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Oxygen Consumption of Laccase-Mediator-Systems (LMS)

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Abstract

Crude oil as a non-renewable resource is presenting challenges for the future in many industrial sectors. Due to the dwindling of these resources, costs are increasing negatively affecting the wood based panels industry, which uses mainly petrochemical resins as components for binding agents. In addition harmful formaldehyde emissions arising from conventionally produced wood composites are demonstrable. In order to substitute these conventional binding agents for wood based panels, in particular medium density fiberboards (MDF), Laccase-Mediator-Systems (LMSs) were investigated in several researches. A popular and reliable method used to test the suitability of LMSs is the measurement of their oxygen consumption on wood fibers in aqueous suspension. This enzymatic catalyzed oxidation was the object of this study. The mediators 4-hydroxybenzoic acid (HBA), vanillic acid (VanA), vanillic alcohol (VAl), ethylvanillin (EVan), acetovanillone (AVan), ferulic acid (FA), caffeic acid (CA) and guajacol (Gu) were tested as possible components of the LMSs. The study showed that all of the LMSs have oxidized wood fibers more efficiently than laccase on its own. Among the different mediators, vanillic alcohol, guajacol and caffeic acid in LMSs have shown the fastest O_2 consumption.

Keywords: enzymatic binder, laccase, Laccase-Mediator-System, LMS, MDF, mediators, medium density fiberboard

1. Introduction

Wood's stability arising from its main components lignin, cellulose and hemi-celluloses is a paradigm for the biotechnological production of wood based panels. In the search for natural binding agents for wood composites such as medium density fiberboard (MDF), lignin plays an important role. By activating the lignin on wood fiber surfaces with oxidative enzymes for example, adhesive properties can be induced (Kües et al., 2007). The most important oxidative enzyme in the present case is laccase which is naturally involved in each biosynthesis and degradation of lignin (Kharazipour & Hüttermann, 1998). It belongs to the group of the polyphenol oxidases and is able to catalyze the oxidation of phenolics and other phenolic compounds by utilizing oxygen (Baldrian, 2006; Widsten, Hummer, Heathcote, & Kandelbauer, 2009). In this process oxygen reacts to water under formation of phenoxy radicals (Felby, Nielsen, Olesen, & und Skibsted, 1997). The major disadvantage of laccase is their lower redox potential in comparison to other oxidative enzymes such as peroxidases (Li, Feng, & Eriksson, 1999). In order to improve this, specific redox-molecules acting between laccase and lignin can be added to the reaction (Kües et al., 2007). These redox-molecules are called mediators.

The mediator is oxidized by laccase and following this, it is able to oxidize lignin and even non-phenolic compounds of lignin by itself (Figure 1) (Kües et al., 2007; Bourbonnais & Paice, 1990).

This Laccase-Mediator-System (LMS) enables a faster reaction time due to its increased redox-potential and a higher spectrum of substrates (Kües et al., 2007). The generating of wood fibers by using thermo-mechanical pulping is also beneficial to the reaction. Due to the high temperature of this process of beyond 170 °C, the lignin of middle lamellae plasticizes and encrusts after cooling down on the fiber surfaces (Kharazipour & Hüttermann, 1998; Euring, 2008). In this state the fiber is completely surrounded by lignin which is able to be activated in further steps (Euring, 2008; Euring, Rühl, Ritter, Kües, & Kharazipour, 2011). The formation of radicals during the incubation with LMS and the following hot pressing of incubated fibers to fiberboards, generates a natural adhesive effect. Euring (2008), Euring, Rühl, Ritter, Kües, and Kharazipour (2011), Euring, Trojanowski, and

Kharazipour (2013) achieved with LMS bonded MDF, most of the required European standards for wood based panels. It can be concluded that LMSs are suitable alternatives for conventional binding systems such as urea-formaldehyde resins. For this reason, various mediators were tested for this current paper by measuring their oxygen consumption on wood fibers as a method to evaluate their reactivity and thus their suitability as mediator for a potential industrial application.

