

Enzymatic Modification of the Interaction Between Lignin and Recycled Cellulose Fibers



LUND
UNIVERSITY

Manfred Klug

Department of Chemical Engineering
Master Thesis 2019

Enzymatic Modification of the Interaction Between Lignin and Recycled Cellulose Fibers

Master Thesis

by

Manfred Klug

Department of Chemical Engineering
Lund University

June 2019

Supervisor LTH: **Postdoctoral Fellow Henrik Almqvist**
Supervisor Smurfit Kappa: **Senior Project Manager Ronald Bredemo**
Examiner: **Professor Gunnar Lidén**

Picture on front page: Reactor setup at Smurfit Kappa, Piteå. Photo by Manfred Klug, edited by Max Viklund.

Department of Chemical Engineering
Lund University
Box 124
SE-221 00 Lund
Sweden

© 2019 by Manfred Klug. All rights reserved.
Printed in Sweden by Media-Tryck
Lund 2019

Acknowledgements

I would like to thank my examiner Professor Gunnar Lidén for believing in me and helping me get this project as my master's thesis project. I would also like to extend my gratitude to my supervisors Henrik Almquist and Ronald Bredemo for always being available to answer my questions and help when needed.

Special thanks also to Anders Arkell and SunCarbon for the help with everything lignin related, to Klaas Van Der Vlist for showing much interest in the project and to MetGen for providing the enzymes. Thanks to everyone at Department of Chemical Engineering in Lund and Smurfit Kappa in Piteå for the hospitality and all the fun and interesting discussions during the coffee breaks. This project would not have been possible without you.

I would also like to thank my family for your constant support during the ups and downs of my studies. Thank you for showing an interest in everything I've been doing, even the most obscure subjects.

Last but not least, thanks to everyone at the Guild of Chemical Engineering and Biotechnology at TLTH for providing well needed distractions from my studies. I would not have made it this far without the friends I've made or without everything I've learned from being involved in all kinds of extracurricular activities. These five years have been the best years of my life thanks to you.

Abstract

The pulp and paper industry have been a growing market during the last decades. Due to higher environmental concerns among people, the recycling of papers has become an even more important part of the industry. Recycled paper fibers however are of lower quality than virgin fibers. This report aims to analyze the possibility to use a laccase enzyme to modify the interaction between lignin and recycled pulp fibers in order to improve the recycled papers strength under humid conditions.

Experiments were carried out in order to characterize the effect of the enzyme on lignin interaction with the paper fibers and pulp. Several experiments were made, e.g. drop tests to measure the absorptivity of the paper, burst and compression strength tests to measure how sturdy the paper was, and moisture content tests to measure how much water the paper absorbed from the surrounding air. Tests were also carried out where sugars were added to the process, to find out if the presence of carbohydrates would improve the integration of lignin.

The results indicate that the lignin reacts fast in the presence of the enzyme, and also indicate that an interaction between the paper fibers and the lignin results from these reactions. The drop tests show that a treatment with enzymes and lignin increase the absorption time with around 20 seconds, and both the burst and compression strength tests show an increase in strength, normalized by the surface weight, by 0.5 MN/kg and 2 kNm/kg respectively. However, the significance of these improvements needs to be validated by further experiments. The addition of carbohydrates seems to only have a small effect on some of the different tests, but these effects are most likely not significant. More experiments to tests other types of lignin, carbohydrates and enzymes should be carried out on order to optimize the procedures and validate the results.

Keywords

Lignin; Pulp and Paper; Recycling; Biotechnology; Enzyme technology; Laccases, Chemical Engineering.

Sammanfattning

Pappers- och pappersmassaindustrin har ökat markant under de senaste årtiondena. På grund av att samhället har blivit mer miljömedvetet har återvinning av papper blivit en allt viktigare del inom industrin. Återvunna pappersfiber är dock av lägre kvalitet än nylagda fibrer. Syftet med denna rapport är att analysera möjligheterna att använda ett enzym av typen lackas för att modifiera interaktionen mellan lignin och återvunnen pappersmassa och därigenom förbättra papprets styrka under fuktiga förhållanden.

Experiment gjordes för att karaktärisera enzymets effekt på lignininteraktionen med pappersfibrerna och pappersmassan. Ett flertal olika experiment gjordes, till exempel dropptest för att mäta papprets absorptionsförmåga, spräng- och kompressionsstyrka mättes för att undersöka papprets robusthet och fukthaltsmätningar gjordes för att se hur mycket fukt pappret absorberade från omgivningen. Tester med tillsats av olika sockerarter utfördes också för att se om kolhydraters närvaro i blandningen ökade ligninets integrationsförmåga.

Resultaten indikerar att ligninet reagerar mycket fort i närvaro av enzymet, och de tyder också på att någon form av interaktion mellan pappersfibrerna och ligninet sker som ett resultat av dessa reaktioner. Dropptesterna visar att enzym- och ligninbehandlingen ökar absorptionstiden med cirka 20 sekunder, och behandlingen ökar även spräng- och kompressionsstyrkan, när dessa normaliserats mot papprets ytvikt, med 0.5 MN/kg respektive 2 kNm/kg. För att avgöra om detta är signifikanta skillnader behöver dock fler experiment göras. Tillsatsen av kolhydrater verkar bara ha en mindre effekt, och denna är högst troligt inte signifikant. Fler experiment med olika typer av lignin, kolhydrater och enzymer bör utföras för att optimera processen och validera resultaten.

Nyckelord

Lignin; Papper och Pappersmassa; Återvinning; Bioteknik; Enzymteknologi; Lackaser; Kemiteknik.

List of abbreviations and symbols

- A Area of test piece
- Abs Absorptivity
- ABTS 2,2'-azine-bis(3-ethylbenzothiazoline-6-sulfonic acid)
- B Maximum hydraulic pressure
- b Initial width of sample piece
- $CaCO_3$ Calcium carbonate
- F 10,000/test area
- \bar{F}_C Mean maximum compression force
- g Grammage
- H_i Height of the i -th elution time
- LCC Lignin Carbohydrate Complex
- M Molar, mole/l
- M_i Molecular weight of the i -th elution time
- M_w Molecular weight
- \overline{M}_w Average molecular weight
- m Mass of test piece
- m_0 Mass of test piece before drying
- m_d Dry mass of test piece
- m_w Wet mass of test piece
- N Number of samples
- NaOH Sodium hydroxide
- Na_2S Sodium sulfate
- P Mean bursting strength
- RH Relative Humidity
- SEC Size Exclusion Chromatography
- w_{H_2O} Moisture content
- wt% Weight percent
- σ_C^b Compression strength

Table of Contents

1. Introduction	1
1.1. Aim	1
2. Background	2
2.1. Composition of wood.....	2
2.2. Pulp and paper production	4
2.2.1. The Kraft process	5
2.2.2. The effect of residual lignin in paper.....	7
2.2.3. Recycled paper	8
2.3. Black liquor treatment and lignin purification	9
2.4. Laccases	10
2.4.1. Laccase applications	10
3. Material & Methods	12
3.1. Chemical and solution preparations	12
3.1.1. Lignin preparation	12
3.1.2. Homemade pulp preparation.....	13
3.2. Enzymatic treatment of lignin.....	13
3.2.1. Laccase treatment of lignin	14
3.2.2. Effects of pH.....	14
3.2.3. Hemicellulose crosslinking.....	14
3.3. Paper treatment and homemade pulp experiments	15
3.3.1. Paper treatment experiments	15
3.3.2. Homemade pulp experiments.....	16
3.3.3. Carbohydrate crosslinking experiments	17
3.4. Pulp from papermill experiments	17
3.5. Analysis and evaluation methods.....	19
3.5.1. Size Exclusion Chromatography	20
3.5.2. Water absorption drop test on treated paper and homemade pulp.....	20
3.5.3. Surface weight tests on paper made from recycled pulp.....	21
3.5.4. Thickness tests on paper made from recycled pulp	21
3.5.5. Bendtsen porosity tests on paper made from recycled pulp.....	22
3.5.6. Cobb ₆₀ test on paper made from recycled pulp	22
3.5.7. Bursting strength test on paper made from recycled pulp	23
3.5.8. Compression strength test on paper made from recycled pulp	24
3.5.9. Moisture content.....	25
4. Results & Discussion	26
4.1. Enzymatic treatment of lignin.....	26
4.1.1. Lignin preparation	26

4.1.2.	Enzymatic reaction	26
4.1.3.	Effects of pH.....	28
4.1.4.	Hemicellulose crosslinking.....	29
4.2.	Paper treatment and homemade pulp experiments	31
4.2.1.	Drop test on treated paper.....	31
4.2.2.	Drop test on homemade pulp	33
4.3.	Pulp from papermill.....	35
4.3.1.	Surface weight	35
4.3.2.	Thickness tests	36
4.3.3.	Bendtsen porosity test.....	37
4.3.4.	Burst strength	38
4.3.5.	Compression strength	39
4.3.6.	Cobb ₆₀ test	40
4.3.7.	Moisture content.....	41
5.	Conclusion	43
6.	Future work.....	44
7.	References.....	45
Appendix I	i
Appendix II	viii
Appendix III	xii

1. Introduction

In today's more environmentally aware society there is a great desire to find new and more ecofriendly materials, as well as to improve the ones we have become dependent on. One such material which could be improved is paper and cardboard. Paper has contributed significantly to the development of the human civilization; for spreading information with books and newspapers, for the storage of food and resources with packaging as well as for the personal hygiene with toilet paper and napkins. This combined with the fact that paper is made from renewable resources and can be recycled makes it one of the most important materials available. A problem however, is that when paper is recycled the cellulose fibers are partly degraded, meaning that the new paper made from recycled material is often of lower quality and is more easily damaged (Hamzeh et al. 2012; Sixta 2006)

In order to improve the quality of said recycled paper, one can look at how the cellulose is protected within the native plant cell. It is there bound together with lignin and hemicellulose to form a stable and resistant complex which keeps the cells rigid and intact. This complex is formed with the help of a certain type of oxidizing enzyme called laccase. Using this enzyme to recreate a more native-like fiber network by reestablishing the interaction between cellulose, lignin and possibly hemicellulose could give the recycled paper improved properties (Morozova et al. 2007; Saake & Lehnen 2007).

1.1. Aim

The aim of this master thesis project was to investigate and analyze the possibility of enzymatically modifying the interaction between technical lignin and cellulose fibers in order to increase the strength of the recycled paper used for corrugated cardboard in humid environments.

The project took place between January and June 2019, and was a collaboration between Smurfit Kappa, Piteå, Sweden and the Department of Chemical Engineering at the Faculty of Engineering, Lund University, Sweden; also involving the partners MetGen OY, Kaarina, Finland, providing enzyme expertise and SunCarbon, Lund, Sweden, providing expertise on lignin purification.

2. Background

2.1. Composition of wood

Forests cover around 30 % of our planet's land area and are an important part not only of the ecosystem but also the economy of many countries in the world, not least Sweden and Finland. Wood and wood related products are of interest in today's society since forests are a renewable source of material as well as a sink for carbon dioxide. Wood is normally divided into two different groups: softwoods, from monocot trees such as pine and spruce, and hardwood, from dicot trees such as birch and oak. The two different groups have a rather different cell structure and composition (Data.worldbank.org, 2019, Schmitt, Koch and Lehnen, 2014).

Chemically, wood consists mainly of three macromolecular components: cellulose, lignin and different kinds of hemicelluloses. They are bound together in the cells forming the so-called Lignin Carbohydrate Complex (LCC), an illustration of which can be seen in figure 2.1. The amount of the different components differs between softwoods and hardwoods, with cellulose being the most abundant at around 40 – 44 %. The amount of hemicellulose in softwoods and hardwoods is often around 25 – 30 % and 30 – 35 % respectively. The amount of lignin varies between 25 – 32 % and 18 – 25 % (Schmitt, Koch & Lehnen 2014).

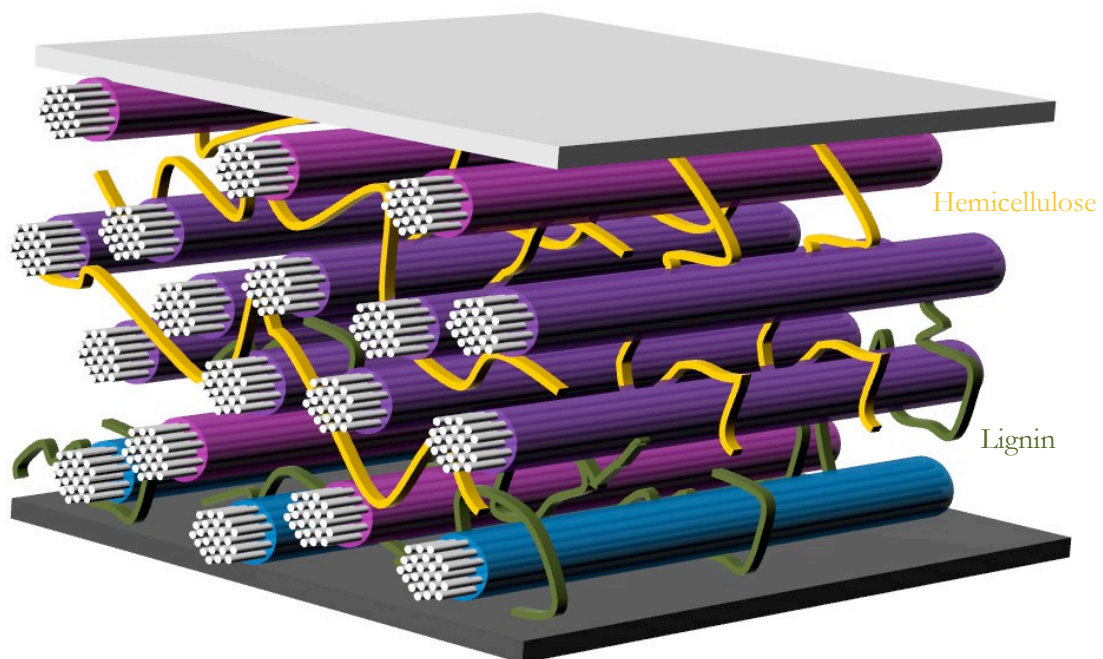


Figure 2.1. Illustration showing the structure of a plant cell, and an example of the lignin carbohydrate complex. The lignin (green), hemicellulose (yellow) and cellulose (grey) are covalently bound together (Palmqvist 2014). Used with author permission.

Cellulose is a long repeating unbranched chain of cellobiose, i.e. two glucose molecules bound together, seen in figure 2.2. The chains are usually around 3,500 – 7,500 cellobiose units long. Cellulose chains form hydrogen bonds between each other, forming sheets of crystalline fibrils that are incredibly sturdy (Ragnar et al. 2014; Schmitt, Koch & Lehnen 2014).

