent and can offer the wood-based product industry a chance to establish environmentally acceptable production methods.

Lignin occurs in quantities of approximately 50 million tons worldwide as a byproduct of cellulose manufacture (15 million tons of lignin-sulfonates and 35 million tons of kraft-lignin) (Puls, 2009). In fact lignin is a mass product for which there are hardly any uses. According to Glasser (2009) only 2% of the technical lignins worldwide are used in industrial processes, although they belong to the most inexpensive of raw materials (approx. 0.15 €/kg). The remaining lignins are burnt for energy generation, or not used at all. For this and other reasons, technical lignins which are available widely and in large quantities, are the most promising polyphenol for the development of binding agents.

It makes sense to use these technical lignins where the natural lignins are already present on the wood fibers due to the high content of phenol groups. This is amongst others, the case with fibers which have been through a thermo-mechanical pulp process (TMP). The middle-lamella lignins in the wood fibers are incrustated on the fiber's surface after the thermo-mechanical process, an effect which is recognized as a significant chemical transformation (Sixta, 2006). The laccase can activate these surface lignins through oxidation resulting in free lignin fractions, which when hot pressed to fiber board cause the fibers to adhere to one another creating a fiber to fiber bond (Hüttermann et al., 2001, Kharazipour et al., 1997, 1998). The utilization of technical lignins and mediators as catalyzers should accelerate and intensify this process, which should lead to a more potent adhesion of the wood fibers (Euring, 2008).

This study will investigate formaldehyde free wood fiber products, specifically medium density fiber-boards (MDF). These wood fiber products should be produced without the addition of conventional, petrochemical binding agents, using a Lignin-Laccase-Mediator-System (LLMS). The use of Laccase-Mediator-Systems has already been comprehensively tested. The results showed that when using an LMS of laccase and 4-hydroxycbenzoic acid, not all mechanical and physical properties were satisfactory (Euring et al., 2011, 2013). The use of technical lignins in LLMS activates additional bonding strength. The wood fiber surface lignins which have been activated with the laccase and mediator should be supported by the use of technical lignin components. For the first time this study will test the applicability of the mediators caffeic acid (CA) and vanillyl alcohol (VA) in the production of MDF.

LLMS should make a stronger bond between the individual wood fibers possible. The implementation of LLMS should be integrated seamlessly into the conventional fiberboard production process. In preliminary stage to the production and testing the physical-technological properties of the MDF, analyses of lignin molecular weight through gel permeation chromatography (GPC) and also of the redox potential of Laccase-Mediator-Systems using a potentiostat, will take place. Those analyses are helpful to understand LMS or LLMS actions. The results and experiences e. g. the optimal LMS or LLMS concentrations will directly be transferred to the MDF production

## **2. Materials and Methods**

### *2.1 Materials*

#### *2.1.1 Wood fibers*

The TMP (thermo-mechanical pulping) -fibers originate from the company Gutex (Waldshut-Tiengen) and consist of 80% spruce and 20% fir fibers.

### 2.1.2 Lignin

Borremet Ca 120 (Calcium-Ligninsulfonate, Borregaard LignoTech, Germany)

### 2.1.3 Laccase

Novozym 51003 (from the company Novozymes, Bagsvaerd, Denmark) fermented from *Trametes vilosa* in recombination with *Aspergillus oryzae*, at hand in liquid form and brown in color due to stabilizer. Its specific activity was measured by determining the oxidation of ABTS (Matsumura et al. 1986). The activity of the laccase stock was about 1,000 U/ml.

### 2.1.3 Mediators

Caffeic acid (CA): from the company Sigma-Aldrich (Seelze, Germany) with a chemical purity of 95%.

Vanillyl alcohol (VA): 4-Hydroxy-3-methoxybenzyl alcohol, from the company Sigma-Aldrich (Seelze, Germany) with a chemical purity of 98%.

### 2.1.4 McIlvain Buffer

Composed of 0.2 M di-potassium hydrogen phosphate ( $K_2HPO_4$ ) and 0.1 M citric acid ( $C_6H_8O_7$ ), buffered to pH 6.0. Both components were from AppliChem, Darmstadt, Germany.

### 2.1.5 Sodium Acetate Buffer

To carry out the potentiostatic trial, a sodium acetate buffer was used in order hold a pH level of 6 constant throughout the tests. The buffer was made by producing 0.1 M sodium acetate (NaAc) solution. Subsequently the pH value was brought to 5 using acetic acid.

## 2.2 Methods

### 2.2.1 Determination of the molecular weight of the lignin using Gel-Permeation-Chromatography (GPC).

Gel-permeation chromatography was used to determine the molecular weight distribution of the lignin prior to, and following, incubation with laccase or respectively laccase and mediator. The measurements were made using a high pressure liquid chromatography (HPLC) unit from Waters. The HPLC method used here is referred to as Gel-permeations chromatography as reference to the use of gel columns in the HPLC instrumentation. In this case it is a special method of column chromatography which is suitable in particular for separating molecules and therefore for establishing molecular weights.

