# Phenolics as Mediators to Accelerate the Enzymatically Initialized Oxidation of Laccase-Mediator-Systems for the Production of Medium Density Fiberboards

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Crude oil as a non-renewable resource is creating new challenges in many industrial sectors. Unsteady costs of crude oil at present and expected increases in the future are due to its limited availability as a finite resource, and these costs negatively impact the industry for wood-based panels, which use petrochemical resins in binding agents. Furthermore, wood panels that are conventionally bonded using urea formaldehyde diffuse formaldehyde into the surrounding air. To achieve independence from petrochemical products and harmful formaldehyde emissions, alternatives for their substitution are in demand. An alternative approach is the enzymatic activation of lignin located on the surface of thermomechanical pulp (TMP) fibers. The present study shows the results of internal bond strength (DIN EN 319 1993), modulus of rupture (DIN EN 310 1993), and thickness swelling (EN 317 2003) of medium-density fiberboards (MDF) bonded with laccase-mediator-system (LMS). Caffeic acid (CA), 4-hydoxy benzoic acid (HBA), and vanillic alcohol (VAl) were used as mediators. The physical and technological properties of MDF, such as internal bond strength, modulus of rupture, and thickness swelling, mostly fulfilled the European standards.

*Keywords: Enzymatic binder; Laccase; Mediator; Laccase-Mediator-System; LMS; Medium density fiberboard; MDF*

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## INTRODUCTION

The wood-based panel industry is one of the main consumers of bonding agents, which are mostly based on petrochemical components (FAO 2011). This industry is therefore dependent on crude oil availability and prices, which have recently driven up the sale prices of wood composites (EUWID 2010). Given that adhesives represent 30% of the total manufacturing costs, the higher expenses for bonding agents affect the costs of medium density fiberboard (MDF) production (Erb 2006; Schöpper 2006).

Formaldehyde emissions are also a serious problem in MDF bonded by urea or phenol-formaldehyde (Weber *et al.* 2012). Since April 2015, formaldehyde emissions have been classified as possibly carcinogenic and mutagenic according to European standards (EG 1272/2008 2008; EU 605/2014 2014). As a result, formaldehyde emissions are now officially considered to be harmful for human beings. Thus, the MDF industry is searching for more environmentally friendly and sustainable alternatives (Müller *et al.* 2007). One possibility is using enzymes for the activation or radicalization of surface lignin in wood fibers in MDF (Kües *et al.* 2007).

The main components of wood are cellulose, hemicelluloses, and lignin. Lignin is the second highest naturally occurring organic matter after celluloses, and its annual production is up to $2 \times 10^{10}$ tons stored in biomass (Strasburger *et al.* 2014). To generate wood fibers through thermomechanical pulping (TMP) of woodchips, middle-lamella lignin is exposed to high temperatures exceeding the glass-transition point, and therefore becomes plasticized. While cooling down, plasticized lignin encrusts the surface of wood fibers as a glassy, inactivated layer (Kharazipour and Hütterman 1993; Widsten 2002; Sixta 2006). It is a copolymer consisting of irregular linked phenylpropanoide units, which is responsible for the stability of solid wood (Nultsch 2001). Lignin and its phenolic components are substrates for the oxidative enzyme laccase (Baldrian 2006). Laccase polymerizes and depolymerizes lignin naturally (Solomon *et al.* 1996), and it is synthesized by white rot fungi such as *Trametes versicolor* (Fuchs 2007). The resulting main products are highly reactive phenoxy radicals (Felby *et al.* 1997).