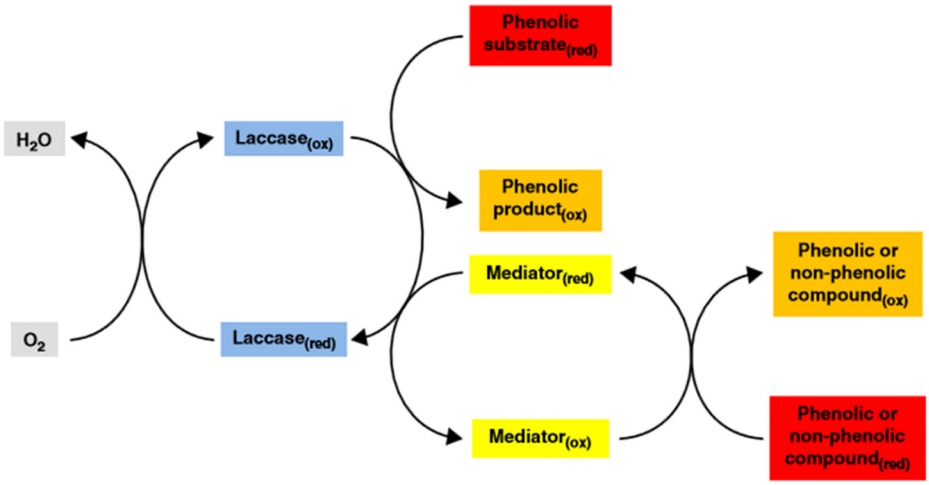

Figure 1. Simplified representation of laccase reactions with phenolic enzyme substrates and Laccase-Mediator-System (LMS) in conformity with Kües (2015)

2. Material and Methods

2.1 Material

2.1.1 Wood Fibers

Manufactured from pine wood (*Pinus sylvestris* L.) by using thermo-mechanical pulping (TMP). Here the arithmetic average fiber length of pine wood is declared as 2.2 mm (Krug, 2010).

2.1.2 Laccase

Novozym 51003 (Novozymes company, Bagsvaerd, Denmark) fermented from *Trametes vilosa* in recombination with *Aspergillus oryzae.* This was at hand in liquid form and a stabilizer was added to increase the durability. Due to this stabilizer the solution got a brown coloration. The product Novozym 51003 was fermented and mixed according to an in-house recipe.

2.1.3 Mediators

Acetovanillone (AVan): 4-hydroxy-3-methoxybenzyl alcohol, Alfa Aesar company (Karlsruhe, Germany), with a chemical purity of 98%.

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

Ethylvanillin (EVan): 3-ethoxy-4-hydroxy-benzaldehyde from Sigma-Aldrich (Seelze, Germany) with a chemical purity of 99%.

Ferulic acid (FA): 4-hydroxy-3-methoxy-cinnamic acid from Merck company (Darmstadt, Germany) with a chemical purity of 99%.

Guajacol (Gu): 2-methoxyphenol, Alfa Aesar (Karlsruhe, Germany), chemical purity 98+%.

4-hydroxybenzoic acid (HBA): from Alfa Aesar (Karlsruhe, Germany) with a chemical purity of 99%.

Vanillic alcohol (VAl): 4-hydroxy-3-methoxybenzyl alcohol, Merck (Darmstadt, Germany), chemical purity of ≥ 98%.

Vanillic acid (VanA): from Alfa Aesar company (Seelze, Germany) with a chemical purity of 98%.

2.1.4 McIlvaine Buffer (McIlv)

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.

2.2 Methods

The consumption of oxygen is a parameter for the quality of the enzymatic activity related to the oxidation. This oxygen consumption was measured with an O_2 electrode (InPro 6800® series 12/220 by Mettler-Toledo, Urdorf, Switzerland) following the methods of Widsten (2002) and Grönqvist et al. (2005) (Figure 2). Reactions took place in a room with a constant temperature of 20 °C under normal air pressure.

First of all the mediator was dissolved in one liter of buffer while stirring and heating at 80°C. The heating treatment enables a better solution of the mediator but benefits a partial degasification causing a decreased saturation. The amount of the mediator is applied to a defined amount of wood fibers. For reactions in the flask with a maximum volume of 123 ml, 1 g of absolutely dry (atro) fibers was added. The fibers were treated with 10 mM of mediator. Afterwards the buffer-mediator-mixture was ventilated for 0.5 h to achieve a 100-percent saturation regaining the initial state, depending on the specific oxygen capacity of water. In order to verify this, the oxygen content of the buffer-mediator-mixture was measured.

After that all components (mediator dissolved in buffer and wood fibers) were added to the flask. To achieve precise results the flask was filled up to the brim, ousting last deposits of entrapped air. In order to get stable oxygen starting values a calibrating phase of the O_2 electrode was required. Then laccase with an activity of 100 U/ml was added per cannula to start the redox reaction and measurement. The current enzymatic activity of laccase was determined by using the ABTS-test developed by Yamamoto, Numata, Kawano, Shin, and Murao (1986). The oxygen content after calibrating was set as 100% saturated and the following O_2 uptake was calculated relatively to this content. A magnetic stirrer (DRAGONLAB, Bejing, China) kept the suspension with average stirring rates in motion. In intervals of 10 seconds the consumption data was recorded by the software LabView 7.1 (National Instruments, Austin, USA) involving an O_2 Transmitter 4100 e (Mettler-Toldeo, Urdorf, Switzerland) over an incubation time of 180 minutes. Every sample was repeated three times. Below an overview is shown for different samples:

- buffer (123 ml)
- buffer + fibers (1g atro)
- buffer + mediator (10 mM)
- buffer + laccase (100 U/ml)
- buffer + mediator + fibers
- buffer + laccase + mediator
- buffer + laccase + fibers
- buffer + laccase + mediator + fibers

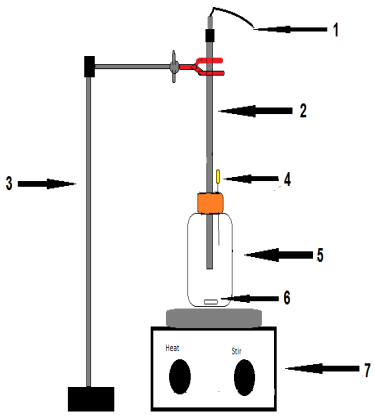

Figure 2. Experimental setup for oxygen consumption tests with 1= access to O_2 transmitter; 2= O_2 electrode; 3= tripod; 4= cannula for adding laccase; 5= flask; 6= magnetic stirring rod; 7= magnetic stirrer

3. Results and Discussion

3.1 Acetovanillone

With acetovanillone acting in the LMS the highest volume of 98% of O_2 was taken up after 180 minutes. In the same time period 90% was consumed by the tests with laccase and fibers alone. Acetovanillone and laccase

consumed 15% of O_2. In the case of the references, no sample consumed more than 20 %. The grey shading above the curve represents the positive standard deviation. The curve itself is calculated as an average value of three repetitions (Figure 3).

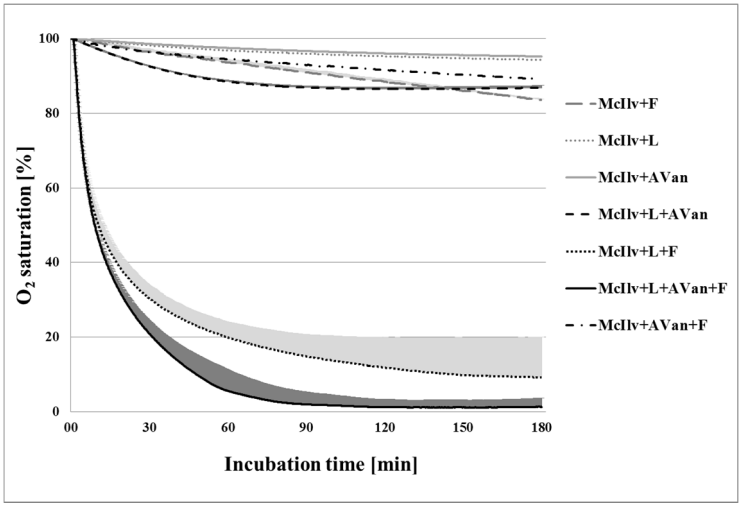

Figure 3. Oxygen consumption versus time for laccase action determined by reduction of oxygen saturation. Comparison of the samples treated with acetovanillone and references; McIlv+F = buffer and fibers McIlv+L = buffer and laccase; McIlv+AVan = buffer and acetovanillone; McIlv+L+AVan = buffer, laccase and acetovanillone; McIlv+L+F = buffer, laccase and fibers; McIlv+L+AVan+F = buffer, laccase, acetovanillone and fibers; McIlv+AVan+F = buffer, acetovanillone and fibers. The grey shading above the curve represents the positive standard deviation. The curve itself is calculated as an average value of three repetitions.

3.2 Caffeic Acid

The LMS with caffeic acid consumed all of the O_2 content within the first 30 minutes. 43% of O_2 was used when caffeic acid and laccase were tested together. 90% was consumed by the tests with laccase and fibers after 180 minutes (Figure 4).