The HPLC instrumentation is comprised of the following:

Waters e2695 Separation Module, Waters 2489 UV-Detector,

GPC- columns: SHODEX KF-803, SHODEX KF-804, SHODEX KF-G

Computer for recording and analyzing the results, Software: Waters Empower 3 Personal

Eluent (mobile phase): water/acetonitrile 80 : 20 (v : v), pH 7.8

Standards: Polystyrene Standards with molecular weights from 2000–150000 Daltons

Sample preparation was as follows:

0.5 g Ca-Ligninsulfonate were added to 27 ml McIlvain buffer (pH 6.0). Depending on the type of sample either 10 U/ml laccase or 10mM mediator were added to the buffer solution. The samples were incubated for 15, 30 and 60 minutes in order to compare the molecular weights. Following the incubation time the samples containing laccase were stopped through being briefly brought to the boil.

Subsequently 20  $\mu$ l from each sample was taken, put into vials, and placed into the GPC, where measurement of the samples took place. The previously recorded values for the polystyrene standards were used as reference values for the evaluation software in calculation of the lignin molecular weights.

Measurements of both controls and samples took place in a UV detector at a wavelength of 254 nm. The eluent used was a water/acetonitrile mixture (80 : 20, v : v, pH 7.8). All results were recorded and calculated using software from Waters, and can be portrayed as spectra using the weight average molecular weight (Mw), the number average Molecular weight (Mn) and the poly-dispersity (Mw/Mn).

### 2.2.2 Electrochemical measurements of LMS by means of Potentiostat

Cyclic voltammetry is an electrochemical method of investigation used to analyze redox reactions. Not only simple parameters, such as redox potential, but also reaction kinetic parameters such as electron transfer stages and

chemical reactions which are potentially taking place, can be recorded and analyzed by means of cyclic voltammetry.

For the purpose of this study the electrochemical measurements were used solely to analyze the redox potential of the mediators caffeic acid and vanillyl alcohol with laccase, in order to assess their reaction-kinetic suitability.

The potentiostat used was a PCSTAT101 from Metrohm Autolab B.V. from Utrecht in the Netherlands. It exhibits a three-electrode system with a carbon working electrode and a reference electrode which is immersed in a 3-molaric potassium chloride solution. The software used was also from Metrohm Autolab B.V. in the version 1.10.4.

For the analysis of the mediators caffeic acid and vanillyl alcohol with a potentiostat, the mediators were first dissolved in a sodium acetate buffer and diluted to a concentration of 0.2 mM. The laccase used in the trials diluted 100 times. For the samples with lignin 2 mM Ca-Ligninsulfonate were added to evaluate the effect of the lignin within the LLMS expecting changing redox-potentials. The measurements were made in a range from 100 mV to 1000 mV with a feed velocity of 5 mV/s. The output potential was held for 10s, before the voltage change occurred. 5 mL of mediator solution was used in each measurement. At first three measurements as a control were taken using pure mediator solution. In order to record the interaction between mediator and laccase, the laccase solution was dripped onto the working electrode. After the solution had completely dried out, a measurement was taken using a 5 ml mediator solution and the same parameters as with the pure mediator solution. Here it is important to note that after each trial the electrode was cleaned completely. Each measurement was repeated three times. The results were reported in a voltage-current strength diagram.

### 2.2.3 Production of LLMS bonded MDF

LLMS bonded MDF were produced under the following procedural conditions:

- Wood fiber amount per treatment: 20 kg
- Laccase concentration: 100 U/ml per 0.001 kg absolutely dry fibers
- Mediator concentration: 10 mM per 0.001 kg absolutely dry fibers
- Ca-Ligninsulfonate: 6% referred to 1 kg absolutely dry fibers
- Raw density: 750 kg/m<sup>3</sup>
- Board dimensions: 600 mm x 800 mm
- Board thickness: 10 mm
- Produced at an outset moisture content of the incubated fibers of 10-15%
- Comparison of the resulting physical/mechanical properties with those of the untreated fibers and those of MDF produced with UF resin.
- Press temperature: 190 °C
- Press Time: 12 s/mm

The LLMS treated wood fibers were directly transferred from the Blender to the tube dryer to guarantee an uninterrupted production of the fibers to a fiber mat formation and then pressing to MDF (equivalent to the industrial process of MDF).

The MDF were produced using the following steps.

#### 1) Blending of fibers with LLMS

The combination of fibers and each LLMS in buffer solution (McIlvain) was achieved using the Blender process in the MDF pilot plant of the Biotechnikum (Büsgen-Institute, Goettingen).