When lignin is activated by oxidative processes, it can be used as a binder for wood-based products. Technical lignins from paper manufacturing in conjunction with an enzymatic redox-system were used by Haars *et al.* (1989) as a two-component natural binder. When TMP wood fibers are used, the applied enzyme reacts directly with the lignin on the fiber surface as a one-component system (Kües *et al.* 2007). However, one disadvantage is the long incubation time for laccase-only bonded MDF in comparison to other oxidative enzymes due to its limited redox potential and large molecular size (Li *et al.* 1999; Euring 2008). To handle this issue, specific phenolic mediators have been added that ensure faster reactions within the first 20 to 30 min, enabling a lower incubation time for more cost-effective industrial applications (Euring 2008; Euring *et al.* 2011, 2012, 2014, 2015; Kirsch *et al.* 2015). Mediators, such as caffeic acid (CA), 4-hydroxy benzoic acid (HBA), and vanillic alcohol (VAl) are low-molecular compounds that act as diffusible electron carriers, enhancing the oxidative reaction of laccase (Bourbonnais and Paice 1990; Potthast *et al.* 1995; Potthast 1998). Laccase-mediator-system (LMS) is an effective activator of wood fiber surface lignin. Its main benefits include a higher range of substrates due to the mediators' ability to oxidize even non-phenolic compounds of lignin and an accelerated reaction time (Bourbonnais and Paice 1990; Kües *et al.* 2007). Previous studies showed LMS can be used as an alternative binding agent in MDF production, owing to the achieved standards of modulus of rupture, internal bond strength, and thickness swelling, and it can easily be integrated into existing systems (Euring 2008, 2013; Euring *et al.* 2011). Because all of the materials used for this kind of MDF manufacture are near-natural and sustainable, recycling can easily be realized in the form of composting (Hüttermann *et al.* 2001; Euring 2008).

The aim of this study was to use novel mediators for proper LMS in order to produce fiberboards with a view to remain competitive with the conventional production of MDF. The mediators were chosen based on oxygen consumption measurements from previous studies where they tested positively in enhancing oxidation (Euring *et al.* 2011; Kirsch *et al.* 2015). The fiberboards were produced in a semi-dry process in which the LMS is sprayed directly onto wood fibers for enzymatic activation. This process is characterized by 12% to 45% fiber moisture before pressing (Lampert 1966). In this study, the target moisture levels of 15% to 20% were achieved by not drying the LMS-incubated fiber material.

Because of the high moisture values, the pressing time was increased up to 52.5 s/mm and 60 s/mm to determine possible impacts on physical and technological properties. Properties such as modulus of rupture, internal bond strength, and thickness swelling were tested. The results showed conformation to the required standards.

## EXPERIMENTAL

### Materials

*Wood fibers*

Wood fibers were manufactured from 80% spruce and 20% fir wood by thermo-mechanical pulping (TMP) (Gutex, Waldshut-Tiengen, Germany).

*McIlvaine buffer (McIlv)*

This buffer was used in all experiments and was 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 (AppliChem, Darmstadt, Germany).

*Laccase*

In this study, Novozym 51003 (Novozymes, Bagsvaerd, Denmark) fermented from *Trametes vilosa* in recombination with *Aspergillus oryzae* was used. It was in liquid form and brown colored due to stabilizer.

*Mediators*

The following substances were used as mediators: caffeic acid (CA), 3-(3,4-dihydroxyphenyl)-2-propenoic acid (Sigma-Aldrich, (Seelze, Germany) with a chemical purity of 95%; 4-hydroxybenzoic acid (HBA) (Alfa Aesar, Karlsruhe, Germany) with a chemical purity of 99%; and vanillic alcohol (VAl), 4-hydroxy-3-methoxybenzyl alcohol (Merck, Darmstadt, Germany) with a chemical purity of ≥ 98%.

*Hydrophobic agent*

Hydrowax of the type 138 with a solid content of 60% was used (Sasol, Johannesburg, South Africa).

### Methods

*Production of MDF on a pilot scale*

MDF was produced at an institutional pilot plant (Binos, Springe, Germany). Fiber gluing took place in a blender system. After passing through the blender unit, the fibers were transported using airflow into a tube dryer and subsequently transferred into the fiber bunker. From there, integrated spreader units dispersed a thick, homogenous fiber mat onto the form conveyer. Afterwards, the fibers were manually pre-pressed without heat. The final stages were curing by hot pressing the prepared boards and conditioning in a climate chamber (Siempelkamp, Krefeld, Germany) for 24 h.