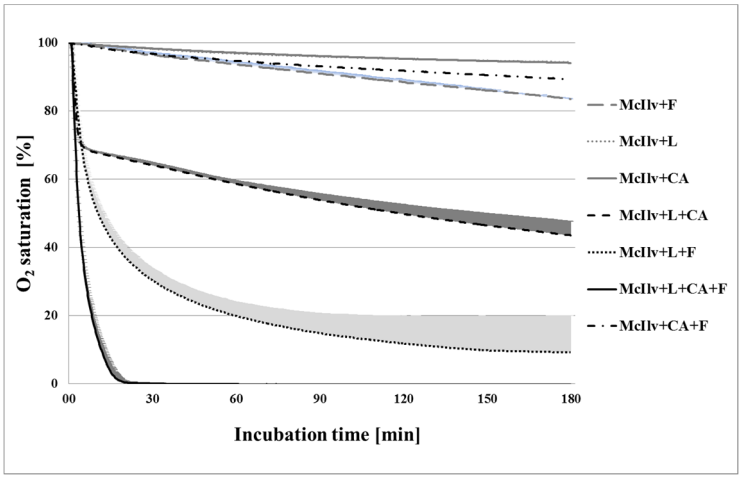

Figure 4. Oxygen consumption versus time for laccase action determined by reduction of oxygen saturation. Comparison of the samples treated with caffeic acid and references; McIlv+F = buffer and fibers McIlv+L = buffer and laccase; McIlv+CA = buffer and caffeic acid; McIlv+L+CA = buffer, laccase and caffeic acid; McIlv+L+F = buffer, laccase and fibers; McIlv+L+CA+F = buffer, laccase, caffeic acid and fibers; McIlv+CA+F = buffer, caffeic acid and fibers. The grey shading above the curve represents the positive standard deviation. The curve itself is calculated as an average value of three repetitions.

3.3 Ethylvanillin

After 180 minutes the LMS with ethylvanillin used up all O_2 in the suspension, more than samples with laccase and fibers. 90% was consumed by the tests with laccase and fibers. 15% was consumed when mediator and laccase were tested. The references consumed less than 20% (Figure 5).

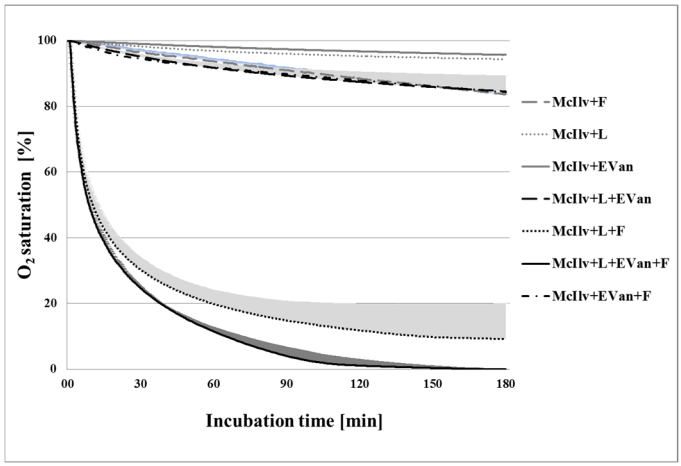

Figure 5. Oxygen consumption versus time for laccase action determined by reduction of oxygen saturation. Comparison of the samples treated with ethylvanillin (EVan) and references; McIlv+F = buffer and fibers McIlv+L = buffer and laccase; McIlv+EVan = buffer and ethylvanillin; McIlv+L+EVan = buffer, laccase and ethylvanillin; McIlv+L+F = buffer, laccase and fibers; McIlv+L+EVan+F = buffer, laccase, ethylvanillin and fibers; McIlv+EVan+F = buffer, ethylvanillin and fibers. The grey shading above the curve represents the positive standard deviation. The curve itself is calculated as an average value of three repetitions.

3.4 Ferulic Acid

An O_2 consumption of nearly 100% within 180 minutes was monitored when ferulic acid was acting in a LMS. 90% was consumed by the tests with laccase and fibers. 34% was taken up with sample ferulic acid and laccase. The references can be associated with the results of acetovanillone or caffeic acid (Figure 6).

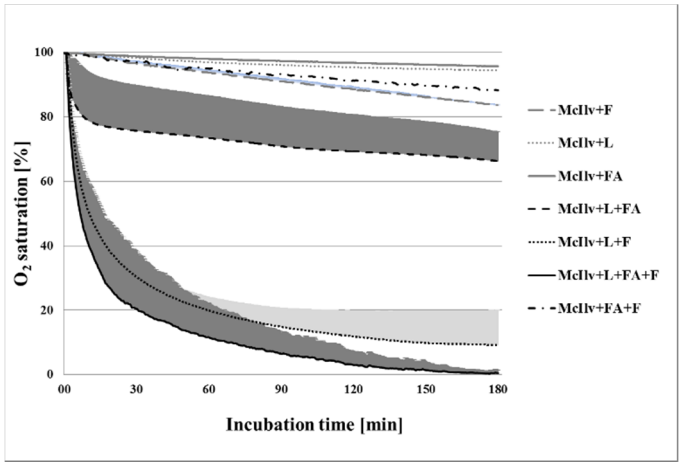

Figure 6. Oxygen consumption versus time for laccase action determined by reduction of oxygen saturation. Comparison of the samples treated with ferulic acid (FA) and references; McIlv+F = buffer and fibers McIlv+L = buffer and laccase; McIlv+FA = buffer and ferulic acid; McIlv+L+FA = buffer, laccase and ferulic acid; McIlv+L+F = buffer, laccase and fibers; McIlv+L+FA+F = buffer, laccase, ferulic acid and fibers; McIlv+FA+F = buffer, ferulic acid and fibers The grey shading above the curve represents the positive standard deviation. The curve itself is calculated as an average value of three repetitions.