#### 2) Drying of the treated fibers

As known from previous studies a fiber moisture content of 10-15% was aimed for in the drying process, in order to support the natural binding forces, but also to avoid a loss of MDF quality associated with higher moisture levels (>15%) in the following hot-pressing process (Euring, 2008, Felby et al., 2011, Euring et al. 2011). The drying temperature was adjusted so that the incubation process with LLMS would not be stopped or interrupted.

#### 3) Fiber Mat formation

Monolayer fiber mats were generated.

#### 4) Pre-pressing and hot-pressing

Hot-pressing of the fiber mat followed in the hot press at 190 °C for 12 s/mm board thickness after pre-pressing. Both temperature and time were determined as optimal parameters for pressing of LLMS bonded MDF in pilot-scale. Each type of MDF was replicated six times.

### 5) Determining the physical-technological properties of the pilot scale MDF

Determination of the following properties was carried out after a conditioning phase of each MDF in a standard climate:

- Determination of bending strength (EN 310, 1993), sample size: 5 x 20 x 0,1 cm<sup>3</sup>, 6 samples/MDF
- Determination of internal bond strength (EN 319, 1993), sample size: 5 x 5 x 0,1 cm<sup>3</sup>, 8 samples/MDF
- Determination of the hygroscopic behavior of the material (thickness swelling and absorption) (EN 317, 1993), sample size: 5 x 5 x 0,1 cm<sup>3</sup>, 8 samples/MDF

Significant difference was calculated by using Tukey's HSD (Honestly Significant Difference) test with a significance level of  $P < 0.05$ .

### 6) Production of the reference MDF boards.

The boards used as references were produced in dry-process using the same process parameters and target sizes, as used in the production with LLMS treated wood fibers. The reference boards were produced with inactivated LLMS (cooking for 10 minutes), only with laccase, with technical Lignin and laccase, with simple LMS and with 10% UF resin like in a typical MDF in Europe. Lastly the physical-technological properties were analyzed and compared.

## 3. Results and Discussion

### 3.1 GPC Measurements

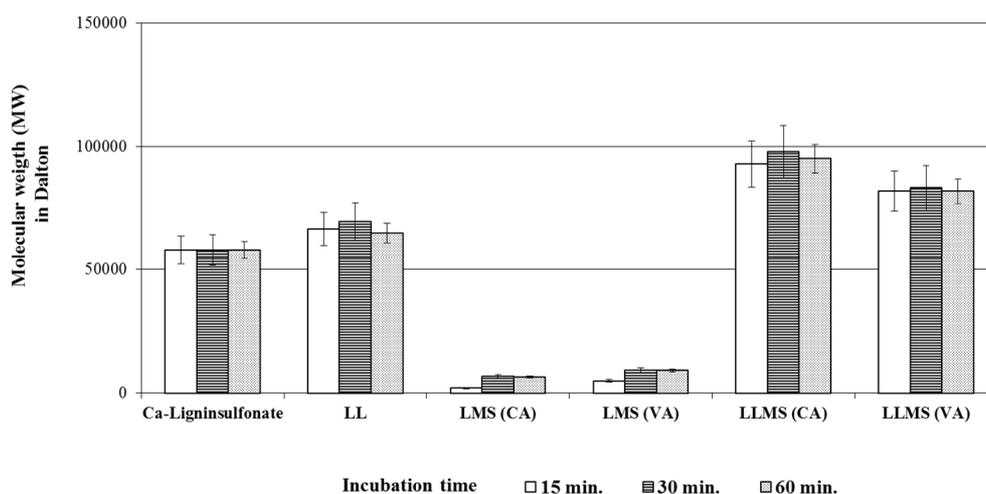


Figure 3. Average molecular weight (Mw) of Ca-Ligninsulfonate with the mediators caffeic acid and vanillyl alcohol (Laccase concentration 10 U/ml, mediator concentration 10 mM in McIlvain buffer, pH 6,0) after incubations of 15, 30 and 60 minutes. Average values from 6 trial series. Sample identifiers: Ca-Ligninsulfonate = Ca-Ligninsulfonate in buffer solution, LL = Ca-Lignosulfonate + Novozym 51003 in buffer solution; LMS (CA): Novozym 51003 + caffeic acid in buffer solution; LMS (VA): Novozym 51003 + vanillyl alcohol in buffer solution; LLMS (CA): Ca-Ligninsulfonate + Novozym 51003 + caffeic acid in buffer solution; LLMS (VA): Ca-Ligninsulfonate + Novozym 51003 + vanillyl alcohol in buffer solution