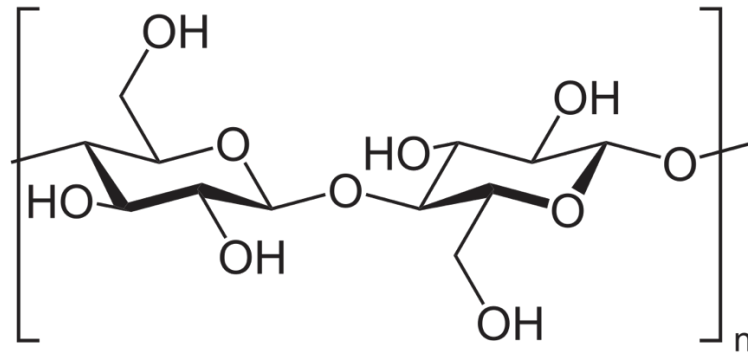


Figure 2.2. Cellobiose which builds up the cellulose chains. n equals between 3,500 and 7,500 (en.wikipedia.org, 2019).

Hemicelluloses differ from cellulose by the fact that the hemicellulose chains are much shorter, often branched and consist of different monosaccharides. These different monosaccharides include pentoses, such as xylose and arabinose, as well as hexoses, such as galactose and mannose. The most common types of hemicelluloses are called xylans and glucomannans. Xylans are more abundant than glucomannans in hardwoods, while glucomannans are more abundant in softwoods. Hemicellulose is covalently bound to lignin in the plant cell and works as a glue keeping the components together. An example of galactoglucomannan can be seen in figure 2.3, where the glucomannan is also bound to galactose (Ragnar et al. 2014; Schmitt, Koch & Lehnen 2014; Tarasov, Leitch & Fatehi 2018).

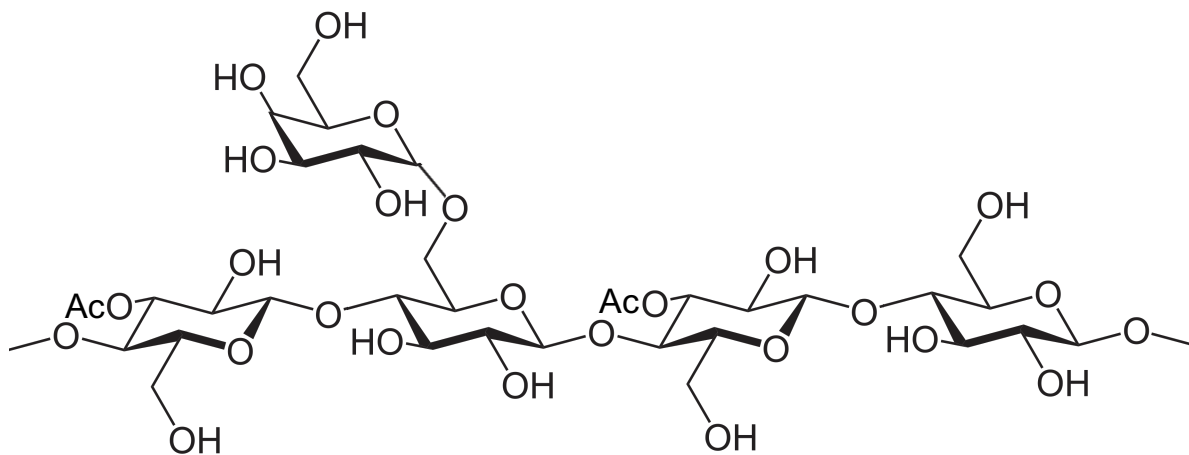


Figure 2.3. Simplified depiction of softwood galactoglucomannan, where the glucomannan is also bound to galactose. Adapted from Schmitt, Koch & Lehnen (2014).

Lignin, an example of which is shown in figure 2.4 is a complex three dimensionally cross-linked phenolic macromolecule. It is mainly built from three hydroxycinnamyl alcohols, namely sinapyl alcohol, coniferyl alcohol and p-coumaryl alcohol. These aromatic molecules, collectively called monolignols, create different lignin units called syringyl, guaiacyl and p-hydroxyphenyl units respectively. The amount of lignin and the fraction of the units it is composed of varies between types of biomass. Softwoods have a relatively high fraction of lignin with guaiacyl as the dominating unit, while the lignin content in hardwoods is slightly lower and has a larger fraction of syringyl (Abdelaziz et al. 2016; Yuan et al. 2011).

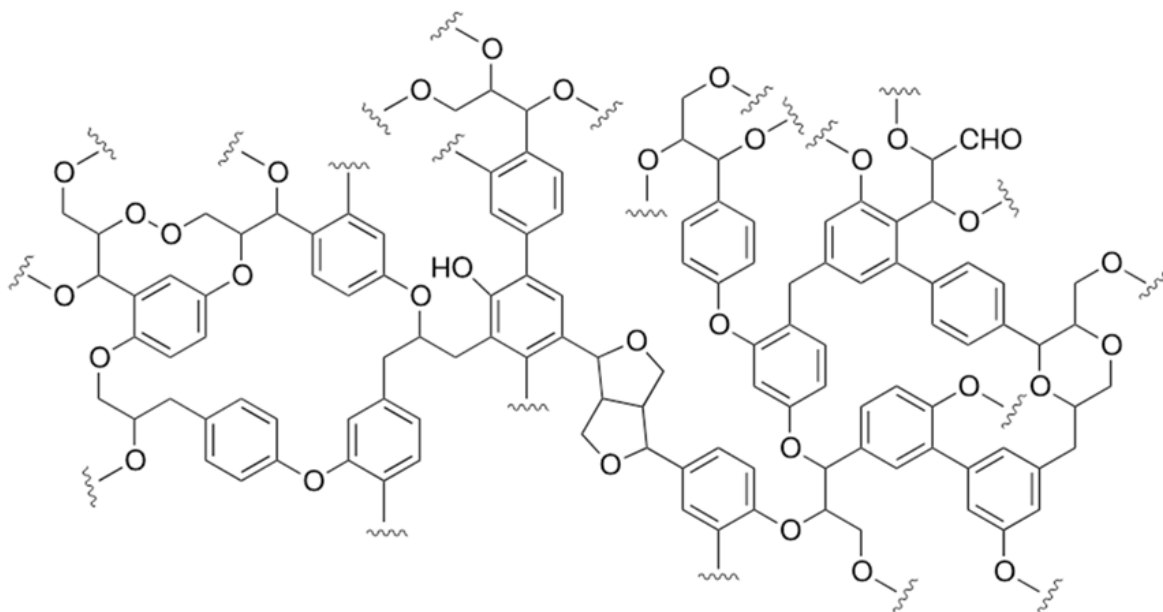


Figure 2.4. Simplified depiction of an example of lignin (Lidén, Sterner & Gorwa-Grauslund 2015). Used with author permission.

Lignin is an important part of terrestrial plants since it is a hydrophobic compound, while hemicellulose and cellulose, depending on their structures, are highly hydrophilic. This means that lignin enables the transport of aqueous solutions inside the xylem of the plants without them losing their rigidity. This property is probably also what lead to larger plants being able to grow on land. The lignin synthesis inside the plant cell is thought to be done by radical reactions between the monolignols, catalyzed a type of enzyme called laccases. The radicals recombine randomly, providing the irregular and complex structure of lignin (Saake and Lehnen, 2007; Abdelaziz *et al.*, 2016).

When creating pulp and paper from wood, the structure of the lignin, as well as the interactions in the LCC changes, and there is a large difference between the native lignin and the lignin found in processed biomass. One should therefore make a clear distinction between processed lignin, which here will be called technical lignin, and native lignin. Since it is very difficult, almost impossible to isolate lignin native state, the structure of native lignin, and it's interactions with the carbohydrates, is still largely unknown (Abdelaziz *et al.* 2016; Gellerstedt & Henriksson 2008).

2.2. Pulp and paper production

The art of creating paper can be traced back to ancient China, where different plants were used to create a primitive paper for writing. Different processes for creating paper were developed during the years, using among other things linen and cotton as a source of fibers. The demand for paper increased over time however, and a cheaper and more available fiber source needed to be found. It was during the latter half of the 19th century that wood started to be utilized and the chemical pulping processes took form (Sixta 2006).

The two dominating methods of chemical pulping are the Sulfate process, also known as the Kraft process, and the Sulfite process. The largest difference between the two methods is that the Kraft process is performed under alkaline condition, and the Sulfite process is performed under acidic conditions. During the earlier half of the 20th century, the Kraft process became the dominating process for producing paper. This was mainly due to the possibility to reuse the cooking chemicals, the acceptance of many different raw materials in the process and the stronger paper resulting from the process. As of 2014, around 95% of all manufactured pulp from chemical pulping came from the Kraft process (Ragnar et al. 2014; Sixta 2006).

Figure 2.5 shows a photo of a Kraft paper mill.



Figure 2.5. Aerial photograph of the Smurfit Kappa paper mill in Piteå, Sweden (Smurfitkappa.com, 2018)

Different types of wood give different properties to the paper. Hardwood generally has shorter fibers than softwood and is often used for finer papers of higher quality. The fibers from softwood are longer and the paper made from it often has higher tensile strength and is therefore often used for packaging and liner (Ragnar et al. 2014).

For this project, sheets of papers and pulp made by recycled paper in a Kraft pulp mill was used.

2.2.1. The Kraft process

There are many steps in the creation of paper using the Kraft process. The process can both be continuous or made in batches. A simplified flow chart of a Kraft process can be seen in figure 2.6. Both hardwood and softwood can be used, and the properties of the paper created by the different types of wood differ. It begins with the debarking and chipping of wood. The woodchips are then impregnated with the cooking chemicals, known as white liquor, and are heated to around 130 – 170 °C under pressure. The cooking chemicals in the Kraft process are a mixture of water, sodium hydroxide (NaOH) and sodium sulfide (Na₂S). During the impregnation, also called the cooking, most of the chemical reactions in the process occur, meaning that the LCC is broken down (Borg 1989).

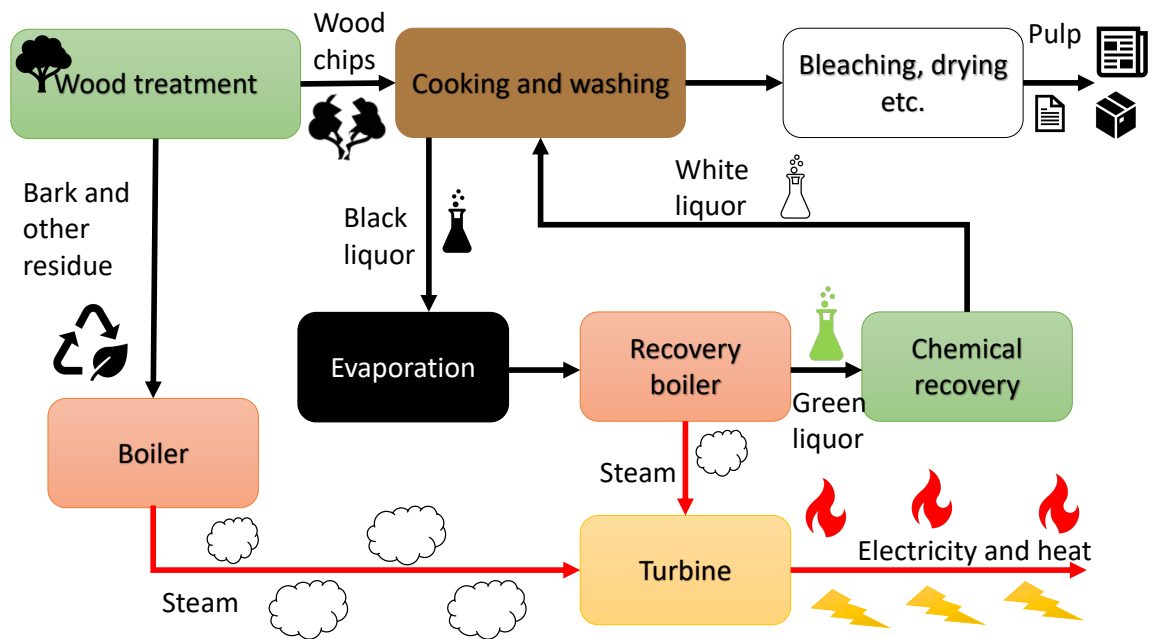


Figure 2.6. Flow chart illustrating the Kraft process.

The chemical reactions mentioned above can be divided into three parts: the initial phase, bulk phase and final phase. During the cooking, the hydroxide and hydrosulfide ions help to form nucleophiles that break the α -aryl and β -aryl ether bonds in the lignin, i.e. the bonds keeping the subunits together. Most of the lignin reacts and is removed during the bulk phase, while the initial phase and final phase only account for around 20 % and 10 % respectively (Ragnar et al. 2014; Sixta 2006).

The cooking reactions are not specific, meaning that both cellulose and hemicellulose also are partly degraded. This mainly occurs during the initial phase. This in turn means that the process needs to be stopped in time to achieve both as high delignification as possible, but also the highest possible yield of the fibers. Glucomannan is especially susceptible to degrading during Kraft cooking (Ragnar et al. 2014; Sixta 2006)

After the impregnation, the chemicals and dissolved lignin is separated from the wood chips. The lignin, degraded carbohydrates and chemical mixture, now called black liquor, move on to the recovery boiler where the chemicals are recycled. The treated wood chips, also known as pulp, is sieved and washed to remove impurities and wood chips that weren't impregnated properly. Depending on which type of paper the pulp is to be used for, said pulp is then bleached. Finer papers for writing or printing for example are bleached extensively, while pulp used for carboard or corrugated paper usually is not. Finally, the pulp slurry is sprayed onto a moving wire bed. In some cases, additives are added to the pulp to improve strength or stiffness of the final product. This process is known as sizing. Depending on the type of paper and the desired properties of the product, different types of additives could be used. One such additive is starch, which can improve the processability, tensile strength and tearing strength of the paper. The water in the slurry is removed by suction and vacuum as well as heat drying and pressing. After it is dried, the paper is rolled up on giant rollers and is then stored until it is sold (Bajpai 2015; Biricik, Sonmez & Ozden 2011).

Figure 2.7 shows an example of a paper machine.

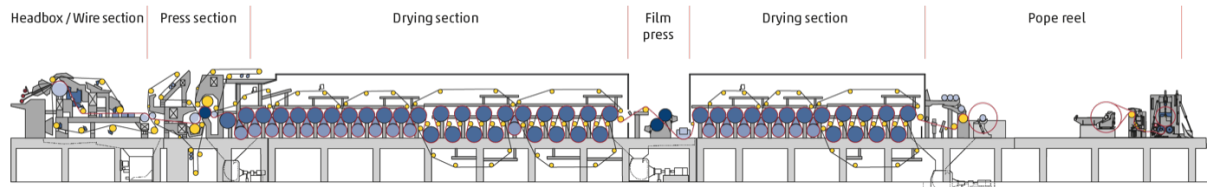


Figure 2.7. Example of a paper machine for the production of corrugated cardboard (Pkvarel.de, 2019).