For each treatment, 15 kg of absolutely dry (atro) wood fibers were blended with 7.5 kg of LMS per spray process. A buffer solution with laccase activity of 200 U/mL with or without 10 mM of a particular mediator (CA, HBA, and VAl) per g of wood was sprayed onto wood fibers at 22 °C. For all treatments, 1% of hydrophobic agent was used for the first time in LMS bonded MDF.

Each treatment was replicated three times. The control samples were wood fibers treated with buffer solution, buffer plus enzyme without mediator, or just the mediator solution. Furthermore, specimen bonded *via* inactivated laccase (cooked for 10 min) was used as an additional reference with an enzymatic activity of 0 U/mL (derived by ABTS-test, following Matsumura *et al.* 1986). After blending, the fibers passed directly through the tube dryer (165 °C) to obtain the target moisture of 15% to 20% without deactivating enzymatic reactions completely. The incubated wood fibers were transferred into the fiber bunker and from there immediately spread (time span of ≤ 30 min) as a fiber mat onto the form conveyer. The time span of spraying till formation of a fiber mat lasted about 30 min, which can be considered as total incubation time. Incubation tests of Kirsch *et al.* (2015) showed that enzymatic oxidation of lignin on fiber material is maximized after half an hour when starting the reaction, especially for the mediators vanillic alcohol and caffeic acid. Finally, the fibers were pressed in a laboratory hot-press at 200 °C for 52.5 s/mm and 60 s/mm to inactivate the enzyme. MDF with a target size of 8 mm and raw density of 850 ± 50 kg/m³ with dimensions of 600 mm by 450 mm were produced according to EN 316 (2009).

The conditioning process occurred in the climate chamber at 20 ± 2 °C and 65 ± 5% humidity for 24 h. Next, MDF samples were sanded and trimmed to their final size. The modulus of rupture (MOR), internal bond strength (IB), and thickness swelling (TS) were tested according to EN 310 (1993), EN 319 (1993), and EN 317 (2003), respectively. A universal testing machine (Zwick/Roell ZO10, Ulm, Germany) running at 10 kN was equipped with testXpert V10.11 software (Zwick/Roell, Ulm, Germany).

## RESULTS AND DISCUSSION

### Modulus of Rupture

Samples for MOR were analyzed by a three-point bending test conforming to EN 310 (1993).

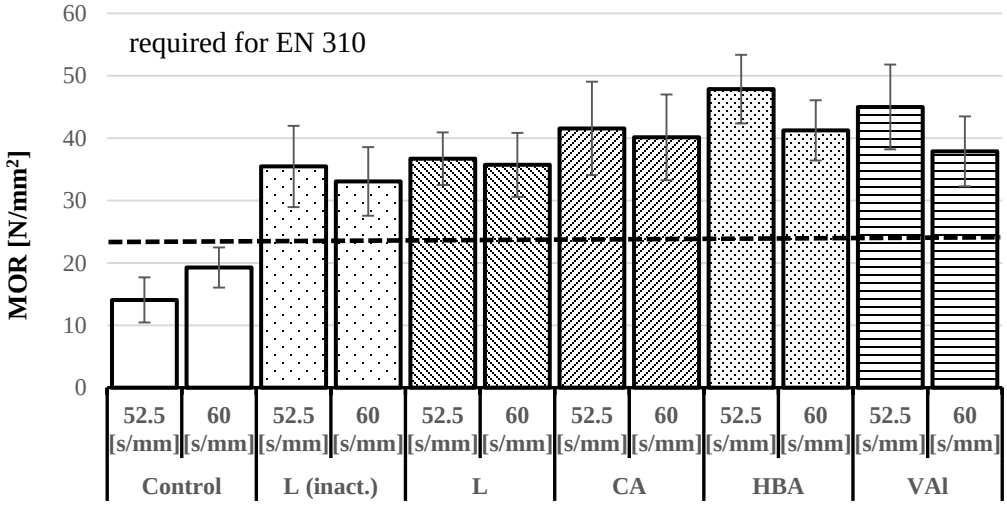


**Fig. 1.** MOR results for MDF samples. Control, buffer; L (inact.), inactivated laccase; L, laccase; CA, caffeic acid; HBA, 4-hydroxybenzoic acid; and VAl, vanillic alcohol. Each result is represented as an average with eliminated outliers of three repetitions. The standard deviation is shown as error bars.