For the first time in the course of this study lignin molecular weights were determined using a water-based eluent in a GPC instrumentation unit. Up till now GPC runs of lignin were only carried out using dangerous solvents such as tetrahydrofuran (THF) (Euring, 2008, Majcherzyk & Hüttermann, 1996). During the trials of Ca-Ligninsulfonate treated with laccase and laccase mediator with incubation times of 15, 30 and 60 minutes, which were successful right from the start, it was observed that both treatment methods resulted in an increase in lignin molecular weight (see Figure 3). Untreated Ca-Ligninsulfonate kept a molecular weight of 55,000 Dalton throughout the entire analysis time, while in the case of incubation with laccase the weights increased to over 70,000 Dalton with the highest value being reached after 30 minutes. It can be assumed that the increase in molecular weight is due to oxidation of the phenol group of the lignin to phenoxy radicals (Ortner et al., 2015),

which would then be available to facilitate the subsequent bonding of the fibers to MDF. After treatment with LLMS (CA) and LLMS (VA) even higher lignin molecular weights were achieved, which can be associated to a higher effectiveness of LLMS than laccase alone. With results almost as high as 100,000 Daltons after 30 minutes incubation with LLMS containing caffeic acid, this is the most effective LLMS. The GPC tests with LMS (CA) and LMS (VA) resulted in only low molecular weights because here there is no lignin present. However, the detected molecular weights are a result of the partly phenolic structures of the mediator. The fact that all laccase, LMS or LLMS samples showed molecular weights are highest at 30 minutes incubation and sink after 60 minutes incubation can be explained by the fact that phenoxy radicals possibly react with each other and are deactivated. Thus it can be seen that the optimal incubation time is that of 30 minutes, especially when considering the process of MDF board production which usually requires 30-40 minutes from the gluing to fiber mat formation and pressing (Euring et al., 2011).

### 3.2 Cyclic Voltammograms of LLMS

The tests with caffeic acid show a significant change in the curve after addition of laccase (Figure 4). The electric current is elevated during oxidation and a shift into the negative range occurs during reduction. The cyclic voltammogram of the tests with caffeic acid show a significant interaction between laccase and mediator (CA and LMS (CA)). It can be seen that there is a reduction of current during the oxidation process and on the other hand a shift of the reduction peak into the negative. Together with Ca-Ligninsulfonate the currents become more intensive caused by the presence of lignin (Lignin + CA and LLMS (CA)). The oxidative effect of laccase is indicated by these results. Furthermore, the oxidation and therefore the radical formation are partly reversible. There is virtually a reversible electron transfer present, and the so formed radicals are partially stable.

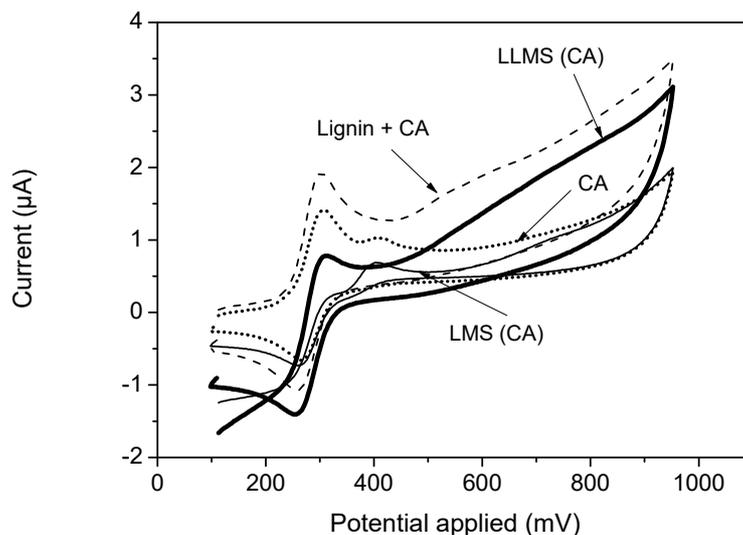


Figure 4. Cyclic voltammograms with CA = caffeic acid in buffer solution, LMS (CA) = Novozym 51003 + caffeic acid in buffer solution, Lignin + CA = Ca-Ligninsulfonate + caffeic acid in buffer solution, LLMS (CA) = Ca-Ligninsulfonate + Novozym 51003 + caffeic acid in buffer solution

The potentiostatic test of vanillyl alcohol as mediator shows a decrease of measured current during oxidation and stronger negative current during the reduction process independently if in presence of lignin (Figure 5). The cyclic voltammographic results of the mediator vanillic acid, display similar results to those with caffeic acid. The oxidative effect of the laccase on the mediator can be recognized by the decreased current during the oxidation process. The change in current during the reduction is however slightly weaker than with caffeic acid, which indicates less stable radicals (Diaz Gonzalés et al., 2009).