In order to make the process economical, it is necessary to recover the cooking chemicals from the black liquor. This is done by burning the liquor in a recovery boiler, which not only makes it possible to recover the chemicals, but also provides energy for the mill. The amount of dry solids in the liquor is often around 10 – 18 weight percent (wt%), where 40 – 50 % of these dry solids are cooking chemicals. The remaining dry solids are dissolved lignin and hemicellulose that was degraded in the impregnation step. In order to recover the chemicals efficiently, the black liquor is concentrated in multi stage evaporators. This makes it possible for the combustion to generate more steam, which is in turn used to generate electricity in a turbine. When the black liquor is combusted in the recovery boiler, it is the lignin and hemicellulose that burns, while the cooking chemicals are smelted and pour out of the boiler floor. The smelt is now called green liquor and is recausticized into white liquor by adding calcium carbonate (CaCO_3). This process is illustrated in figure 2.8 (Ragnar et al. 2014).

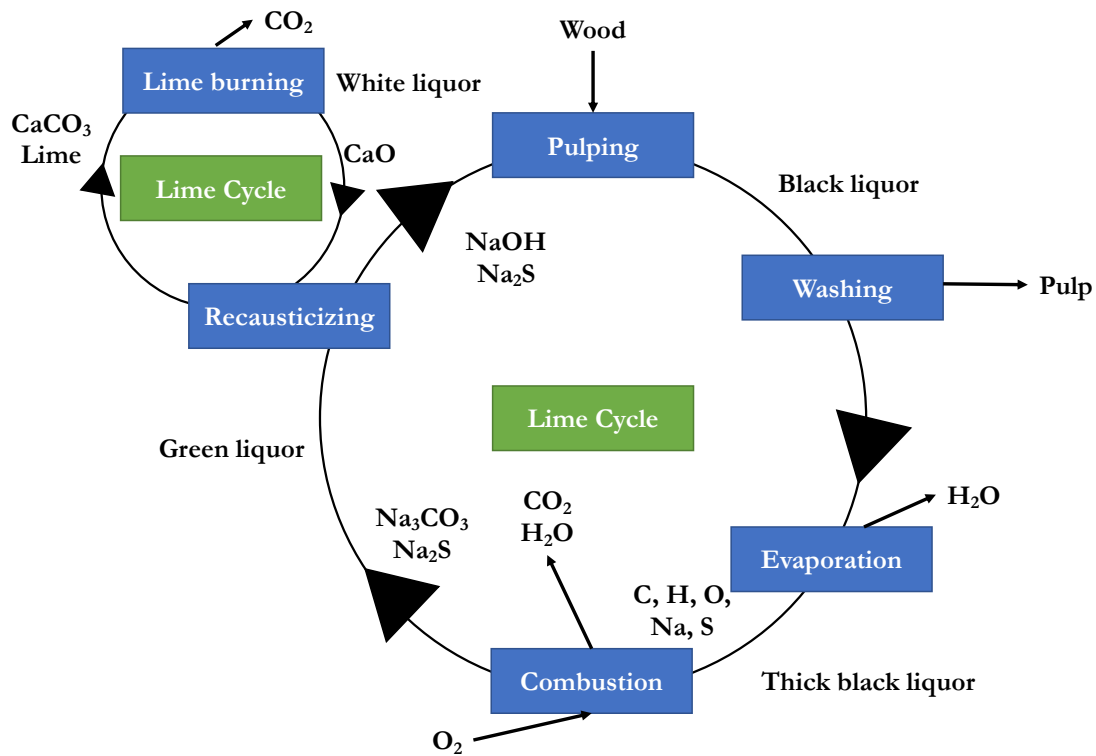


Figure 2.8. Chemical recovery cycle, adapted from Ragnar et al. (2014).

2.2.2. The effect of residual lignin in paper

Most of the lignin is separated from the wood chips during the impregnation. However, it is both difficult and expensive to remove it all without lowering the fiber yield too much. The amount of lignin that remains in the paper has an effect on its properties. There are different reasons why all the lignin is not removed during the cooking process. There might be steric hindrances for the dissolution, meaning that for example a large cluster of cellulose blocks the cooking chemicals from reaching the lignin, or that new bonds are formed during the extreme conditions of the cooking, which make the lignin harder to dissolve. The most important reason is probably the fact that the covalent bonds in the LCC keep the lignin tightly bound to the fibers (Ragnar et al. 2014).

The amount of lignin in paper can be estimated by a number of different methods. One of these is the kappa number. This measurement is based on how many milliliters of 0.02 M potassium permanganate one gram of pulp can consume at 25 °C before being saturated, i.e. it gives an estimate of the amount of oxidizable structures that remain in the pulp. If a paper has a kappa number of 1 unit, it corresponds to approximately 0.15 % residual lignin (Dence 1992; Ragnar et al. 2014; Sixta 2006).

Another method used to measure the lignin content is by the Klason Lignin determination. In this method, the pulp is dissolved in sulfuric acid. The cellulose and hemicellulose will be dissolved by the acid, while the lignin remains insoluble. This insoluble lignin is called Klason Lignin (Ragnar et al. 2014; Sixta 2006).

Lignin remaining in the paper will affect its properties, which can be positive or negative depending on what the paper should be used for. If there is residual lignin in the paper, it may interfere with the hydrogen bonds forming between the cellulose fibers, which makes the paper more brittle. The lignin may also hinder the fibers from swelling, making it stiff. The stiffness of the fibers, and by extent the stiffness of the paper has been shown to be optimal at a kappa number of around 30 – 60. Another factor that can be affected by the lignin content is the mechano-sorptive creep stiffness, which is the deformation of the paper that occurs by different forces under variations in relative humidity. The wet strength of the paper, i.e. how resistant the paper is to breakage when wet, has been shown to increase with the addition of lignin (Antonsson 2007, 2008; Maximova 2004).

2.2.3. Recycled paper

Since the introduction of chemical pulping, the development in paper production in the world has increased significantly. Between 1950 and 2003, the amount of produced paper in the world increased almost eightfold, from 43.8 million tons in the fifties to 340 million tons in the early two thousands. Together with the increase in production, there has also been an exponential increase in the usage of recycled paper. The use of recycled paper fibers is often encouraged, and in many countries even required. It is today regarded by society to be an environmentally friendly process since it preserves forests as well as avoids unnecessary landfilling. Figure 2.9 shows an example of collected waste paper. The use of recycled paper has become such an important part of paper production that the total amount of recycled fibers in Europe exceeded the total amount of virgin fibers in 2001, a trend that was still seen as late as 2017 (CEPI 2017; Sixta 2006).



Figure 2.9. Collected paper for recycling in Italy (en.wikipedia.org, 2019).

Before the recycled fibers can be utilized and mixed with the virgin fibers it needs to be pretreated, and since recycled fibers come from many different sources, it means that they may need to be pretreated differently. In some papermills, the newly made paper is cut into smaller pieces before being delivered to the customer. The leftover pieces of paper can then be recycled directly in the factory without needing much pretreatment since they haven't been used for anything else. On the other hand, paper that for example comes from households may need to be deinked before they can be processed into new paper. This deinking process provides an environmental problem since it produces a relatively high amount of waste and sludge (Sixta 2006).

Another problem with the recycling is that it cannot be done an infinite number of times. Each time paper is recycled the fibers lose some of their properties and there is more discoloration of the final product. Effects of recycling include loss in wet flexibility, water retention and conformability. The length of the fibers is shortened when the paper is recycled as well, which has a negative impact on the quality of the new paper. Since more and more recycled paper is used in paper and pulp production, there is a desire to improve the quality of the recycled fibers (Hamzeh et al. 2012).

2.3. Black liquor treatment and lignin purification

Black liquor, being a by-product in the paper mill, needs to be taken care of. After the evaporation stage described in section 2.2.1, the high content of inorganic solids and lignin in the concentrated black liquor gives it a high viscosity. This could lead to precipitation, fouling and clogging in the system. Also, a higher concentration of organic compounds in the black liquor could overload the evaporators and recovery boiler, which in turn could become a bottleneck in the process. Instead of upgrading the recovery boiler and evaporator system, which could be very expensive, the lignin can be separated from the black liquor, which both decreases the load on the system, as well as providing a source of lignin which can then be used for other purposes (Arkell 2018).

There are different methods to produce lignin from the black liquor. A way to do this is to lower the pH of the liquor, which precipitates the lignin. One of these methods is the LignoBoost method. A newer method that utilizes precipitation by lowering the pH, filtrating the lignin and then resuspending the filtered lignin in sulfuric acid in order to remove inorganic materials (Gellerstedt 2015).

Another method for the separation of lignin from black liquor is the SunCarbon process. This is a process where the lignin is separated from the black liquor through different steps of ultrafiltration. The black liquor from the pulping is first split into two streams where the first stream is evaporated and then incinerated in the recovery boiler to be recycled. The second stream however is filtered through a filter to produce a retentate and a permeate. Most of the organic material remains in the retentate, and the permeate is devoid of it. The permeate is then passed through another filter with lower cutoff in order to retain smaller molecules. The second permeate mainly consists of cooking chemicals. The lignin in the first retentate stream can be depolymerized and then combined with the second retentate stream in order to give a homogenous lignin stream which is suitable for different applications (Arkell 2018).

The lignin used in this project was produced with the SunCarbon process made from black liquor from the Smurfit Kappa paper mill in Piteå, Sweden. The black liquor was a mixture of around 70 % softwood, 30 % hardwood and is taken out from the above-mentioned homogenous lignin stream.

2.4. Laccases

Laccases are a type of enzymes found in plants, fungi and bacteria and are one of the so-called lignin modifying enzymes. They specifically catalyze the reduction of molecular oxygen to water and are thought to help with both the degradation and building of organic polymers, such as lignin. Laccases were one of the earlier enzymes to be discovered and dates back to the 19th century where they were found in the Japanese lacquer tree. The name laccase comes from the fact that one of the earliest applications of laccases were to extract lacquer from said trees. Laccases are abundantly found in organisms that live on and of trees, for example the tree rot fungi *Trametes versicolor*, or different plant pathogens (Kaczmarek et al. 2017; Morozova et al. 2007; Rodríguez-Couto 2018).

The fact that lignin is a complex and irregular molecule indicate that it does not form by specific enzymes catalyzing the formation of each bond in the plant cell, but rather that it is a radical chain reaction catalyzed by a mediator system. Due to the structure of lignin, enzymes have no possibility to interact with it directly, simply because the lignin molecule cannot fit in the active site of the enzyme. Instead, the enzyme oxidizes a smaller molecule, a so-called mediator, and that mediator then carry the charge and react with the lignin (Morozova et al. 2007; Oinonen et al. 2015).

Laccases have been proven to have a greater activity when synthetic mediator compounds are added to the reaction mixture. Examples of known mediators are 2,2'-azine-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), syringaldehyde and 2,6-dimethylphenol. With these types of mediators, the radical chain reaction loop is closed, meaning that the mediator is not consumed and can instead be reused indefinitely, see figure 2.10 (Morozova et al. 2007).

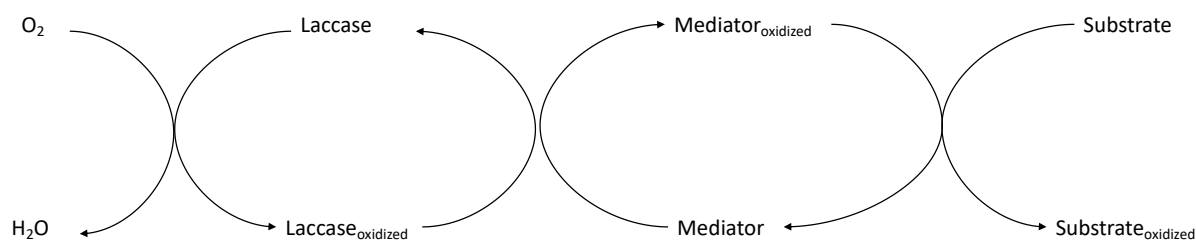


Figure 2.10. Oxidation path of a laccase catalyzed reaction with a reusable mediator. Adapted from Morozova et. al (2007).

2.4.1. Laccase applications

Laccases have many applications in biotechnology. Among other things, they are used to help keep wine fresh and taste better, they are used in the bleaching of denim products, they are used in different types of organic synthesis and they are used in the pulp and paper industry (Mate & Alcalde 2017).

In the pulp and paper industry, laccases have the possibility to be used for a variety of things. For example, the addition of laccases during the bleaching of the pulp has proven to reduce the need of chemicals to achieve the same bleaching effect. This in turn reduces toxic contaminants. Other studies have been done which uses laccases to graft different types of substances on the surface of paper packaging. In a study by Elegir et al. (2008), laccases were used to graft phenolic antimicrobial molecules to paper made from softwood Kraft pulp, which lead to a packaging that was resistant to certain types of microbes (Elegir et al. 2008; Kaczmarek et al. 2017).

A study by Kudanga et al. (2010) showed the possibility to use laccases to introduce different fluorophenols and alkylamines to the surface area of packaging. The covalent bonding of these types of substances increased the hydrophobicity of the surface, which in turn decreased the biodegradability of the material, increasing its lifespan (Kaczmarek et al. 2017; Kudanga et al. 2010).

Since laccases take part in the production of lignin in the plant cell, one application for the enzyme is to use it to degrade the lignin into smaller molecules which can then be used for something else. The fact that lignin is one of the larger byproducts in the pulp and paper industry, there is a large desire to valorize it in some way. Different types of specialty laccases have been developed in order to optimize the degradation of lignin under different conditions (Hämäläinen et al. 2018).

Another way to use laccases to valorize lignin is to use their ability to build up lignin to incorporate the molecule back into pulp. The inherent water repellent nature of lignin could then help to increase the moisture resistance of paper. In a study by Aracri *et al.* (2010), laccases were used to incorporate monomeric phenols in pulp created from flax and sisal. If monomeric phenols can be integrated with pulp, there could be a possibility to also integrate larger phenolic compounds, such as larger lignin molecules. Another study, by Oinonen *et al.* (2015), showed that there is a possibility to use laccases to increase the molecular weight (M_w) of hemicelluloses that have phenolic compounds attached to them. This could indicate that hemicelluloses can be used as a bridge to bond lignin to the cellulose fibers in papers (Aracri et al. 2010; Oinonen et al. 2015).

3. Material & Methods

3.1. Chemical and solution preparations

3.1.1. Lignin preparation

The lignin was kindly provided by SunCarbon AB, produced from black liquor containing about 70% softwood and 30% hardwood. The black liquor came from the Smurfit Kappa papermill in Piteå, Sweden.