There was no significant effect when using the extended pressing time of 60 s/mm. All MOR values of different MDFs for both pressing times were within the prescribed standard of at least 23 N/mm², with the sole exception of the control sample with MOR 14.06 N/mm² and 19.25 N/mm². At a pressing time of 52.5 s/mm, the LMSs of CA (41.55 N/mm²), HBA (47.85 N/mm²), and VAl (44.99 N/mm²) had the highest MOR. Laccase-bonded MDF had a tensile strength value of 36.07 N/mm². The samples bonded with inactivated laccase and buffer had MOR values of 35.46 and 14.06 N/mm².

At a pressing time of 60 s/mm, the optimum results for MOR were obtained by HBA (41.23 N/mm²), followed by CA (40.12 N/mm²) and VAl (37.87 N/mm²). MDF bonded by laccase and inactivated laccase reached values of 35.72 N/mm² and 33.05 N/mm². In general, the results of the inactivated laccase and active laccase samples are very similar. This issue is caused by the natural adhesive effect of this protein when no extra incubation time (no more than 30 min) was used. Boards bonded by buffer only showed the lowest MOR (19.25 N/mm²). Thus, better results were obtained by adding laccase or laccase in conjunction with mediators supported by a plasticizing effect by hot pressing. The achieved values exceeded the MOR requirements. Samples bonded with buffer and the relevant mediator performed in a similar way to the control and did not meet the required standard (data not shown).

## Internal Bond

IB tests were carried out according to the standard EN 622-5 (1997), by testing samples with loads perpendicular to the panel plane. Here, prescribed values of at least 0.65 N/mm² should be achieved; 14 samples of each MDF were analyzed. Figure 2 displays the IB of MDF pressed at 52.5 s/mm and 60 s/mm and bonded with buffer, laccase, or LMS. In addition, boards were produced from fibers exclusively incubated with buffer in combination with the particular mediator (data not shown).