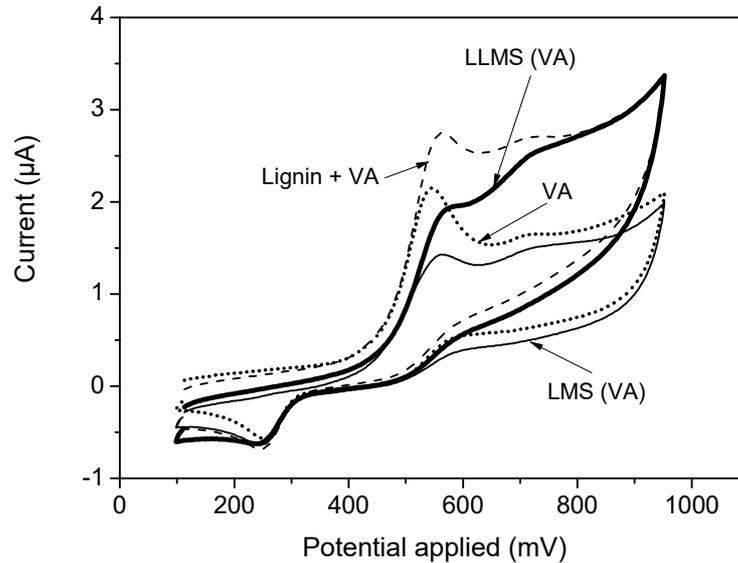


Figure 5. Cyclic voltammograms with VA = vanillyl alcohol in buffer solution, LMS (VA) = Novozym 51003 + vanillyl alcohol in buffer solution, Lignin +VA = Ca-Ligninsulfonate + vanillyl alcohol in buffer solution, LLMS (VA) = Ca-Ligninsulfonate + Novozym 51003 vanillyl alcohol in buffer solution

Based on the higher stability of the radicals, caffeic acid would appear to possess a higher potential to function in LLMS (Siebert, 2015).

### 3.3 Physical-Technological Properties of MDF Made with LLMS in Pilot Scale

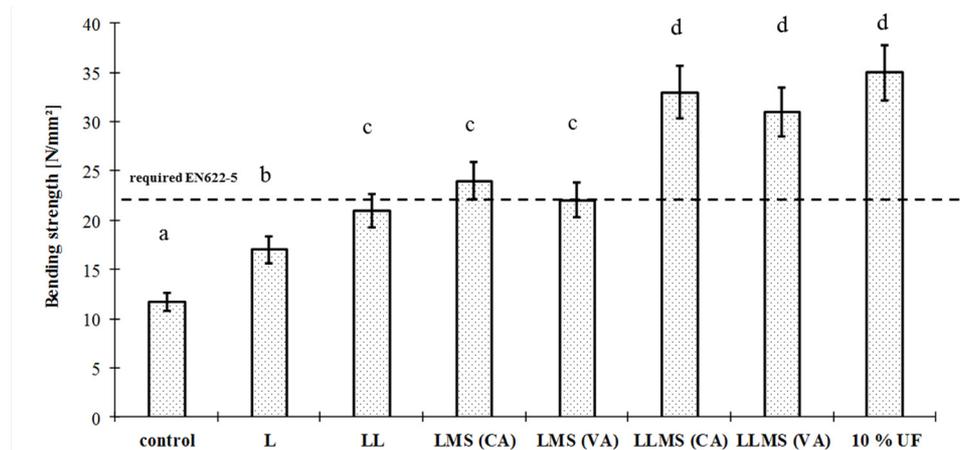


Figure 6: Bending strengths of MDF (raw density 750/kg/m<sup>3</sup>, board thickness 10mm), control (not activated LLMS), L = Novozym 51003, LL = Ca-Ligninsulfonate + Novozym 51003, LMS (CA) = Novozym 51003-caffeic-acid-system, LMS (VA) = Novozym 51003-vanillyl alcohol-system, LLMS (CA) = Ca-Ligninsulfonate-Novozym 51003-caffeic acid-system, LLMS (VA) = Ca-Ligninsulfonate-Novozym 51003-vanillyl alcohol-system, 10 % UF = reference with 10 % (in absolutely dry fibers) urea formaldehyde (UF)-resin. Means marked with different letters (a, b, c, d) are significantly different at P < 0.05

The resulting bending strengths of the MDF differed depending on the treatment of the fibers (Figure 6). In both LLMS, (CA) and (VA), the required bending strength norm of at least 23 N/mm<sup>2</sup> was exceeded by far, and almost equaled that of the control MDF with 10% UF. When applying only LMS, the bending strengths fluctuate around the required norm. The control boards, the laccase and also the laccase-Ligninsulfonate bonded MDF did not reach the required norm value.