The lignin was provided as a powder. For the enzyme to react with it, it needed to be dissolved in an aqueous solution. Since lignin is not soluble at low pH, this solution needed to be alkaline. The method of preparing the lignin was based on a method used by Hämäläinen et al. (2018). The lignin was dissolved at 100 g/l in 0.25 M NaOH and was mixed at room temperature for 30 minutes. It was then centrifuged at 6,000 g for 20 minutes. The supernatant was transferred to a beaker where the pH was lowered to 11, 10, 9 and 8 with hydrochloric acid (HCl). The pellets from the centrifugation were dried and weighed to determine the amount of undissolved lignin.

The lignin solution with the different pH values were then filtered through pre weighed filter papers to remove the lignin that precipitated after the change in pH, illustrated in figures 3.1 and 3.2. Since there was a risk of the filters breaking, two were placed on top of each other. Samples from the permeate was taken for analysis, and the rest was stored for future experiments.



Figure 3.1. Foaming occurred when the lignin was filtrated.



Figure 3.2. Filter cake of precipitated lignin.

The used filter papers, together with the retentate, were dried in an oven at 105 °C for 24 hours and were then weighed to determine the amount of lost lignin after the lowering of pH. Each pH value was adjusted for in three different beakers, providing a triplicate of tests on the composition of the final solution.

The lignin solution was prepared twice. Once for the experiments described in sections 4.1. and 4.2., and once for the experiments described in section 4.3.

3.1.2. Homemade pulp preparation

Sheets of paper made from recycled pulp, with a weight of 90 g/m² was provided by Smurfit Kappa. 15 g of these papers were cut into pieces and mixed with 0.5 l of water in an ordinary kitchen blender, which gave a final concentration of 30 g/l. The pulp was then transferred to a flask, which is shown in figure 3.3, and stored for later experiments.

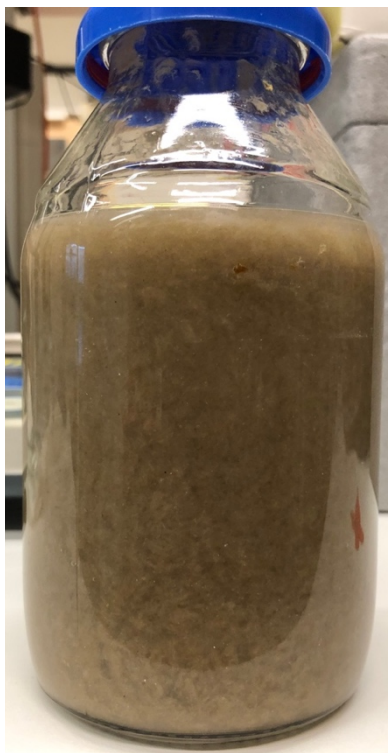


Figure 3.3. Homemade pulp stored in a 500 ml jar with a concentration of around 30 g/l.

3.2. Enzymatic treatment of lignin

The enzyme used in the experiments was the MetZyme® LIGNO™, which was kindly provided by MetGen OY.

Several different experiments were performed with the enzyme and lignin. They all followed a basic structure however, which is described below.

The concentration of lignin for the enzymatic treatment was decided on to be 25 g/l. This meant that 2.5 ml of the concentrated lignin solution was diluted together with the enzyme to a final volume of 5 ml. The experiments took place in a 50 ml Erlenmeyer flask in order to assure sufficient aeration, shown in figure 3.4. The final volume was corrected with deionized water. Experiments were performed in order to find out how much enzyme that were to be added.

The flasks were placed in a Kuhner Shaker X (Birsfelden, Schweiz) at a constant temperature and a shaker speed at 200 rpm, see figure 3.5. The reaction took place for a predetermined time, and samples for analysis were taken at even intervals throughout the experiment. Since water evaporated during the reaction due to the relatively high temperature, the flasks were weighed before sampling and deionized water was added to the flasks to compensate for evaporation.

The samples were instantaneously diluted five times in 0.1 M NaOH and placed on ice in order to stop the reaction after the sampling was done.

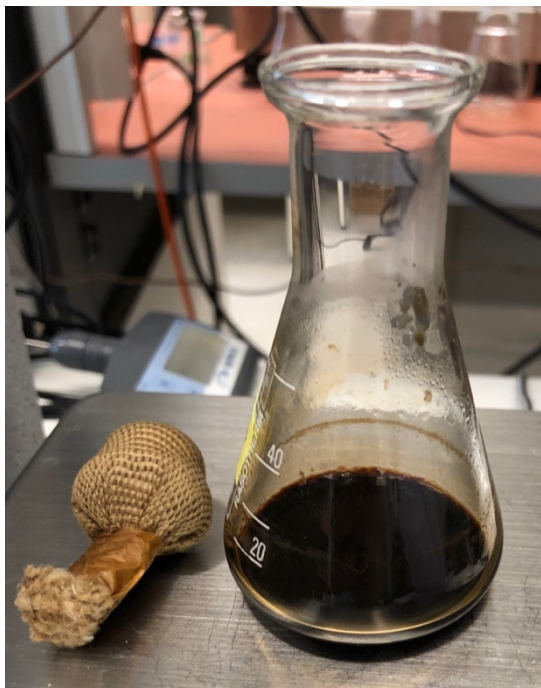


Figure 3.5. Flask in which the reaction took place.



Figure 3.4. Four flasks in the shaker oven.

3.2.1. Laccase treatment of lignin

In order to decide on how much enzyme that should be used in the upcoming experiments, the product specification sheet from MetGen was consulted. The temperature was set to 60 °C, the shaker to 200 rpm and the lignin solution with pH 10 was used, according to recommendations. Four different enzyme concentrations were used: 10 µl, 100 µl, 200 µl and 500 µl. The reaction time was set to 6 hours with sampling for analysis taking place once each hour.

3.2.2. Effects of pH

To see how the pH affected the activity of the enzyme and the effect on the lignin, different values of pH were tested with 100 µl of the enzyme solution. The temperature was set to 60 °C, the shaker speed to 200 rpm and the reaction took place for 4 hours with sampling for analysis taking place once each 30 minutes.

3.2.3. Hemicellulose crosslinking

A hemicellulose solution which was filtrated from the process water from thermomechanical production of pulp from spruce was kindly provided by Johan Thuvander at the Department of Chemical Engineering at Lund University. It was mixed with the lignin and enzyme so that both the lignin and hemicellulose got a final concentration of 12.5 g/l. The temperature was set to 60 °C, the shaker speed to 200 rpm and the reaction took place for 4 hours with sampling for analysis taking place once each 30 minutes.

3.3. Paper treatment and homemade pulp experiments

For the paper treatment and homemade pulp experiments, sheets of paper made by recycled pulp with a weight of 90 g/m² which were kindly provided by Smurfit Kappa were used.

3.3.1. Paper treatment experiments

The paper was cut in strips and weighed before the coating experiments. A reaction mixture with 12.5 g/l pH 10 lignin and 12.5 g/l hemicellulose was prepared and the strips were lowered into the mixture and secured so that a part of the strip would stay submerged the other would stay dry, even when the flask was shaken in the oven. The reaction took place for 4 hours, at 60 °C and a shaker speed at 200 rpm. Samples of the mixture for analysis were taken at the start and at the end of the experiment.

The strips of paper were then washed thoroughly with deionized water and dried in an oven overnight. The experiment setup can be seen in table 3.1 below and figure 3.6. Duplicates were performed.

Table 3.1. Combination of parameters in the mixtures used for the paper coating experiments. Mixture 1 contained only lignin. Mixture 2 contained lignin and enzyme. Mixture 3 contained lignin and hemicellulose. Mixture 4 contained lignin, hemicellulose and enzyme. Mixture 5 contained hemicellulose and enzyme.

	Lignin	Hemicellulose	Enzyme
1	X	-	-
2	X	-	X
3	X	X	-
4	X	X	X
5	-	X	X



Figure 3.6. Setup of the paper treatment experiments. Part of the paper was in contact with the lignin solution and part of it never came in contact with it.

3.3.2. Homemade pulp experiments

The homemade pulp was mixed with the lignin and hemicellulose to achieve a final concentration of around 10 g/l pH 10 lignin and hemicellulose, 0.1 g/l enzyme solution and 6 g/l pulp. The volume of pulp and lignin had a 1:1 correlation, the volume of pulp and hemicellulose had a 1:2 correlation and the volume of pulp and enzyme had a 1:0.1 correlation. The reaction took place for 4 hours, at 60 °C and shaker speed at 200 rpm. Samples were taken at the start and at the end of the experiment.

The experimental setup can be seen in table 3.2. Samples for analysis were taken at the start and at the end of the experiment.

Table 3.2. Combination of parameters in the mixtures used for the homemade pulp experiments. Mixture 1 contained only pulp. Mixture 2 contained pulp and lignin. Mixture 3 contained pulp, lignin and enzyme. Mixture 4 contained pulp, lignin, hemicellulose and enzyme. Mixture 5 contained pulp, lignin and hemicellulose.

	Pulp	Lignin	Hemicellulose	Enzyme
1	X	-	-	-
2	X	X	-	-
3	X	X	-	X
4	X	X	X	X
5	X	X	X	-

To remove the unreacted lignin from the solution, the mixture was centrifuged for 10 minutes at 4,000 g so that the pulp sedimented. The supernatant was then removed, and the pulp was washed. This was repeated three times. The washed pulp was then filtered so a small sheet of paper was created. The sheet was placed under a weight and dried in an oven at 105 °C for 24 hours. Figure 3.7 shows two homemade sheets of paper.



Figure 3.7. Papers from homemade pulp. The one on top has not been treated with anything, and the one below was treated with lignin and enzyme.

3.3.3. Carbohydrate crosslinking experiments

In addition to the hemicellulose, other carbohydrates were tested. These were two different hexoses: glucose and mannose; two different pentoses: xylose and arabinose and one disaccharide: sucrose. For these experiments, 12.5 g/l of pH 10 lignin solution was used, and the different carbohydrates also had a concentration of 12.5 g/l. The reaction took place for 4 hours at 60 °C, and samples were taken at the start and at the end of the experiment.

3.4. Pulp from papermill experiments

Pulp from recycled paper directly from the mill was provided by Smurfit Kappa Piteå, Sweden. When the pulp was taken from the mill, a bacteriocidal substance was added to avoid the pulp from spoiling.

The experiments were similar to those described in section 3.3.2. but with a few modifications.

A larger volume of 1 liter was used. In order to create a sheet of paper with a surface weight of 100 g/m² for analysis, the final mass of dry pulp used needed to be 3.47 g. Using a pulp with a concentration of 40 g/l, this meant that 43.375 ml, or approximately 75 g of pulp, was needed for each sheet.

In order to use the same volume correlations as described in section 3.3.2, this meant that 43.375 ml lignin solution, 86.75 ml carbohydrate solution and 4.3375 ml of enzyme was added. This gave a final concentration of approximately 4.3375 g/l lignin and carbohydrate.

A lignin solution with pH 10 was used, and sucrose was used as a carbohydrate in these experiments due to its availability. All sheets of paper for these experiments were treated with the enzyme.

Enough solution to create two sheets of paper was prepared in a one-liter container. The container was placed in a water bath keeping 60 °C and was stirred using a mechanical stirrer in order to ensure sufficient aeration. The experimental setup is shown in figure 3.8.



Figure 3.8. Experimental setup for the experiments with the papermill pulp.

Nine different mixtures, seen in table 3.3, were used for these experiments. Enough of each mixture was made to get four sheets of paper for each treatment.

Table 3.3. Experimental setup of the experiments using the papermill pulp. With a final volume of 1 l, 43.375 ml of lignin solution, 86.75 ml of carbohydrate solution and 4.3375 ml of enzyme solution were added to reach the desired concentrations.

Mixture number	Initial lignin conc. [g/l]	Final lignin conc. [g/l]	Initial sucrose conc. [g/l]	Final sucrose conc. [g/l]
9	0	0	0	0
8	0	0	12.5	2.16875
7	0	0	25	4.3375
6	25	2.16875	0	0
5	25	2.16875	12.5	2.16875
4	25	2.16875	25	4.3375
3	50	4.3375	0	0
2	50	4.3375	12.5	2.16875
1	50	4.3375	25	4.3375

Sheets of paper were then made from the treated pulp. The pulp was poured in a sheet former, seen in figure 3.9. In the sheet former, the pulp was diluted with water and then stirred. The water was then drained which caused the pulp to form a sheet on a copper filter. The sheet was placed under a couple of blotting papers and a weight was used to press out excess water.



Figure 3.9. Sheet former used for forming sheets. Copper wire used to collect the pulp seen in the right corner.

The wet blotting papers were removed and exchanged for new ones, and the sheet was placed on a metal plate. More sheets were made the same way and the plates were placed in a pile. When the pile was of sufficient size, they were placed under 0.55 MPa of pressure for 5 minutes. The wet blotting papers were once again removed and exchanged for new ones, and the pile was then pressed at 0.55 MPa for another 2 minutes. After the pressing, the sheets were first stored in a special condition chamber at 23°C and 35 % relative humidity (RH) for 8 hours and were then conditioned at 23 °C and 50% RH for 24 hours. Figure 3.10 show a sheet of paper after pressing, and 3.11 show the sheets being conditioned.



Figure 3.10. Sheet of paper made from recycled pulp. Seen on top of a pile with other sheets after being pressed at 0.55 MPa.



Figure 3.11. The sheets of paper made from recycled pulp being conditioned at 23 °C and 50% RH.

3.5. Analysis and evaluation methods

In order to see if the treatment could prove useful in future commercial uses, some standard paper tests following ISO standards were performed. These tests are described in sections 3.5.3 – 3.5.9 and which standard used for which analysis method can be seen in table 3.4.

Table 3.4. Numbers of the different ISO standards that the tests were based on.

	Weight	Thickness	Bendtsen porosity	Cobb ₆₀	Burst strength	Compression strength	Moisture content
ISO	536	534	5636-3	535	2758	9895	287

3.5.1. Size Exclusion Chromatography

Size Exclusion Chromatography (SEC) was performed using a HPLC system (Waters Corporation, Milford, Massachusetts, United States) and a Superdex 200 column (GE LifeSciences, Chicago, Illinois, United States), UV detector (Waters Corporation, Milford, Massachusetts, United States) measuring at 280 nm and a RI detector (Shimadzu, Kyoto, Japan). 10 polystyrene sulfonate standards (Polymer Standard Service, Mainz, Germany) ranging between 1 kDa and 1,000 kDa were used as molecular weight standards, enabling estimation of molecular sizes in the SEC chromatograms based on retention times. Average molecular weight of each sample could be calculated using equation 1:

$$\overline{M}_W = \frac{\sum(H_i M_i)}{\sum H_i} \quad (1)$$

Where H_i is the height of the i -th elution time and M_i is the molecular weight of the i -th elution time. The calibration, calculations and plotting were done using MatLab R2016a.