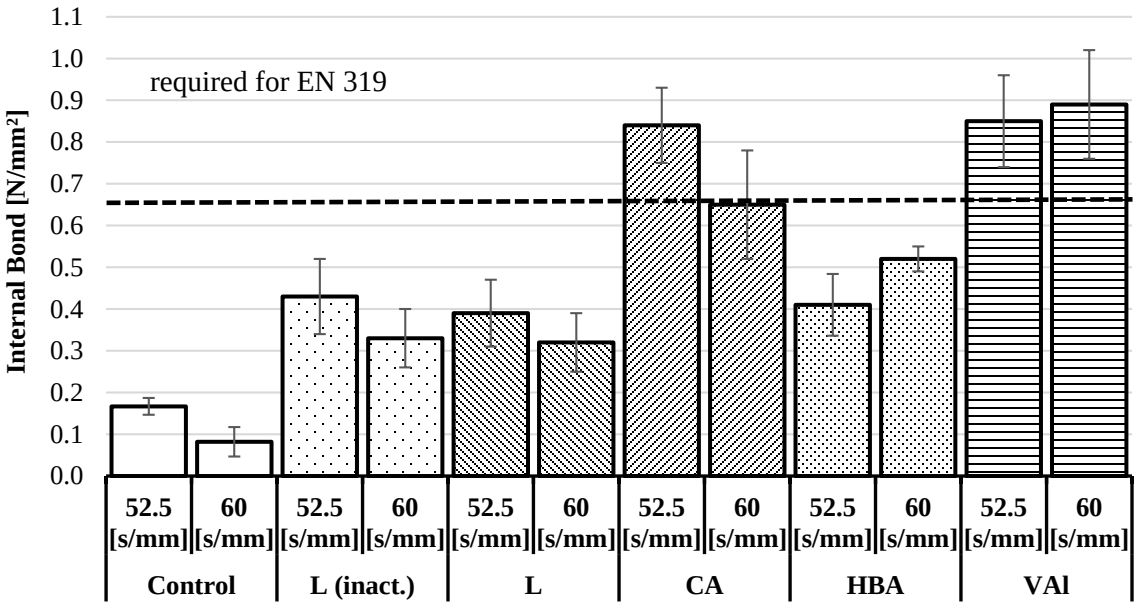


**Fig. 2.** IB results for MDF samples. Control, buffer; L (inact.), inactivated laccase; L, laccase; CA, caffeic acid; HBA, 4-hydroxybenzoic acid; and VAl, vanillic alcohol. Each result is represented as an average with eliminated outliers of three repetitions. The standard deviation is shown as error bars.

No major differences were observed when using the lengthened press time of 60 s/mm. At a pressing time of 52.5 s/mm, LMS samples using CA and VAl as mediators achieved values of 0.84 and 0.85 N/mm², respectively, and fulfilled the requirements of EN 319 (1993). Inactivated laccase, laccase, and LMS with HBA obtained IB values of 0.43, 0.39, and 0.41 N/mm², respectively. Using buffer only for bonding MDF produced an IB value of 0.17 N/mm².

At a pressing time of 60 s/mm, the required standard values were achieved by LMS bonded MDF with CA and VAl (0.65 and 0.89 N/mm$^2$). However, LMS with HBA showed a slightly less internal bond value of 0.52 N/mm$^2$. When using inactivated laccase and laccase, values of 0.33 and 0.32 N/mm² were obtained. The control sample (0.08 N/mm$^2$) showed the lowest IB value.

The LMS samples of CA and VAl fulfilled the IB standard. One possible explanation for this issue is the high redox potential of these mediators. The effectiveness of these mediators has already been demonstrated in previous reports (Kirsch *et al*. 2015). Laccase has a low redox potential compared with LMS, which is reflected in the IB results. Furthermore, the IB values of inactivated laccase and laccase were very similar, which may reflect the natural adhesive effect of this protein (compare the control IB value).

MDF has been produced using LMS with an activity of 200 U/mL, which meets the required standard (Euring 2008). To further improve the internal bond strength, an increased amount of mediator is not necessarily expedient (Euring 2008).

Another factor that might explain the reduced stability is the use of hydrophobic agents for MDF production (Back 1987). Felby *et al*. (2002) pointed out that enzymatic treatment combined with hydrophobic agents is incompatible due to the paraffin wax property of coating fibers, complicating the access to lignin on the fiber surface. In the present paper, a hydrophobic agent was used for the first time in conjunction with LMS.

The IBs for the reference samples bonded with merely buffer and mediator were comparable to results for control sample (data not shown).

Furthermore, for better explanation testing of detailed chemical bonding will be published in an additional manuscript.

## Thickness Swelling (TS)

TS tests were carried out after 24 h of soaking in water with a consistent water level. According to standard EN 622-5 (1997), samples can exhibit a maximum swelling behavior of 15%. For each MDF and pressing time, eight samples were tested. Figure 3 displays the swelling behavior of MDF pressed at 52.5 s/mm and 60 s/mm and bonded with buffer, laccase, or LMS. Boards were also produced from fibers exclusively incubated with buffer in combination with the respective mediator (data not shown).

There was no significant impact when using the extended press time of 60 s/mm. At a pressing time of 52.5 s/mm, samples of MDF bonded with CA or VAl achieved figures of 18.4% and 18.65%, respectively. The swelling behavior of MDF bonded with inactivated laccase, laccase, and HBA had values of 19.90, 20.20, and 21.40%, respectively. The control sample showed the highest TS value (27.70%).

As shown in Fig. 3, a pressing time of 60 s/mm led to the following TS results. MDF bonded with CA resulted in an average swelling value of 16.4% in conformity with EN 317. In contrast, other LMS bonded MDF achieved values of 20.7% for HBA and 17.45% for VAl. Furthermore, TS values of 19.6% and 19.7% were obtained for inactivated laccase and laccase. The control MDF swelled 31.3%. As for MOR or IB,

samples bonded with buffer and the respective mediator performed similar to control sample (data not shown).