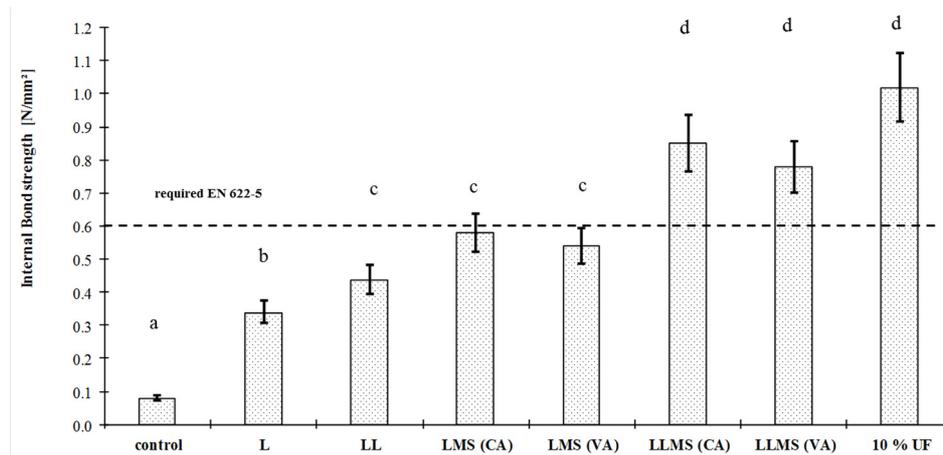


Figure 7. Internal bond strengths of MDF (raw density 750/kg/m<sup>3</sup>, board thickness 10mm), control (not activated LLMS), L = Novozym 51003, LL = Ca-Ligninsulfonate + Novozym 51003, LMS (CA) = Novozym 51003-cafeic-acid-system, LMS (VA) = Novozym 51003-vanillyl alcohol-system, LLMS (CA) = Ca-Ligninsulfonate-Novozym 51003-cafeic acid-system, LLMS (VA) = Ca-Ligninsulfonate-Novozym 51003-vanillyl alcohol-system, 10 % UF = reference with 10 % (in absolutely dry fibers) urea formaldehyde (UF)-resin. Means marked with different letters (a, b, c, d) are significantly different at P < 0.05

The values for internal bond strength of MDF bonded with 10% UF exceed those of the required norm of 0.6 N/mm<sup>2</sup> (Figure 7). The required norm is also reached distinctly with LLMS with the mediator caffeic acid or vanillyl alcohol. In the case of the MDF with the two different LMS, it didn't quite reach the norm values for IB and therefore do not comply with the required norm. The control samples and the laccase bonded MDF fall short of the norm.

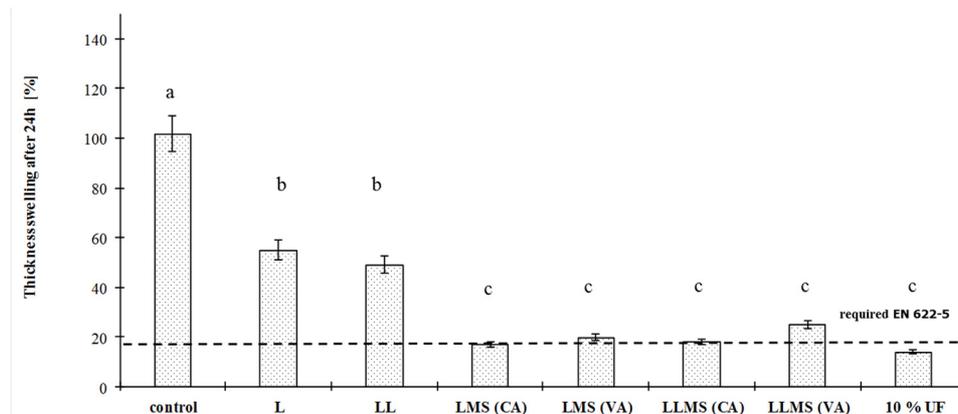


Figure 8. Thickness swellings of MDF after 24 hours in water. (raw density 750/kg/m<sup>3</sup>, board thickness 10mm), control (not activated LLMS), L = Novozym 51003, LL = Ca-Ligninsulfonate + Novozym 51003, LMS (CA) = Novozym 51003-cafeic-acid-system, LMS (VA) = Novozym 51003-vanillyl alcohol-system, LLMS (CA) = Ca-Ligninsulfonate-Novozym 51003-cafeic acid-system, LLMS (VA) = Ca-Ligninsulfonate-Novozym 51003-vanillyl alcohol-system, 10 % UF = reference with 10 % (in absolutely dry fibers) urea formaldehyde (UF)-resin. Means marked with different letters (a, b, c) are significantly different at P < 0.05

Thickness swelling values after 24 h water immersion are still too high in the LMS or LLMS bonded MDF (Figure 8). However the values are almost as good as those with 10% UF bonded MDF. All other MDF in the study showed thickness swelling values which are too high. As has been discovered in earlier studies, the hydrophilic properties of technical lignins have a negative effect on water absorption (Müller et al. 2007). The use of hydrophobic agents would be of assistance in this case. Another possibility could be to choose higher pressing temperatures than 190 °C to improve the physical-technological properties of the boards, but in industrial process temperatures of 200 °C and above are considered uneconomic, as well as too long pressing times (Dunky & Niemz, 2002).

#### 4. Conclusion

The study demonstrates that the Lignin-Laccase-Mediator-System as a natural bonding agent is very suitable for the production of MDF. In both the analytical tests and in the production of LLMS bonded MDF, significant advantages over the previous use of LMS can be seen (Euring et al., 2011, 2013).