3.5.2. Water absorption drop test on treated paper and homemade pulp

Water absorption drop tests were performed on both the treated and nontreated parts of the paper strips. The paper was suspended in the air by two wooden clamps so that the part that was to be tested was not in contact with any surface. A pipette was used to place a drop containing 10 μ l of water on the paper. The time for the water to be absorbed by the paper was measured, from when the drop hit the surface until the visible gloss on the surface had disappeared, see figures 3.12 and 3.13.



Figure 3.12. Drop test performed on treated paper. The left drop is on an untreated area, and the right is on the part of paper dipped in the lignin solution.



Figure 3.13. Drop test performed on paper made from homemade pulp. The top drop has already been absorbed and the bottom drop has just been applied.

3.5.3. Surface weight tests on paper made from recycled pulp

Weight, thickness and porosity tests were done on the paper made from recycled pulp. The weight was determined by cutting each sheet into equally large circles using a form. The circles had a diameter of 200 mm.

The weight per square meter, or grammage could be calculated using equation 2:

$$g = \frac{m}{A} * 10,000 \quad (2)$$

Where

g = Grammage [g/m^2]

m = Mass of test piece [g]

A = Area of test piece [cm^2]

Calculations can be found in Appendix I.

3.5.4. Thickness tests on paper made from recycled pulp

The thickness was measured by using the apparatus seen in figure 3.14. The apparatus measures the thickness of the paper in micrometers by pressing a circular pressure face to another circular pressure face called the anvil. Five measurements on each sheet of paper were performed, giving a total of 20 values for each type of treatment. The tests were done in a conditioned environment at 23 °C and 50 % RH. Calculations can be found in Appendix I.



Figure 3.14. Apparatus measuring the thickness of the paper samples.

3.5.5. Bendtsen porosity tests on paper made from recycled pulp

The air permeance was determined using the ISO standard 5636-3, called the Bendtsen method, which is the mean flow of air through an area under a specific pressure and time, measured in milliliters per minute. It was measured using the apparatus seen in figure 3.15.

One measurement from each side of each sheet was made, giving a total of eight values for each type of treatment. The tests were done in a conditioned environment at 23 °C and 50 % RH. Calculations can be found in Appendix I.

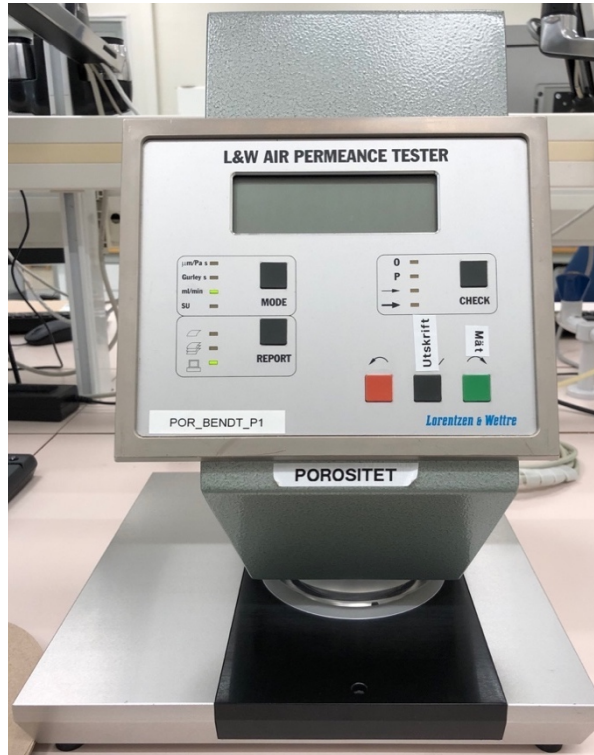


Figure 3.15. Apparatus measuring the air permeance of the paper samples.

3.5.6. Cobb₆₀ test on paper made from recycled pulp

Tests were performed in order to test the water absorptivity, or Cobb₆₀ value, of the paper. In this test, the paper is kept under 1 cm of water for 60 seconds under. The equipment used is shown in figure 3.16.

The sample of paper was weighed and then placed in a form with an area of 25 cm². 25 ml of water was added in order to achieve a 1 cm layer on top of the paper. After 45 seconds, the water was poured off and after 60 seconds, the test piece was blotted using blotting paper and a roller. The test piece was then weighed again, and the Cobb₆₀ was calculated using equation 3:

$$Abs = (m_w - m_d) * F \quad (3)$$

Where

Abs = Absorptivity [g/m²]

m_d = Dry mass of test piece [g]

m_w = Wet mass of test piece [g]

F = 1000/Test area [1/m²]

Two Cobb₆₀ tests were performed on each sheet of paper, giving a total of four values on each treatment. The tests were done in a conditioned environment at 23 °C and 50 % RH. Calculations can be found in Appendix I.

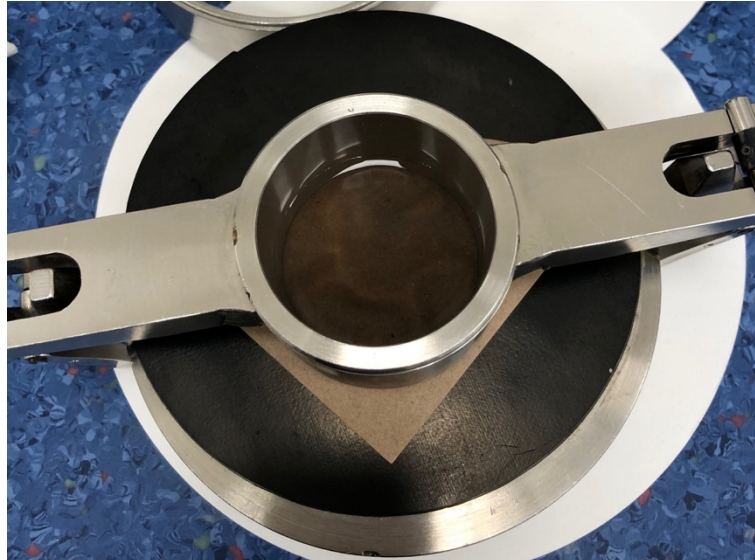


Figure 3.16. Cobb test equipment. The sheet of paper was placed under the ring and 25 ml of water was poured onto it.

3.5.7. Bursting strength test on paper made from recycled pulp

Bursting strength tests were performed on the paper in order to determine how much pressure that could be exerted on it before it broke, i.e. the bursting strength, using the equipment shown in figure 3.17.

The paper was held in place by clamps and a rubber diaphragm was pushed up with hydraulic fluid under a controlled manner until the paper ruptured. The maximum amount of pressure before the breaking point was recorded as the bursting strength of the paper.

The mean bursting strength of paper is calculated using equation 4:

$$P = \frac{B}{N} \quad (4)$$

Where

P = Mean bursting strength [kPa]

B = Mean maximum hydraulic pressure [kPa]

N = Number of samples

Four burst tests were performed on each sheet of paper made from differently treated pulp, two on each side, giving a total of 16 values for each type of treatment. The tests were done in a conditioned environment at 23 °C and 50 % RH. Calculations can be found in Appendix I.



Figure 3.17. Equipment used for the bursting strength tests.

3.5.8. Compression strength test on paper made from recycled pulp

The compression strength of the paper was measured by placing a piece of it between two clamps and pressing them together until structural failure occurs. The distance between the clamps was 0.7 mm and a strip of paper with a width of 15 mm was used. The equipment used can be seen in figure 3.18.

The compression strength was then calculated using equation 5:

$$\sigma_C^b = \frac{\bar{F}_C}{b} \quad (5)$$

Where

σ_C^b = Compression strength [kN/m]

\bar{F}_C = Mean maximum compression force [N]

b = Initial width of sample piece [mm]

Five tests were made on each strip of paper taken from the different treatments of the pulp, giving a total of 20 values for each type of treatment. The tests were done in a conditioned environment at 23 °C and 50 % RH. Calculations can be found in Appendix I.



Figure 3.18. Equipment used for the compression strength tests.

3.5.9. Moisture content

The moisture content of the papers was by drying strips cut out from the differently treated papers at 105 °C until constant mass. The strips were placed in glass containers and weighed before being placed in the oven. They remained in the oven for 24 hours and were then placed in desiccators for 3 hours to cool. The containers were then weighed again, and the moisture content measured in percent was calculated using equation 6:

$$w_{H_2O} = \frac{m_0 - m_d}{m_0} * 100 \quad (6)$$

Where

w_{H_2O} = Moisture content [%]

m_0 = Mass of test piece before drying [g]

m_d = Mass of test piece after drying [g]

The tests were done in a conditioned environment at 23 °C and 50 % RH. Calculations can be found in Appendix I.

4. Results & Discussion

4.1. Enzymatic treatment of lignin

4.1.1. Lignin preparation

The first step in the enzymatic treatment experiments was the dissolution of lignin, which was done twice. There were some losses during both of the batches, primarily since all the lignin did not dissolve. There was also a loss of lignin due to precipitation when the pH was lowered. The total loss of lignin for the first batch can be seen in table 4.1. Calculations can be found in Appendix I.

Table 4.1. Loss of lignin for different pH values.

	Undissolved [g]	Precipitation [g]	Total loss [g]	Actual concentration [g/l]
pH 11	1.95	0.04	1.99	48.01
pH 10	1.95	0.05	2.00	48
pH 9	1.95	5.29	7.24	42.76
pH 8	1.95	6.72	8.67	41.33
Total	1.95	12.1	14.1	-

The undissolved and precipitated lignin was not measured for the second batch, meaning that the exact concentrations used for the experiments in section 4.3. is not known.

4.1.2. Enzymatic reaction

With the lignin prepared, the enzymatic reactions could begin. The first experiment performed was to look at what volume of enzyme should be added in order to achieve a sufficient reaction within a reasonable time frame.

The average molecular weights as a result of the enzymatic treatment of lignin are shown in figure 4.1., and the chromatogram from the SEC analysis of the sample with 100 μ l added is shown in figure 4.2. The average molecular weight was derived from the results of the SEC analyses and was calculated using equation 1 described in section 3.5.1.

Supplemental figures showing the chromatograms of the other experiments can be found in Appendix II.

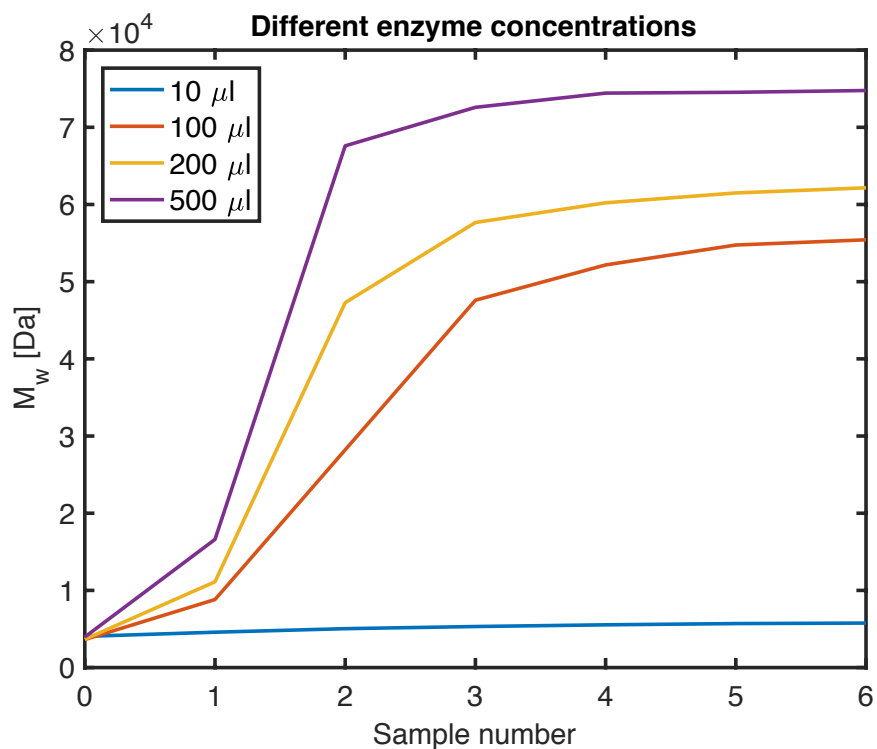


Figure 4.1 The average molecular weight of lignin which was produced from the differently added volumes of lignin. The reaction took place for 6 hours, and samples were taken once each hour. The lignin solutions had a pH of 10.

The first thing to notice from the results is that the enzyme and lignin reacts very quickly. With an enzyme dose of 500 µl was used, the average molecular weight reached a maximum after only two hours. With 200 µl, the molecular weight reached a maximum after about three hours, and with 100 µl, it took almost four. With 10 µl, the reaction was much slower and no major change in average molecular weight was seen in the SEC analyses.

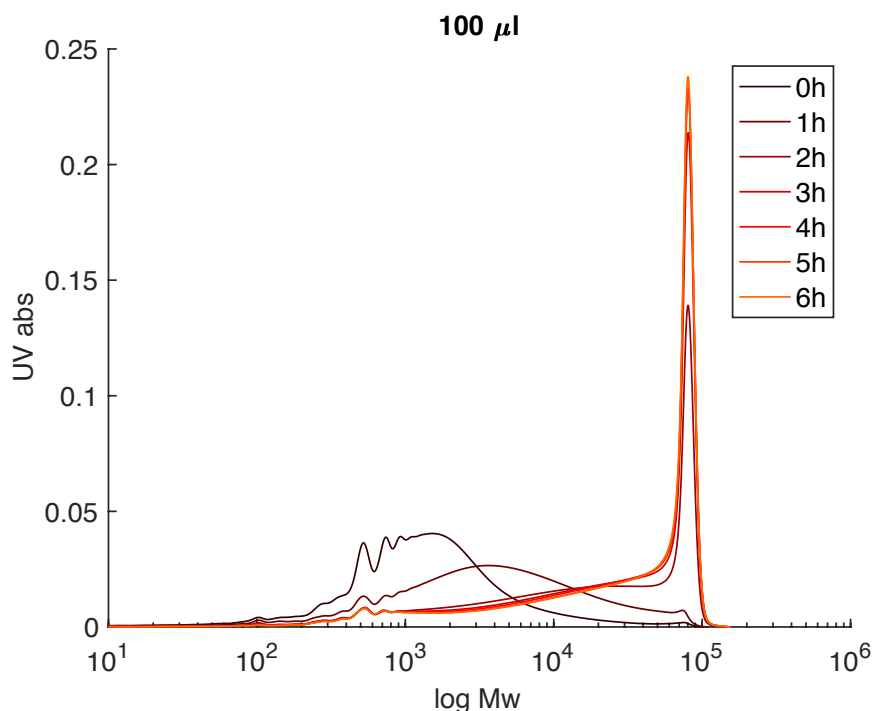


Figure 4.2 SEC chromatogram of the enzymatic reaction when 100 µl of enzyme was added, with UV absorbance vs. logarithmized molecular weight.