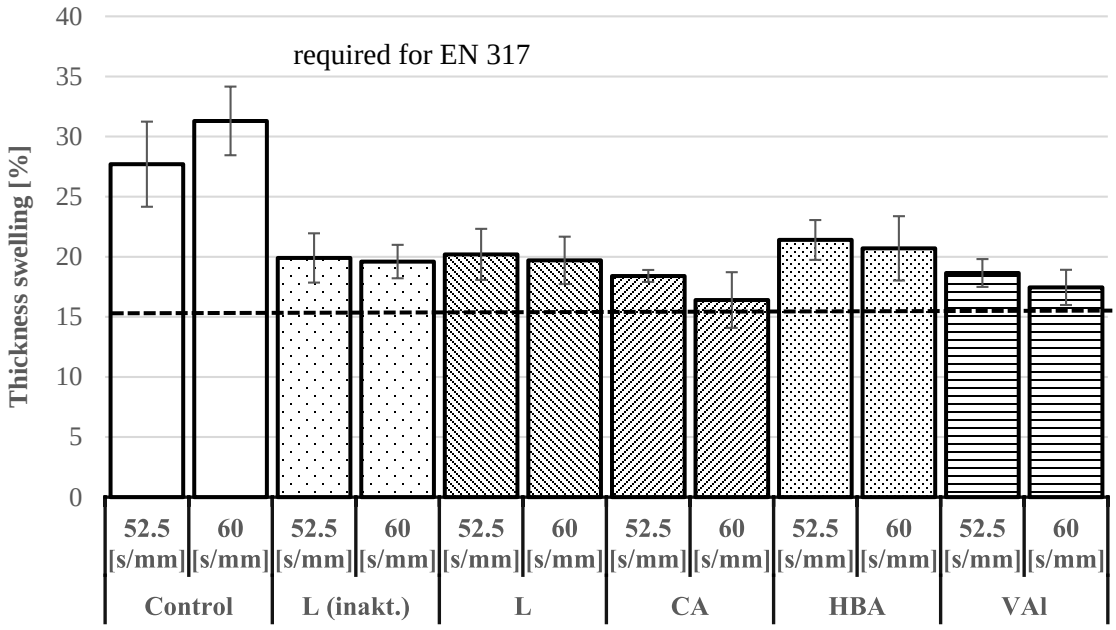


**Fig. 3.** TS results for MDF samples. Control, buffer; L (inact.), inactivated laccase; L, laccase; CA, caffeic acid; HBA, 4-hydroxybenzoic acid; and VAl, vanillic alcohol. Each result is represented as an average with eliminated outliers of three repetitions. The standard deviation is shown as error bars.

In this study, a hydrophobic agent was used in conjunction with LMS for the first time. Although not all samples met the necessary criteria in comparison with Euring (2008), a noticeable improvement was observed by using a hydrophobic agent. In general, the advantage of using the half-dry process is the higher moisture content and, thus, a longer glass transition point ($T_g \geq 90$ °C) during hot pressing, due to the almost waterlogged lignin (Goring 1963; Türk 2014). Furthermore, better heat allocation is achieved through the condensing steam (Erb 2006).

## CONCLUSIONS

1. In summary, the application of LMS showed an improvement with regard to required mechanical properties for MOR. By adding phenolic mediators, the obtained results were even better, which is mirrored in the achieved requirements for MOR and partially for IB and TS. LMSs with CA and VAl showed the most promising results. Good results for HBA were already investigated in previous studies by Euring (2008) and Euring *et al.* (2011, 2013). VAl and CA may now also be taken as serious alternatives. Control samples with merely buffer bonded MDF did not achieve useful results in terms of physical-technological properties.
2. The addition of hydrophobic agents is useful for improved results in TS but reduces the properties for IB, which was confirmed in studies by Back (1987) and Felby *et al.*

(2002). To achieve lower TS values, the amount of hydrophobic agent might be increased.

3. There was no clear improvement in physical-technological properties when using extended pressing times of more than 52.5 s/mm. For higher IB values and reduced pressing times, an industrial press can be utilized instead of a laboratory press.

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