3.5 Guajacol

Like caffeic acid all of the O_2 content was consumed by the sample LMS with guajacol within the first 30 minutes. 90% was consumed by the tests with laccase and fibers. A consumption of 35% was monitored when guajacol was tested with laccase. The references can be seen with again O_2 usages of less than 20% (Figure 7).

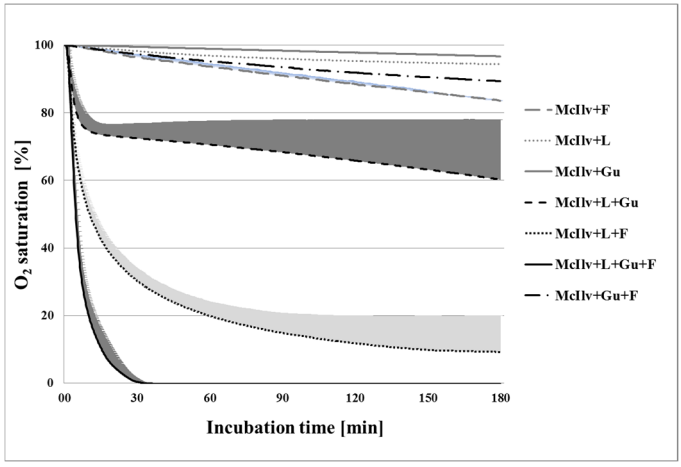

Figure 7. Oxygen consumption versus time for laccase action determined by reduction of oxygen saturation. Comparison of the samples treated with guajacol (Gu) and references; McIlv+F = buffer and fibers McIlv+L = buffer and laccase; McIlv+Gu = buffer and guajacol; McIlv+L+Gu = buffer, laccase and guajacol; McIlv+L+F = buffer, laccase and fibers; McIlv+L+Gu+F = buffer, laccase, guajacol and fibers; McIlv+Gu+F = buffer, guajacol and fibers. The grey shading above the curve represents the positive standard deviation. The curve itself is calculated as an average value of three repetitions.

3.6 4-hydroxybenzoic acid (HBA)

The LMS with HBA with O_2 consumption of 95% uses slightly more O_2 in a direct comparison with sample laccase and fibers (90%). HBA and laccase together had taken up less than 10% ranking in the same area with samples of the references. HBA is, as a consequence, like the combination of buffer, laccase and HBA shows, the least effective of all tested mediators. Nevertheless the combination of buffer, laccase, HBA and fibers shows in comparison to the combination buffer, laccase and fibers an increasing effectivity (Figure 8).

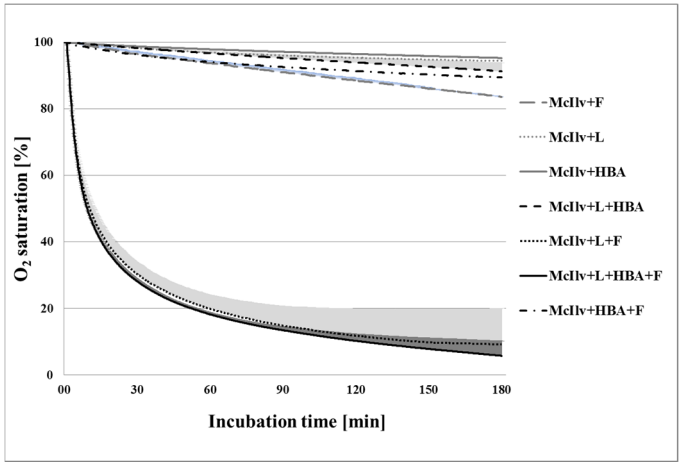

Figure 8. Oxygen consumption versus time for laccase action determined by reduction of oxygen saturation. Comparison of the samples treated with 4-hydroxybenzoic acid (HBA) and references; McIlv+F = buffer and fibers McIlv+L = buffer and laccase; McIlv+HBA = buffer and 4-hydroxybenzoic acid; McIlv+L+HBA = buffer, laccase and 4-hydroxybenzoic acid; McIlv+L+F = buffer, laccase and fibers; McIlv+L+HBA+F = buffer, laccase, 4-hydroxybenzoic acid and fibers; McIlv+HBA+F = buffer, 4-hydroxybenzoic acid and fibers. The grey shading above the curve presents the positive standard deviation. The curve itself is calculated as an average value of three repetitions.