The reason for the average molecular weights reaching a maximum is due to the fact that the column used for the SEC analysis could not separate molecules larger than 5000 kDa, which is evident from figure 4.2. It is not very likely that the radical reactions would stop occurring just because the molecule became 5000 kDa in size. A more reasonable explanation would be, like mentioned, that the column lacked the ability to separate molecules that large.

When using an enzyme dosage of 100 μl , the dynamics of change of molecular size fit nicely within the analyses time frame and suggested what amount of enzyme to use in the ensuing experiments.

4.1.3. Effects of pH

The next step was to find out what effect a change of pH would have on the enzymatic treatment of lignin. The results of these changes are shown in figure 4.3.

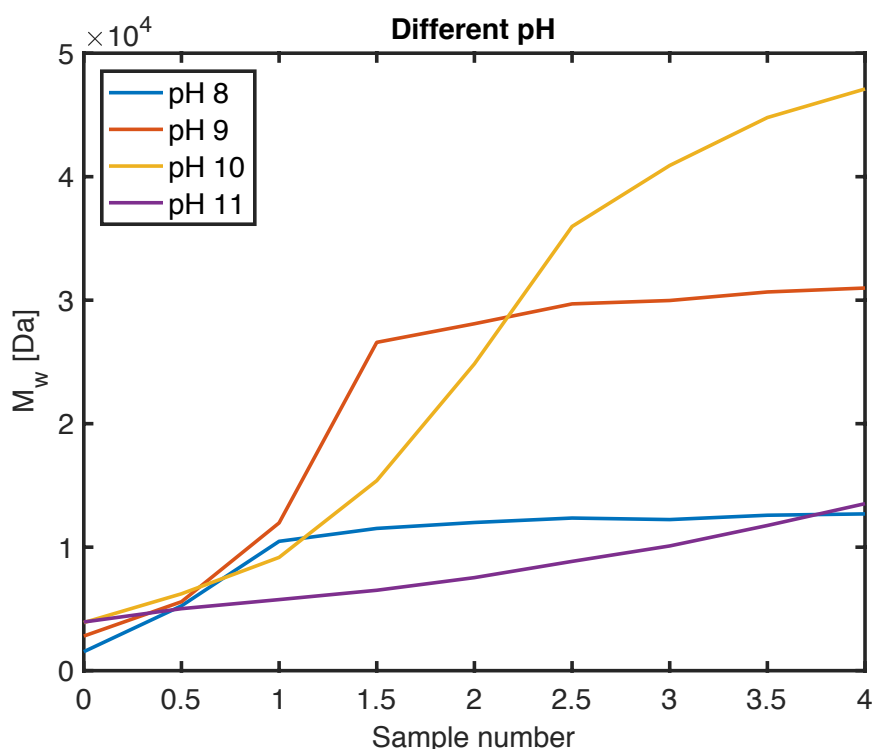


Figure 4.3. Results of the pH experiments. The average molecular weight is plotted against the sample number, i.e. the hours the sampling took place.

As can be seen from the figure above, the average molecular weight increases the most, which indicate the highest enzyme activity at pH 10. It was less active at pH 9 and 11 and almost inactive at pH 8. This agrees well with documentation from the manufacturer and confirms that the pH 10 lignin would be the best to use for the upcoming experiments with cellulose.

The pH 9 solution reaches a maximum after around 1.5 hours and the pH 8 after around 1 hour. pH 11 on the other hand is very slow but continues to increase. Had the reaction been allowed to continue, the same effect seen for the pH 10 might have been seen. This would however have taken too long and would be outside of this projects time scale.

As described in section 4.1.1., there was a lot of precipitation of lignin when the pH was lowered. This meant that there was a lower concentration of lignin in the pH 9 and 8 solution, which in turn could be contributing to the lower average molecular weight. The pH 9 reaction still goes fast, but since it reaches a stop after 1.5 hours, this could mean that the lignin that precipitates when the pH was lowered is needed for the reaction to occur.

4.1.4. Hemicellulose crosslinking

Following the experiments in which only lignin was subjected to enzymatic treatment, the next step was to see how said treatment was affected with the addition of hemicellulose. The results of the modifications are shown in figure 4.4. Supplemental graphs are found in Appendix II.

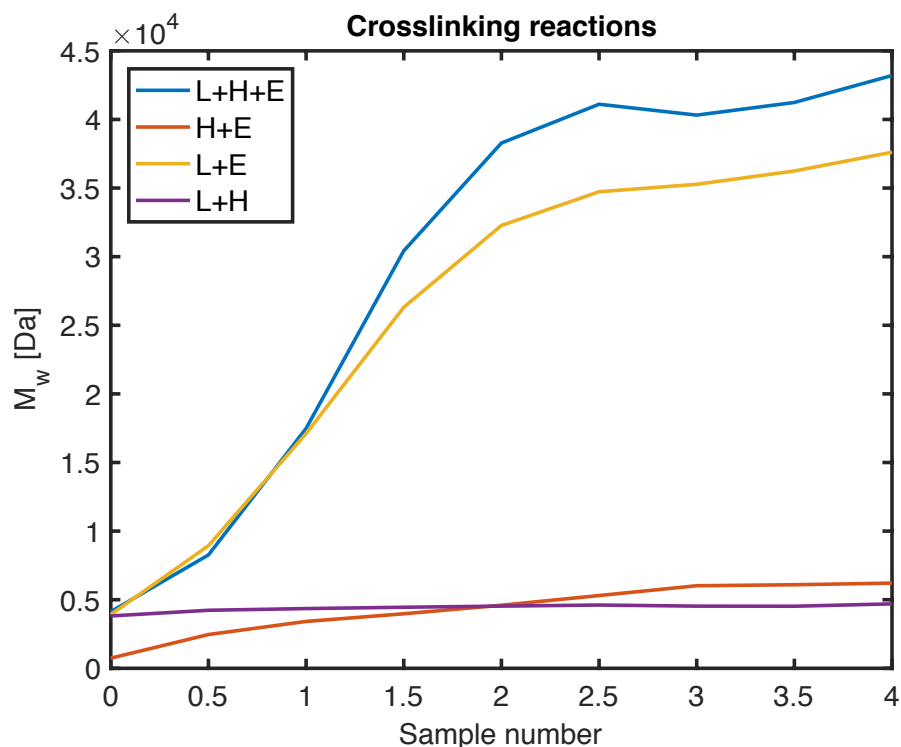


Figure 4.4. Results of the crosslinking experiments with hemicellulose. The average molecular weight is plotted against the sample number, i.e. the hours the sampling took place. L = Lignin, H = Hemicellulose and E = Enzyme.

A couple of things becomes evident from these results. Firstly, the enzymatic reaction is virtually the same when the enzyme reacts with only lignin and when hemicellulose is also present. The average molecular weight is slightly higher when the hemicellulose is present, but it is difficult to determine if this is due to the lignin and hemicellulose binding together, or if it is just measurement differences.

The results do however show that there is no spontaneous reaction between the lignin and hemicellulose, and it can be assumed that all reactions that take place are due to the enzyme.

Since hemicellulose does not show UV absorbance at the wavelength used, 280 nm, these results cannot be used to determine if the enzyme have an effect on the structure of the carbohydrate. In order to determine this, the UV detector was switched to a RI detector instead. Results of which are shown in figures 4.5. and 4.6.

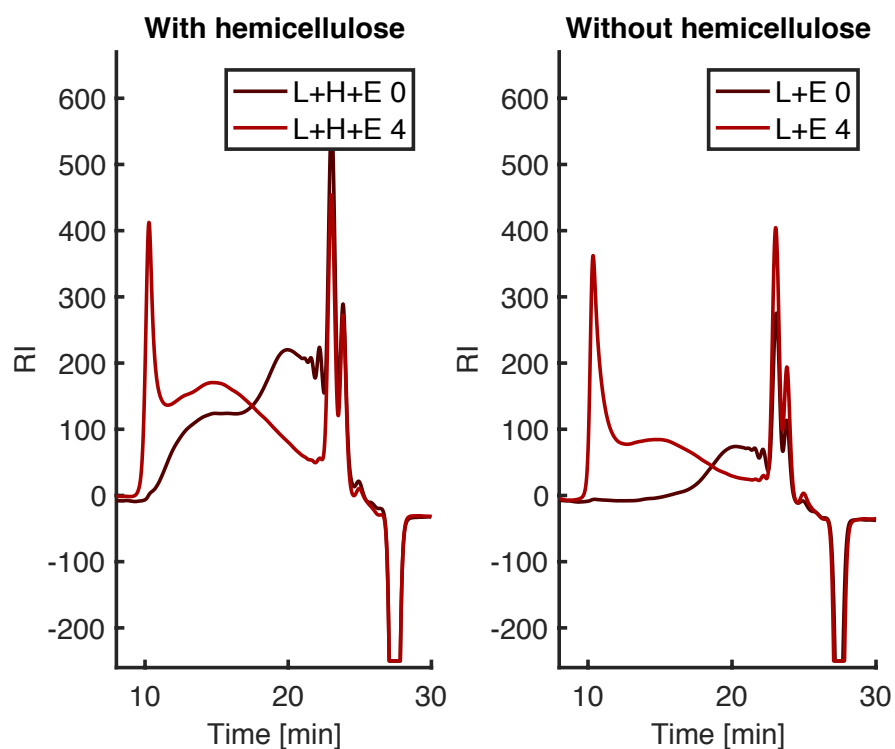


Figure 4.5. RI analysis of the crosslinking experiments, with time on the X-axis and signal on the Y-axis. The left graph shows the first and last samples from the treatment of lignin, hemicellulose and enzyme and the right graph shows the first and last samples from the treatment of only lignin and enzyme.

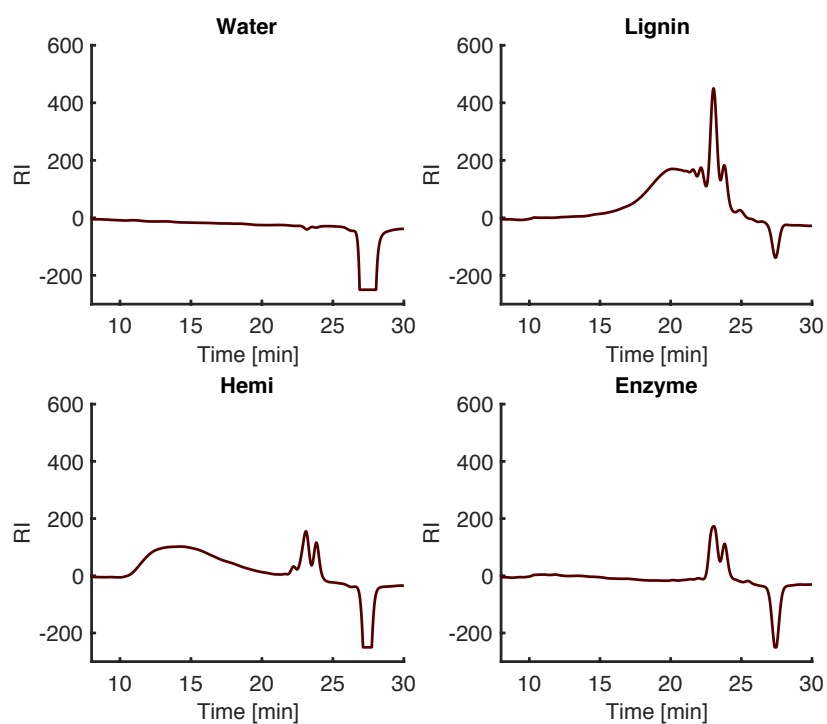


Figure 4.6. RI analysis of only water, lignin, hemicellulose and enzyme. The lignin and hemicellulose were both untreated for this analysis.

By comparing the enzymatically treated samples in figure 4.5. to unreacted lignin, unreacted hemicellulose as well as the enzyme itself in figure 4.6., the RI analysis does shed some light on what may have occurred during the treatment.

Firstly, when comparing the two graphs in figure 4.5. it can be seen that the broad peak present for sample L+H+E 0, that starts at around 12 minutes hemicellulose. Since this peak is not present in the right graph of figure 4.5. but is present in the bottom left graph of figure 4.6. which only shows the hemicellulose, this can be said with confidence.

When the enzyme reacts with the lignin, the molecules become bigger and they will elute from the column faster. This is the reason for the sharp peak at around 10 minutes seen in both graphs in figure 4.5. This is also what gives rise to said peaks tail. The problem is that this tail appears at the same place as the hemicellulose peak, meaning that it is impossible to tell if there has been an increase or decrease in molecular size of the hemicellulose.

The results of the RI analysis could not be calibrated with the standards. This means that this analysis cannot be used to quantify the hemicellulose the same way that the lignin could with the UV analysis. The sample taken at 0 hours in the right graph was diluted twice due to lack of sample. This is the reason why the peaks from this analysis are smaller than the others.

Neither the UV or RI analysis could with certainty show that the lignin and hemicellulose were crosslinked with the reaction of the enzyme. Other analysis methods would need to be performed in order to get a conclusive result.

4.2. Paper treatment and homemade pulp experiments

4.2.1. Drop test on treated paper

When the tests with the lignin and enzymes had been carried out and the optimal reaction conditions was decided, the following part of the project was to see if the lignin could be impregnated into the cellulose of the paper. The easiest way to test this was to dip pieces of paper in the reaction mixture, and then see if there was change in the time it took for a 10 µl drop of water to be absorbed by the paper. Results of these tests are shown in figure 4.7.