The GPC analysis results of LLMS in comparison with the previously investigated LMS already show a significant increase in the lignin molecular weight after 15 minutes incubation, with the highest results after 30 minutes. 30 minutes incubation coincides with the duration of MDF production from blending to the pressing process of the completed product. In production terms therefore, the use of LLMS in the production of MDF is appropriate.

As shown in the positive technological mechanical properties of the so produced MDF, laccase and mediator activated wood fiber surface lignin are supported by the additional phenoxy radicals contributed by the technical lignins, and therefore facilitate stronger bonding of the individual wood fibers. Caffeic acid proved to be more effective than vanillyl alcohol in LLMS. Further tests need to follow in order to optimize the LLMS particularly with regard to thickness swelling properties.

#### Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG), grant EU 124/2-2 to Markus Euring.

#### References

- CARB. (2008). California Air resources Board. Airborne Toxic Control Measure to Reduce Formaldehyde Emissions from Composite Wood Products, April 2008, Final Regulation Order. California Environmental Protection Agency. Retrieved from <http://www.arb.ca.gov/regact/2007/compwood07/fro-final.pdf>
- Díaz González, M., Vidal, T., & Tzanov, T. (2009). Electrochemical Study of Phenolic Compounds as Enhancers in Laccase-Catalyzed Oxidative Reactions. *Electroanalysis*, 21, 2249–2257. <http://dx.doi.org/10.1002/elan.200904678>
- Dunky, M., & Niemz, P. (2002). Holzwerkstoffe und Leime - Technologie und Einflussfaktoren. Springer-Verlag Berlin Heidelberg. <http://dx.doi.org/10.1007/978-3-642-55938-9>
- EG 1272/2008. (2008). Verordnung (EG) Nr. 1272/2008 über die Einstufung, Kennzeichnung und Verpackung von Stoffen und Gemischen. Retrieved from [http://www.gaa.baden-wuerttemberg.de/servlet/is/16495/1\\_1\\_08.pdf](http://www.gaa.baden-wuerttemberg.de/servlet/is/16495/1_1_08.pdf). Accessed 19 Sep. 2015
- EN 310. (1993). Holzwerkstoffe; Bestimmung des Biege-Elastizitätsmoduls und der Biegefestigkeit. Deutsche Fassung, Beuth-Verlag.
- EN 317. (1993). Spanplatten und Faserplatten; Bestimmung der Dickenquellung nach Wasserlagerung. Deutsche Fassung Beuth-Verlag.
- EN 319. (1993). Spanplatten und Faserplatten; Bestimmung der Zugfestigkeit senkrecht zur Plattenebene. Deutsche Fassung, Beuth-Verlag.
- EU 605/2014. (2014). Verordnung (EU) Nr. 605/2014 der Kommission vom 5. Juni 2014 zur Änderung der Verordnung (EG) Nr. 1272/2008/EG des Europäischen Parlaments und des Rates über die Einstufung, Kennzeichnung und Verpackung von Stoffen und Gemischen zwecks Einfügung von Gefahren- und Sicherheitshinweisen in kroatischer Sprache und zwecks Anpassung an den technischen und wissenschaftlichen Fortschritt. Retrieved October 15, 2015 from [http://www.umwelt-online.de/regelwerk/eu/14a/14a\\_0605.htm](http://www.umwelt-online.de/regelwerk/eu/14a/14a_0605.htm)
- Euring, M. (2008). *Einsatz von Mediatoren bei der enzymatischen Aktivierung der fasereigenen Bindefkräfte zur Herstellung von enzymgebundenen, bindemittelfreien Holzwerkstoffe*. Dissertation, Faculty of Forest Sciences and Forest Ecology, Georg-August University Goettingen, 211 pages.
- Euring, M., Rühl, M., Ritter, N., Kües, U., & Kharazipour, A. (2011). Laccase-mediator-systems for eco-friendly production of medium-density fiberboard (MDF) in pilot scale: Physico-chemical analysis of the reaction mechanism. *Biotechnol. J.*, 6(10), 1253-1261. <http://dx.doi.org/10.1002/biot.201100119>
- Euring, M., Trojanowski, J., & Kharazipour, A. (2013). Laccase-Mediator catalyzed modification of wood fibers - Studies on the mechanism and medium-density fiberboard (MDF) making. *For. Prod. J.*, 63(1/2), 54-60. <http://dx.doi.org/10.13073/FPJ-D-12-00075>
- Euring, M., Kirsch, A., & Kharazipour, A. (2015). Hot-Air/Hot-Steam Process for the Production of Laccase-Mediator-System Bound Wood Fiber Insulation Boards. *BioResources*, 10(2), 3541-3552. <http://dx.doi.org/10.15376/biores.10.2.3541-3552>