3.7 Vanillic Acid

In the sample LMS with vanillic acid, 100% of O_2 was taken up after 46 minutes. 90% was consumed by the tests with laccase and fibers at the end of the measurement. Vanillic acid and laccase consumed 37%. The references were localized in the same range in comparison to the other mediators (Figure 9).

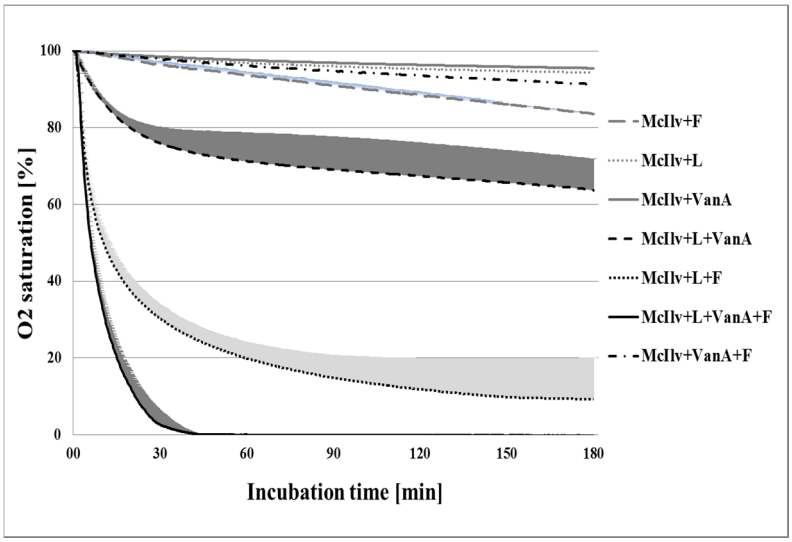

Figure 9. Oxygen consumption versus time for laccase action determined by reduction of oxygen saturation. Comparison of the samples treated with vanillic acid (VanA) and references; McIlv+F = buffer and fibers McIlv+L = buffer and laccase; McIlv+VanA = buffer and vanillic acid; McIlv+L+VanA = buffer, laccase and vanillic acid; McIlv+L+F = buffer, laccase and fibers; McIlv+L+VanA+F = buffer, laccase, vanillic acid and fibers; McIlv+VanA+F = buffer, vanillic acid and fibers. The grey shading above the curve represents the positive standard deviation. The curve itself is calculated as an average value of three repetitions

3.8 Vanillic Alcohol

100% O_2 consumption was monitored within the first 40 minutes with vanillic alcohol as component in an LMS. 90% was consumed by the tests with laccase and fibers. Vanillic alcohol and laccase had taken up 40% O_2 (Figure 10).

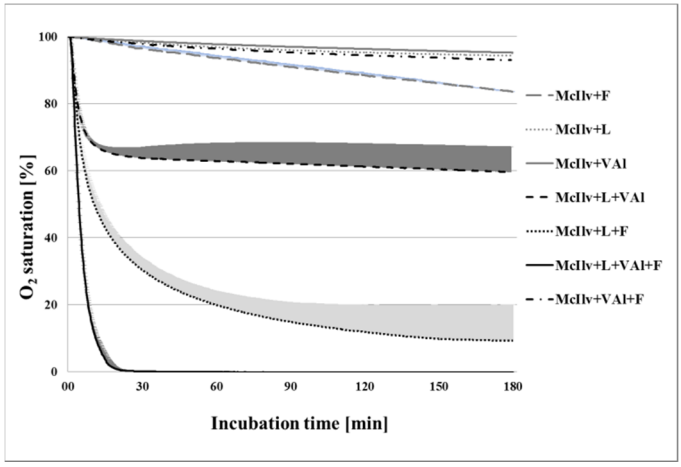

Figure 10. Oxygen consumption versus time for laccase action determined by reduction of oxygen saturation. Comparison of the samples treated with vanillic alcohol (VAl) and references; McIlv+F = buffer and fibers McIlv+L = buffer and laccase; McIlv+VAl = buffer and vanillic alcohol; McIlv+L+VAl = buffer, laccase and vanillic alcohol; McIlv+L+F = buffer, laccase and fibers; McIlv+L+VAl+F = buffer, laccase, vanillic alcohol; McIlv+VAl+F = buffer, vanillic alcohol and fibers. The grey shading above the curve represents the positive

standard deviation. The curve itself is calculated as an average value of three repetitions.