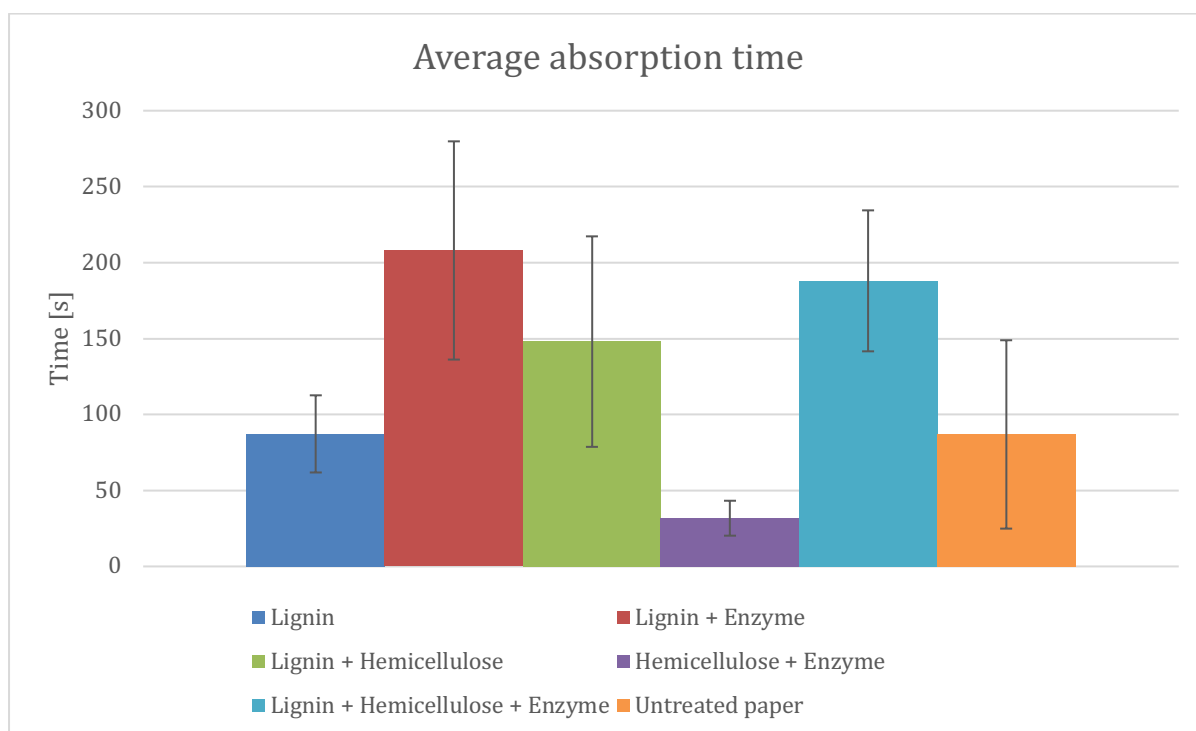


Figure 4.7. Average time for the absorption of a drop of water on differently treated strips of papers together with an average of the time for the absorption of a drop of water on the untreated part of the papers.

Only a small piece of the paper was dipped in the reaction mixture. This meant that one part of said paper never was in contact with the mixture and was thereby not affected by it. The unaffected part of the papers is what is called the untreated paper. These were all bunched together in figure 4.7 above. Below, the different mixtures will be described by the following notations:

- Mixture with only lignin: L
- Mixture with lignin and enzyme: L+E
- Mixture with lignin and hemicellulose: L+H
- Mixture with hemicellulose and enzyme: H+E
- Mixture with lignin, hemicellulose and enzyme: L+H+E

The average absorption time for the differently treated papers varied largely, with the fastest absorption averaging at around 30 seconds, and the slowest at around 3 minutes. The untreated papers average around 1.5 minutes, but there was also a large variance with these tests. Some drops took around 50 seconds to absorb, while others took around 5 minutes. This is also the reason for the large standard deviations which are present on all the treatments.

A small increase of average absorption time was seen when lignin was present in the solution, and it was increased with the presence of enzyme. Since all the papers were thoroughly washed before they were dried in the oven, there should not be any unbound lignin left on the surface of the paper that could cause an increase of the time of absorption.

One very interesting result is the fact that the absorption went really fast with the H+E mixture. This could mean that the hemicellulose binds to the paper, making it more hydrophilic. Another interesting result is the fact that the L+H mixture has a longer absorption time than the L mixture. Since earlier experiments showed that there was no interaction between the lignin and hemicellulose without the enzyme, the effect from these treatments should be the same.

Lastly, the difference between the L+E mixture and L+H+E mixture was very small, with the absorption time being the longest for the L+E mixture. These results indicate that there is little to no effect from the presence of hemicellulose in the reaction mixture.

As mentioned, the standard deviations for most of the tests are very large, as can be seen in figure 4.7. Some drops took under one minute to absorb on the paper, and the next drop on the same paper took over five minutes. It is difficult to explain the reason for this, but one could be the fact that the papers are made from recycled fibers. This means that there is a mix of different types and differently treated papers in the sheet, which could have an effect on the drop. When looking at the papers, it could be seen that there was a difference and even small blotches of color left from whatever the papers were used for before. This could mean that one part of the paper behaves differently than the next, but if enough tests were to be performed, it would even out to an average.

In order to see if some of the measurements were different enough to be considered outliers, and could thereby be discarded, a Grubbs' Z-test was performed. This test compares the value of the suspected outlier with the standard deviation and mean value, and gives a result showing the statistical probability of the data point being an outlier. None of the Z-tests showed an indication that any data point was an outlier, even though some data points differed a lot.

The standard deviations should still be taken into account since they are so large. For instance, the standard deviation for the treated L+H mixture overlaps with the values for the treated L mixture. If the results fluctuate that much, the difference between these mixtures may be smaller than they seem.

4.2.2. Drop test on homemade pulp

After the treatment finished paper, the next step was to see if the treatment had an effect on pulp which was then made into paper. The pulp was made from the same type of paper that was treated in section 4.2.1. and the reactions were carried out the same way. This time, different types of carbohydrates were tested as well. After the pulp was formed into papers, drop tests were performed, the results of which can be seen in figures 4.8 – 4.10 below.

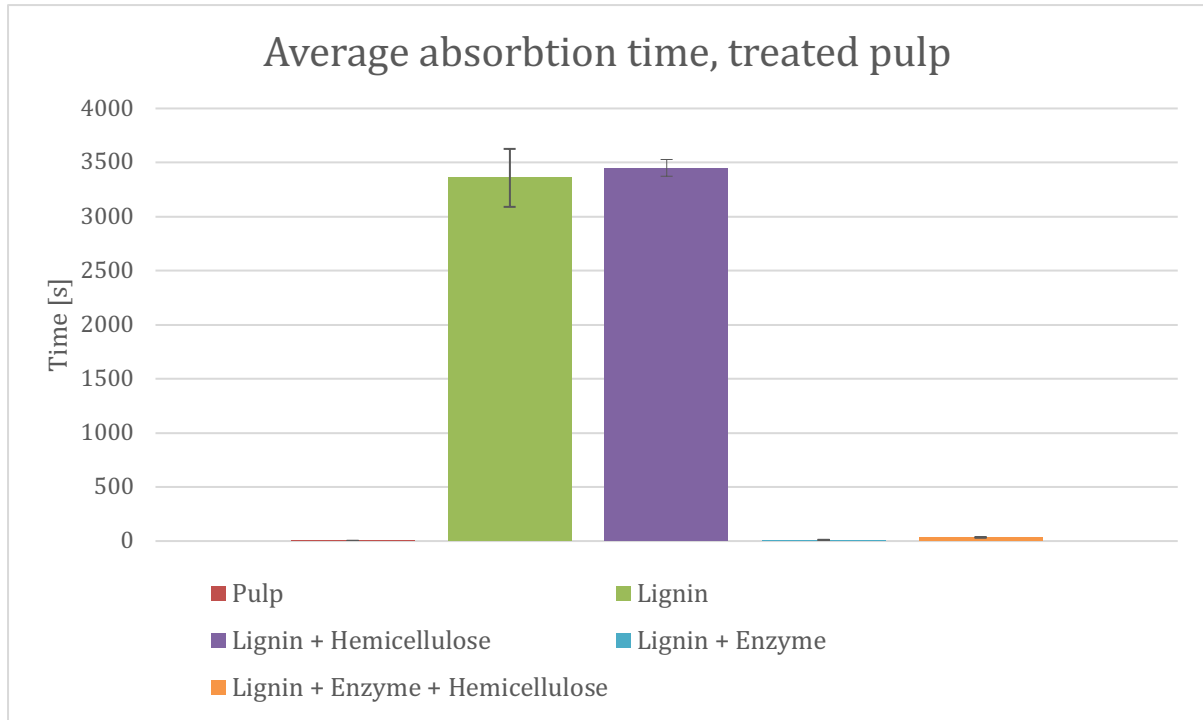


Figure 4.8. Average time for the absorption of a drop of water on differently treated homemade pulp, with standard deviations.

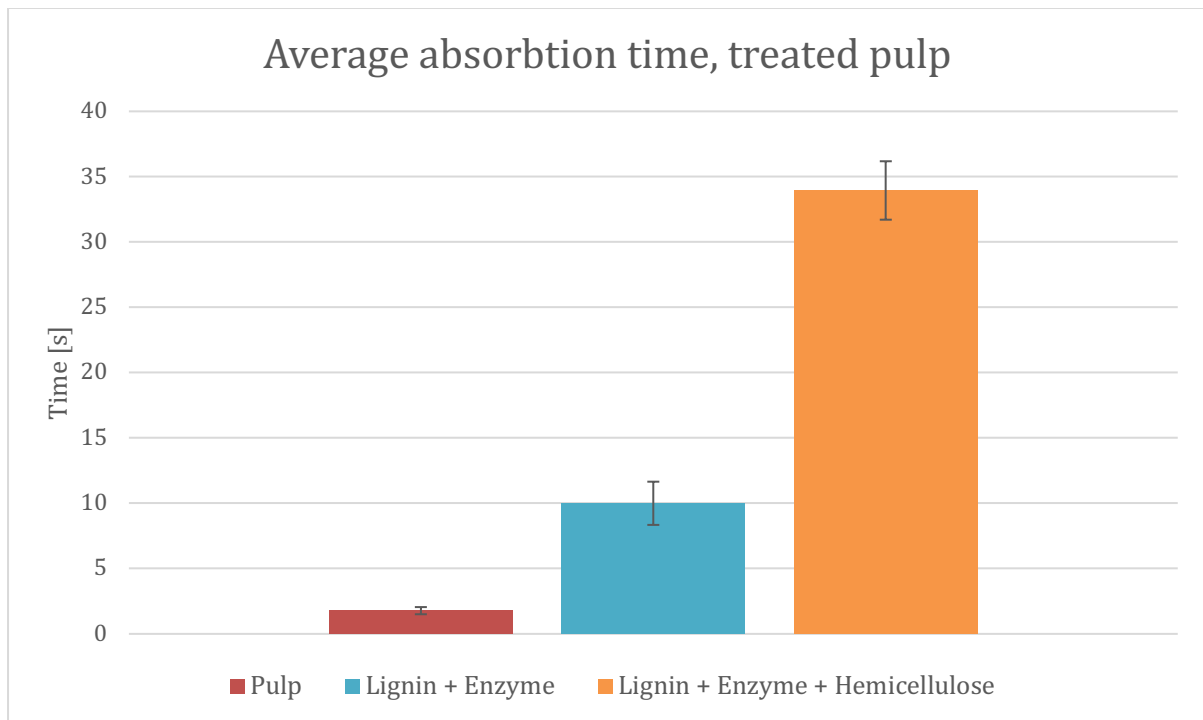


Figure 4.9. Average time for the absorption of a drop of water on differently treated homemade pulp, with standard deviations. Only enzymatically treated papers and control.

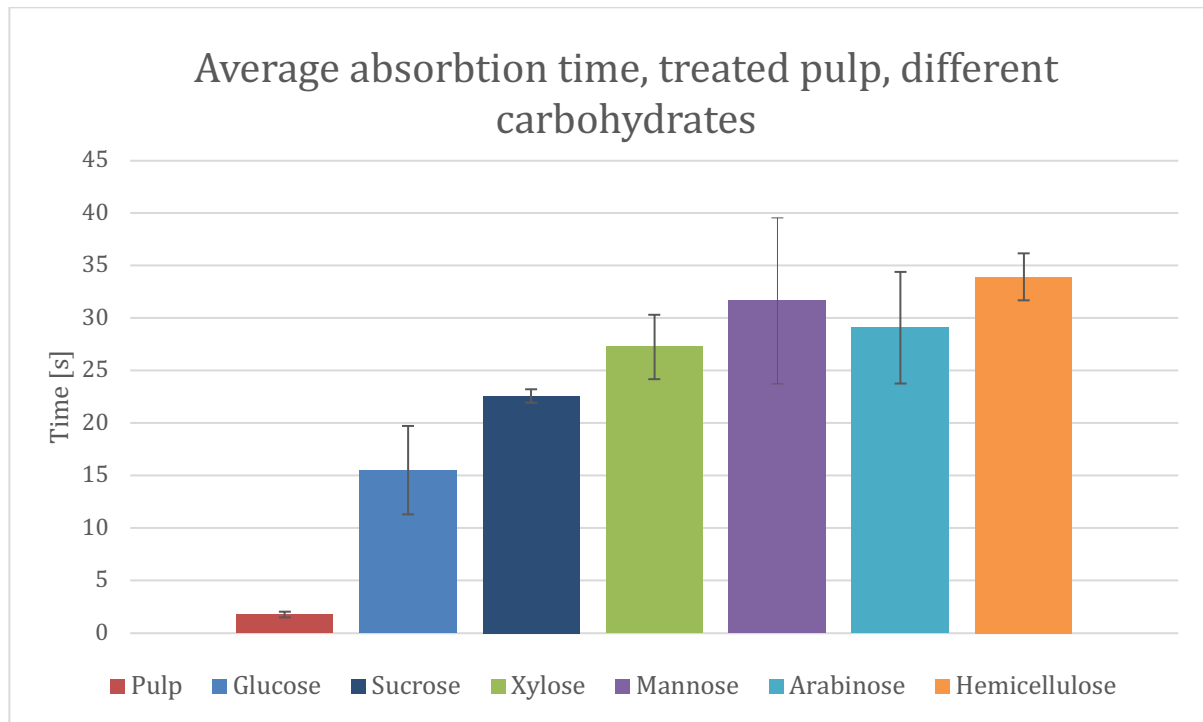


Figure 4.10. Average time for the absorption of a drop of water on homemade pulp, treated with different carbohydrates and lignin, with standard deviations.

Once again, the following notations will be used to describe the different mixtures:

- Mixture with only lignin: L
- Mixture with lignin and enzyme: L+E
- Mixture with lignin and hemicellulose: L+H
- Mixture with lignin, hemicellulose and enzyme: L+H+E

The first thing noticed was that the time it took for a drop of water to be absorbed in the sheets was much higher for the two mixtures without any enzyme present, i.e. L and L+H. So much higher in fact that the main reason for the drop of water disappearing was more likely due to evaporation than absorption. It is not easy explaining the reason for this. One possible reason is that since there was no enzyme present, no reaction increasing the molecular weight is occurring. Therefore, smaller lignin molecules penetrate the fibers and get stuck within the network. This in turn made them harder to wash away. When the mixture was filtrated, the remaining lignin was stuck to the surface, causing a hydrophobic layer which prevented the absorption.

Next noticed result, seen in figure 4.9., is that the pulp treated with the L+H+E mixture has an absorption time that is almost 30 seconds slower than the untreated pulp, and 25 seconds slower than the L+E mixture. This time, there are not an as large standard deviation either, which indicate that these differences did not just occur by happenstance. Interestingly this trend continues with the other carbohydrates, seen in figure 4.10.