- Felby, C., Hassigboe, J., & Lund, M. (2002). Pilot-scale production of fiberboard's made by laccase oxidized wood fibres: board properties and evidence for cross-linking of lignin. *Enz. Microbial Technol.*, *31*, 736-741, [http://dx.doi.org/10.1016/S0141-0229\(02\)00111-4](http://dx.doi.org/10.1016/S0141-0229(02)00111-4)
- Formacare. (2015). REACH Regulation and Formaldehyde. Retrieved October 16, 2015, from <http://www.formacare.org/regulatory-information/reach>
- Glasser, W. G. (2009). Lignin-Retrospect and prospect. In: Stoffliche Nutzung von Lignin. Gülzower Fachgespräche, Nand 31, Fachagentur für Nachwachsende Rohstoffe e.V. (FNR), 42-62
- Hüttermann, A., Mai, C., & Kharazipour, A. (2001). Modification of lignin for the production of new compounded materials. *Appl. Microbiol. Biotechnol.*, *55*, 387-394. <http://dx.doi.org/10.1007/s002530000590>
- IARC (2004): International Agency for Research of Cancer): *IARC classifies formaldehyde as carcinogenic to humans*. Press release nr. 153, 15. April 2004.
- Kharazipour, A., Hüttermann, A., & Lüdemann, H. D. (1997). Enzymatic activation of wood fibres as a mean for the production of wood composites, *J. Adhes. Sci. Technol.*, *11*, 419-427. <http://dx.doi.org/10.1163/156856197X00796>
- Kharazipour, A. (2004). Holz als Werkstoff. Im Jahrbuch 2004/2005 Nachwachsende Rohstoffe, Wirtschaftsfaktor Biomasse, C.A.R.M.E.N, 335-337
- Kharazipour, A., Schindel, K., & Hüttermann, A. (1998). Enzymatic activation of wood fibers for wood composite production. *ACS Symposium Series*, *687*, 99-115. <http://dx.doi.org/10.1021/bk-1998-0687.ch009>
- Majcherzyk, A., & Hüttermann, A. (1996). Size-exclusion chromatography of lignin as ion-pair complex. *J. Chromatogr. A* *764*, 183-191. [http://dx.doi.org/10.1016/S0021-9673\(96\)00909-0](http://dx.doi.org/10.1016/S0021-9673(96)00909-0)
- Matsumura, E., Yamamoto, E., Numata, A., Kawano, T., Shin, T., & Murao, S. (1986). Structures of the laccase-catalysed oxidation products of hydroxybenzoic acids in the presence of ABTS. *Agric. Biol. Chem.*, *50*(5), 1355-1357.
- Müller, C., Kües, U., Schöpfer, C., & Kharazipour, A. (2007). Natural Binders. In U. Kües (Ed.), *Wood Production, Wood Technology, and Biotechnological Impacts* (Chapter 16, pp. 347-382). Universitätsverlag Göttingen.
- Oberdorfer, G. (2008). Formaldehydemissionen aus Holzwerkstoffen. *Holzforschung*, *2008*, *4*, 8-9.
- Ortner, A., Huber, D., Haske-Cornelius, O., Weber, H. K., Hofer, K., Bauer, W., ... Guebitz, G. M. (2015). Laccase mediated oxidation of industrial lignins: Is oxygen limiting? *Process Biochem*, *50*(8), 1277-1283, <http://dx.doi.org/10.1016/j.procbio.2015.05.003>
- Puls, J. (2009). Lignin-Verfügbarkeit, markt und Verwendung: Perspektiven für schwefelfreie Lignine. In: Stoffliche Nutzung von Lignin. Gülzower Fachgespräche, Band 31, Fachagentur für Nachwachsende Rohstoffe e.V. (FNR), 18-41
- Siebert, J. (2015). Untersuchungen zu neuen Mediatoren im Laccase-Mediator-System. Master Thesis. Faculty of Forest Sciences and Forest Ecology, Georg-August University Goettingen, 60 pages
- Sixta, H. (2006). *Handbook of pulp*. 2. Aufl., Wiley-VHC.
- SUBSPORT. (2013) *Specific substances alternatives assessment formaldehyde*. Retrieved November 6, 2015, from <http://www.subsport.eu/wp-content/uploads/data/formaldehyde.pdf>
- Wiechmann, B., & Kohla, M. (2014). Verbände-Positionspapier zur Neu-Einstufung von Formaldehyd in die TA Luft. Retrieved October 29, 2015 from <http://vhi.de/wp-content/uploads/sites/2/2014/07/Verbaende-Positionspapier-Formaldehyd.pdf>
- Yang, A., Zhang, Y. P., Chen, D., Chen, W. G., & Wang, R. (2001). Eye irritation caused by formaldehyde as an indoor pollution – a controlled human exposure experiment. *Biomed. Environ. Sci.*, *14*(3), 229-36.

## Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).