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Lignin polymerization by Laccase

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Abstract

• Lignin represents a significant by-product of the wood pulp industry, with an annual production ranging from 50 to 70 million tons.

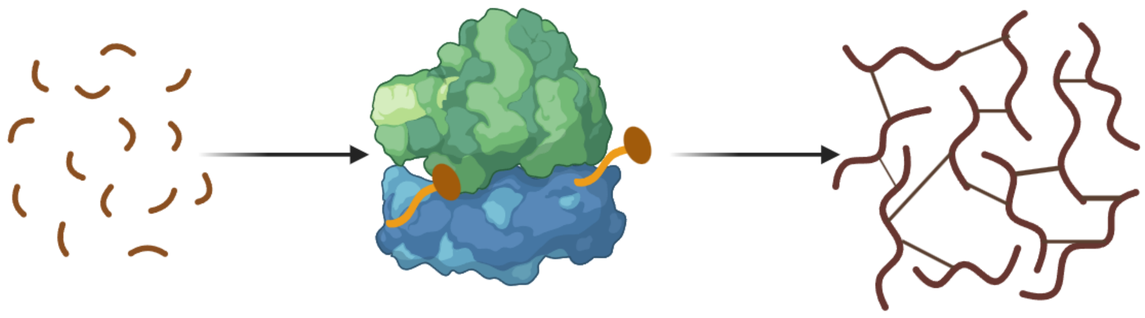
• Just a small fraction, approximately 1-2% of the total lignin generated, is employed for the production of value-added materials like dispersants, resins, additives, vanillin, and more.

• In this study, an enzymatic treatment was applied on Lignin to increase its molecular weight, allowing to unlock its natural structural properties, thereby improving its suitability as a binder in various applications.

Keyword : Biopolymers, biorefinery, enzymatic treatment, laccase, lignin valorization.

Introduction

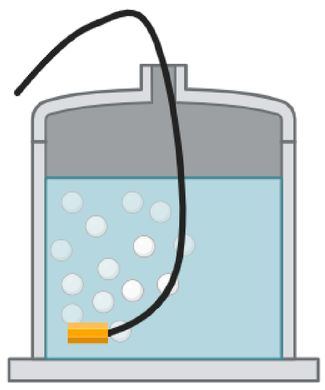
Lignin structure arises from the oxidative polymerization of three monolignols, a process driven by enzymes such as peroxidase and laccases. These enzymes play a vital role in catalyzing the oxidation of monolignols, initiating the production of radicals that subsequently interconnect. This series of radical coupling reactions ultimately yields larger molecular weight compounds.



During wood cooking to attain cellulose fibers, lignin is fragmented and dissolved, leading to a reduction in its binding capability and the break of the wood structure.

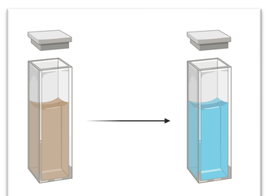
In order to replicate the structural role of lignin in wood within composites, it becomes necessary to polymerize these lignin fragments. By harnessing the natural enzymes that participate in this process, it becomes possible to customize and modify these fragments, thereby altering the properties of the molecule and the lignin-based material.