To allow an easier comparison, the following table shows in comparison the oxygen consumption of the different mediators concerning saturation and time. These figures are calculated as an average value of three repetitions (Table 1).

Table 1. Oxygen consumption versus time for laccase action determined by reduction of oxygen saturation. Comparison of the samples with buffer, fibers, laccase and different mediators. McIlv+L+AVan+F = buffer, laccase, acetovanillone and fibers; McIlv+L+CA+F = buffer, laccase, caffeic acid and fibers; McIlv+L+EVan+F = buffer, laccase, ethylvanillin and fibers; McIlv+L+FA+F = buffer, laccase, ferulic acid and fibers; McIlv+L+Gu+F = buffer, laccase, guajacol and fibers; McIlv+L+HBA+F = buffer, laccase, 4-hydroxybenzoic acid and fibers; McIlv+L+VanA+F = buffer, laccase, vanillic acid and fibers; McIlv+L+VAl+F = buffer, laccase, vanillic alcohol

Sample	**O_2 saturation [%]**							
time [min]	0	5	10	15	30	60	120	180
McIlv+L+AVan+F	100,00	62,74	45,75	36,35	20,42	5,41	1,26	1,31
McIlv+L+CA+F	100,00	35,82	13,77	3,39	0,07	0,00	0,00	0,00
McIlv+L+EVan+F	100,00	63,81	47,06	38,67	24,65	11,51	1,11	0,01
McIlv+L+FA+F	100,00	57,95	40,74	31,97	20,46	11,62	3,02	0,48
McIlv+L+Gu+F	100,00	39,19	18,19	9,50	0,28	0,00	0,00	0,00
McIlv+L+HBA+F	100,00	63,52	47,64	39,88	27,92	18,11	10,23	5,79
McIlv+L+VanA+F	100,00	55,22	32,56	19,62	2,50	0,00	0,00	0,00
McIlv+L+Val+F	100,00	40,48	12,38	3,39	0,14	0,00	0,00	0,00

One noticeable observation is that reactions were most intense immediately after adding laccase except for the test buffer with laccase. The curves illustrate highest reactions within the first 20 to 30 minutes. The more time passes by the more flat the curves become. This should relate to the high initial availability of substrates such as lignin or lignin and mediator and the high initial value of co-substrate O_2 in the suspension. The temperature-considering the Q_{10} temperature coefficient and pH of the suspension are also important. The pH of McIlvaine buffer is therefore applied to the pH optimum of laccase used for this study which was reported to be best at pH 6.0 (Kües et al., 2007).

In most of the cases, lignin and mediator were apparently oxidized by laccase. Only HBA showed a contradictory result when tested together with laccase consuming less than 10% O_2.

Overall, tests with LMSs showed a higher consumption of oxygen than with laccase on fibers on its own. Most of the LMSs (vanillic acid, vanillic alcohol, ethylvanillin, caffeic acid, ferulic acid and guajacol) even consumed the entire available oxygen content of the suspension. This is attributable to the increased spectrum of substrates and higher redox-potential of LMS (Kües et al., 2007; Bourbonnais & Paice, 1990). Similar results were obtained in the study of Euring, Rühl, Ritter, Kües, and Kharazipour (2011) with HBA acting in a LMS. Potthast, Rosenau, Chen, and Gratzl (1995) found out that LMSs can transfer two electrons at the same time due to the mediators` ability as additional electron donor which adequately describes the better redox-potential of LMS.

Among the different mediators in LMSs, vanillic alcohol, guajacol and caffeic acid have shown most rapid O_2 consumption. It can be concluded that these LMSs catalyzed the oxidation of phenolic and non-phenolic compounds in lignin more efficiently than other LMSs. These results were underlined by the results of preparations with laccase and mediator without wood fibers. Here, uptake rates of up to 40% O_2 were monitored. These three mediators should be focused upon when being tested for an application as a binding system for MDF.

4. Conclusion

In exploring the effectiveness and suitability of LMSs, various mediators were tested in this study. A reliable method for this is the measurement of their oxygen consumption on phenolic (laccase) and non-phenolic (mediator)

compounds. This work has shown that mediators such as vanillic acid, vanillic alcohol, ethylvanillin, caffeic acid, ferulic acid and guajacol, which have had the highest oxygen consumption of the tests, are possible alternatives to increase effectiveness of LMS. An important aspect for future studies will be to use the best working mediators to produce MDF in pilot-scale. This is a popular method to determine the effects regarding to technical properties such as modulus of rupture, internal bond and thickness swelling.