Methods

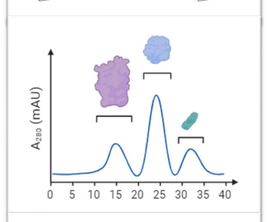


pH11 ; 40ºC: Air bubbling
2 U/mg of lignin

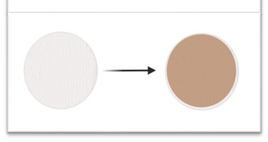
Phenolic content



Molecular weight distribution



Lignin Conversion



Results

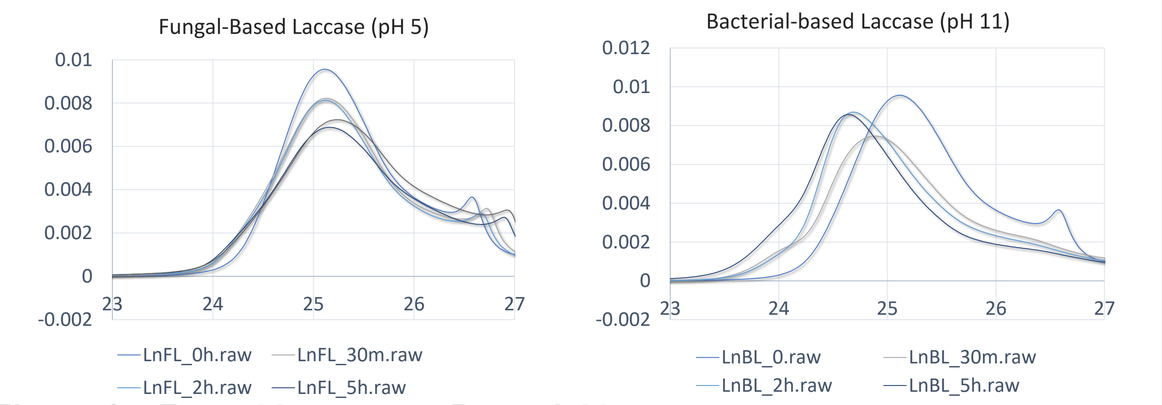


Figure 1 – Fungal laccase vs Bacterial laccase.

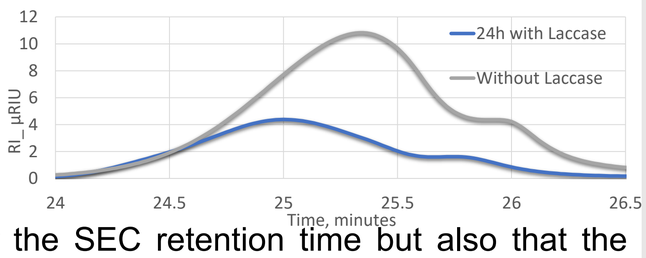
The lignin converted by bacterial laccase-mediated reaction exhibits a decrease in retention time in the Size Exclusion Chromatogram as the reaction time is extended (0-5 hours), indicating an increase in the molecular size of the molecules involved. In contrast, this phenomenon is not observed when using the fungal-based laccase. These findings, coupled with the assessment of phenolic content in the samples and the lignin conversion, underscore the superior performance of the bacterial-derived laccase reaction under the specified conditions.

Table 1 – Substrate conversion (lignin retention in 1.2 µm filter).

Time, hours	BL 1 g/L (%)	BL 17.5 g/L (%)	Ln_ethanol 1g/L (%)	Pre-hydrolysis 7 g/L (%)
0	5.0	6.0	5.0	30.0
1	14.1	11.1	11.3	27.9
2	24.1	14.3	15.6	30.7
5	31.4	24.9	21.0	47.1
8	25.7	15.1	18.8	26.4
24	31.2	12.0	21.0	20.0
72	63.3	9.4	46.0	17.1

- Black liquor 1g/L achieved the higher lignin conversion
- Increasing BL concentration, resulted in enzymatic inhibition
- The pre-hydrolysis sample contains high sugar content, which has an adverse effect on the process of lignin polymerization.

Figure 2 - SEC chromatogram of original lignin and laccase treated lignin



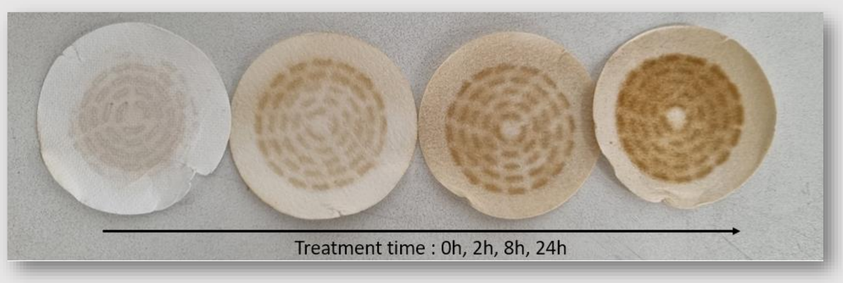
It is important to consider not only the SEC retention time but also that the overall area of the metabolite signal was significantly reduced. Lower retention time indicates a molecule with higher molecular weight and the reduced signal is affected by the retention of the material in the syringe filter used to prepare the SEC samples. Both parameters show the polymerization of lignin by laccase.

The total phenolic content decreased from 27 to 16% with laccase, which is also an identification of the increased molecular weight of lignin, decreasing the value with more condensed structures.

Conclusion

Alkaline Bacteria-based laccase resulted in higher polymerization vs acidic fungal-based laccase.

The phenolic content reduction, the behavior of the lignin signal in the molecular weight analysis and the lignin retention all indicated the efficient polymerization of lignin by the alkaline bacteria-based laccase.



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