Acknowledgements

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References

Baldrian, P. (2006). Fungal Laccases - Occurrence and Properties. *FEMS Microbiology Reviews,* 30(2), 215-42. http://dx.doi.org/10.1111/j.1574-4976.2005.00010.x

Bourbonnais, R., & Paice, M. G. (1990). Oxidation of non-phenolic substrates: An expanded role for laccase in lignin biodegradation. *FEBS Letters, 267*(1), 99-102. http://dx.doi.org/10.1016/0014-5793(90)80298-W

Euring, M. (2008). Einsatz von Mediatoren bei der enzymatischen Aktivierung der fasereigenen Bindekräfte zur Herstellung von enzymgebundenen, bindemittelfreien Holzwerkstoffen (Dissertation). Georg-August-University of Göttingen, Göttingen, Germany. Retrieved from https://ediss.uni-goettingen.de/bitstream/handle/11858/00-1735-0000-0006-B111-9/euring.pdf?sequence=1

Euring, M., Rühl, M., Ritter, N., Kües, U., & Kharazipour, A. (2011). Laccase Mediator Systems for Eco-Friendly Production of Medium-Density Fiberboard (MDF) on a Pilot Scale: Physicochemical Analysis of the Reaction Mechanism. *Biotechnology Journal, 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 Reaction Mechanism and Making of Medium-Density Fiberboard, Göttingen.

Felby, C., Nielsen, B. R., Olesen, P. O., & und Skibsted, L. H. (1997). Identification and Quantification of Radical Reaction Intermediates by Electron Spin Resonance Spectrometry of Laccase-Catalyzed Oxidation of Wood Fibers from Beech (Fagus Sylvatica). *Applied Microbiology and Biotechnology, 48*(4), 459-64. http://dx.doi.org/10.1007/s002530051080

Grönqvist, S., Viikari, L., Niku-Paavola, M.-L., Orlandi, M., Canevali, C.， & und Buchert, J. (2005), Oxidation of Milled Wood Lignin with Laccase, Tyrosinase and Horseradish Peroxidase. *Applied Microbiology and Biotechnology,* 67(4), 489-494. http://dx.doi.org/10.1007/s00253-004-1800-6

Kharazipour, A., & Hüttermann, A. (1998). Biotechnological production of wood composites. In A. Bruce, & J. W. Palfreyman (Eds.), *Forest Products Biotechnology* (chapter 9, pp. 141-150). Taylor & Francis.

Krug, D. (2010). Einfluss der Faserstoff-Aufschlussbedingungen und des Bindemittels auf die Eigenschaften von mitteldichten Faserplatten (MDF) für eine Verwendung im Feucht- und Außenbereich (Dissertation). Hamburg University, Hamburg, Germany. Retrieved from http://ediss.sub.uni-hamburg.de/volltexte/2010/4679/pdf/Detlef-Krug_Dissertation.pdf

Kües, U. (2015). Fungal enzymes for environmental management. *Current Opinion in Biotechnology, 33,* 268-278. http://dx.doi.org/10.1016/j.copbio.2015.03.006

Kües, U., Bohn, C., Euring M., Müller C., Polle, A., & Kharazipour, A. (2007). Enzymatically Modified Wood in Panel Board Production. In U. Kües (Ed.), *Wood Production, Wood Technology, and Biotechnological Impacts* (pp. 433-467). Universitätsverlag Göttingen.

Li, K., Feng, X., & Eriksson, K. E. L. (1999). Comparison of Fungal Laccases and Redox Mediators in Oxidation of a Nonphenolic Lignin Model Compound. *Applied and Environmental Microbiology, 65*(6), 2654-2660

Potthast, A., Rosenau, T., Chen, C.-L., & Gratzl, J. S. (1995), Selective Enzymic Oxidation of Aromatic Methyl Groups to Aldehydes. *The Journal of Organic Chemistry, 60*(14), 4320-4321. http://dx.doi.org/10.1021/jo00119a006

Widsten, P. (2002). Oxidative Activation of Wood Fibers for the Manufacture of Medium-Density Fiberboard (MDF) (Dissertation). Helsinki University of Technology, Helsinkim Finland. Retrieved from https://aaltodoc.aalto.fi:443/handle/123456789/2239

Widsten, P., Hummer, A., Heathcote, C., & Kandelbauer, A. (2009). A preliminary study of green production of fiberboard bonded with tannin and laccase in a wet process. *Holzforschung, 63*, 545-550. http://dx.doi.org/10.1515/HF.2009.090

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. Japan Society for Bioscience, *Biotechnology and Agrochemnistry. Agric. Biol. Chem., 50*(5), 1355-1357. http://dx.doi.org/10.1271/bbb 1961.50.1355