There is not a large difference between the different types of carbohydrates, with the exception of glucose which was around 10 seconds faster than the rest. Hemicellulose, however, seems to be the most effective. The pentoses seem to have a larger effect than the hexoses, which was to be expected. Partly because pentoses generally are more reactive than hexoses, but also since hemicellulose partially consists of pentoses, and the LCC consists of hemicellulose bound to lignin and cellulose.

Another thing that is interesting is that the average time for the water to absorb when treated with sucrose was only around five seconds faster than the treatment of mannose, and also around seven seconds slower than glucose. Since sucrose consist of glucose and fructose, the absorption time should in theory be closer to glucose than mannose. This could indicate that fructose is a better carbohydrate to work with than glucose and is something that could be tried in the future.

The sucrose experiments were also the ones with the lowest standard deviation, only around 0.64 seconds, where the others lie around three to seven seconds. This, together with the fact that sucrose is relatively cheap and easily available means that it could be a good candidate for future experiments and upscaling.

4.3. Pulp from papermill

In order to keep track of the different reaction mixtures used for the papermill pulp they will use the notations seen in table 4.1. for the following sections.

Table 4.1. Experimental setup of the experiments using the papermill pulp.

Mixture number	Initial lignin conc. [g/l]	Initial sucrose conc. [g/l]	Notation	Explanation
9	0	0	LLS	Low lignin, low sugar
8	0	12.5	LLMS	Low lignin, medium sugar
7	0	25	LLHS	Low lignin, high sugar
6	25	0	MLLS	Medium lignin, low sugar
5	25	12.5	MLMS	Medium lignin, medium sugar
4	25	25	MLHS	Medium lignin, high sugar
3	50	0	HLLS	High lignin, low sugar
2	50	12.5	HLMS	High lignin, medium sugar
1	50	25	HLHS	High lignin, high sugar

4.3.1. Surface weight

With the data from the previous experiments, it was time to perform tests on papers made from pulp taken directly from a papermill. After the creation of the sheets of paper, they needed to be weighed in order make sure that they did not deviate to much from 100 g/m², which was the desired weight. The weight was also needed to get comparable results for the strength tests.

The average weight, or grammage of the different treatments can be seen in table 4.2 and figure 4.11.

Table 4.2. Average results and standard deviations of the weight tests.

		LLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Avg	g/m ²	92.85	94.05	94.00	94.00	90.25	92.95	92.05	90.55	98.7
Std	g/m ²	5.203	4.463	2.105	4.868	3.803	1.298	2.390	4.480	3.885

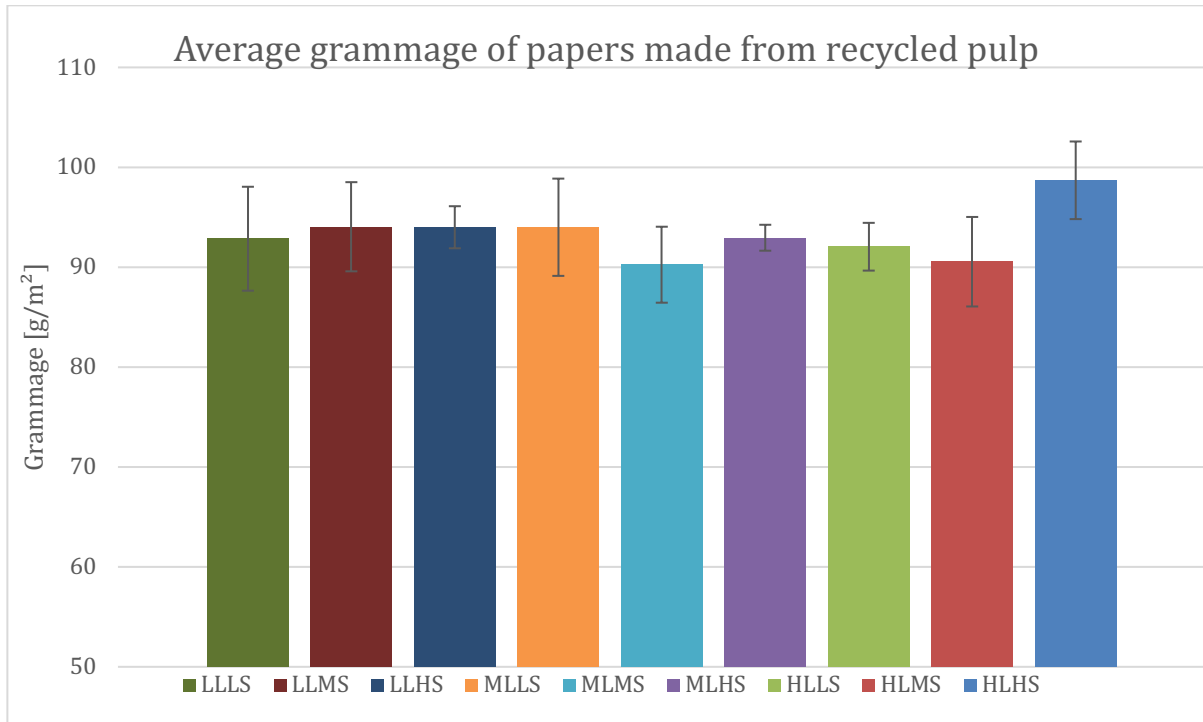


Figure 4.11. Average grammage of the differently treated papers. LLLS = Low lignin, low sugar. LLMS = Low lignin, medium sugar. LLHS = Low lignin, high sugar. MLLS = Medium lignin, low sugar. MLMS = Medium lignin, medium sugar. MLHS = Medium lignin, high sugar. HLLS = High lignin, low sugar. HLMS = High lignin, medium sugar. HLHS = High lignin, high sugar.

The amount of pulp used for the experiments was enough to create sheets of paper with a grammage of 100 g/m². None of the mixtures reached this, but all of the sheets of paper had an average grammage above 90 g/m², with the HLHS mixture having the grammage closest to 100 g/m².

The difference in weight is most likely due to deviations when the pulp was poured into the sheet maker, rather than an indication that the treatment has had an effect.

4.3.2. Thickness tests

The next step was to measure the thickness of the papers, the results of which can be seen in table 4.3 and figure 4.12

Table 4.3. Average results and standard deviations of the thickness tests.

		LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Avg	μm	164.7	170.2	167.1	163.6	157.6	161.9	158.9	157.4	168.3
Std	μm	9.200	10.50	5.500	8.800	7.800	1.002	8.400	8.900	10.00

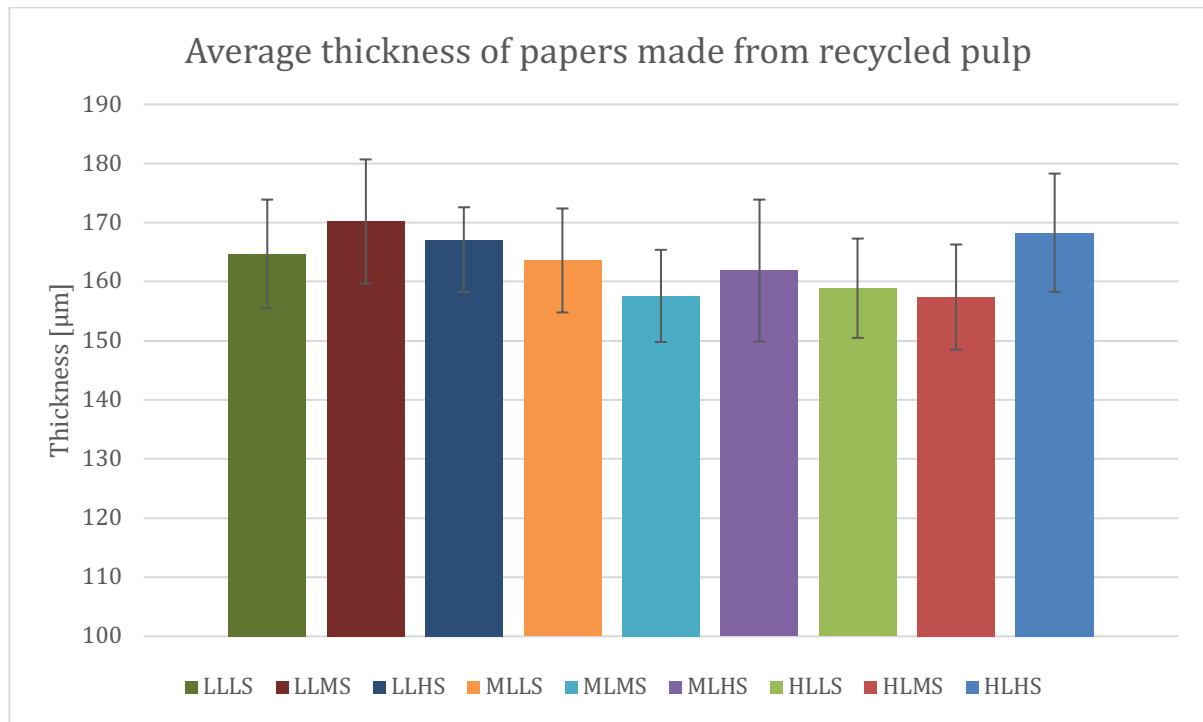


Figure 4.12. Average thickness of the differently treated papers. LLLS = Low lignin, low sugar. LLMS = Low lignin, medium sugar. LLHS = Low lignin, high sugar. MLLS = Medium lignin, low sugar. MLMS = Medium lignin, medium sugar. MLHS = Medium lignin, high sugar. HLLS = High lignin, low sugar. HLMS = High lignin, medium sugar. HLHS = High lignin, high sugar.

The thickness varies between the papers, but all lie around 160 μm. Mixture 8 and 1 were the thickest, and 2 and 5 were the thinnest. These results are fairly consistent with the weight tests which was to be expected since the area of all sheets are the same. This means that if the sheets weigh more, it should also be thicker.

Once again, the differences between the thicknesses is most likely due to human error and no conclusions can be drawn if the treatment had any effect.

4.3.3. Bendtsen porosity test

The next property of the papers to test was the porosity, i.e. how tightly bound together the fibers in the papers were. These tests were done in order to see if the enzymatic treatment affected the way fibers were bound in some way. The results of the testes are shown in table 4.4. and figure 4.13.

Table 4.4. Average results and standard deviations of the Bendtsen porosity tests.

		LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Avg	ml/min	1791	1960	1800	1716	1798	1811	1811	1611	1198
Std	ml/min	374.4	104.7	134.8	73.30	210.4	258.9	226.4	318.3	111.3

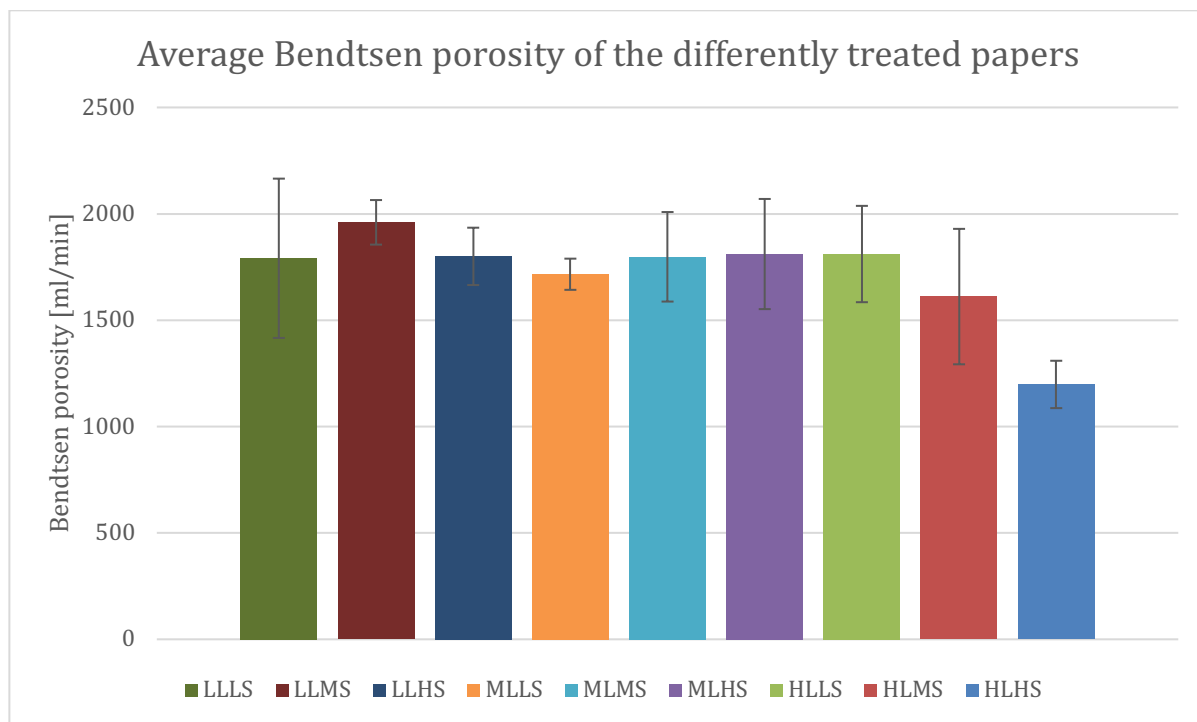


Figure 4.13. Average Bendtsen porosity of the differently treated papers. LLLS = Low lignin, low sugar. LLMS = Low lignin, medium sugar. LLHS = Low lignin, high sugar. MLLS = Medium lignin, low sugar. MLMS = Medium lignin, medium sugar. MLHS = Medium lignin, high sugar. HLLS = High lignin, low sugar. HLMS = High lignin, medium sugar. HLHS = High lignin, high sugar.

The porosity follows the same pattern as the weight and thickness tests, with the exception of the HLHS mixture which has a lower porosity compared to the other papers. The fact that the HLHS mixture has a lower porosity means that the fibers are more tightly bound together which could be an indication of the treatment having an effect. If the enzyme manages to bind the lignin together with the cellulose fibers, this could close the pores of the paper and by extension make the paper less porous.

If that was what happened however, similar results should also be seen in the HLMS, HLLS, MLHS, MLMS and MLLS mixtures as well, since they all were treated with some amount of lignin. Unfortunately, this is not the case, meaning that the difference in porosity most likely comes from the fact that the paper treated with the HLHS mixture is thicker.

Other than this, the porosity does not vary much between the different papers, and the addition of sugar does not seem to have an effect.

4.3.4. Burst strength

With the nondestructive tests out of the way, tests that required that the paper was broken were next in line. The first of these was the burst strength tests. Since all the papers had a different surface weight, they needed to be normalized in order to be fairly compared. This was done by dividing the burst strength value by the grammage. The non-normalized data can be found in Appendix I and II.

The results of the burst strength tests are shown in table 4.5. and figure 4.14.

Table 4.5. Normalized results and standard deviations of the burst strength tests.

		LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Avg	MN/kg	2.577	2.437	2.540	2.803	2.907	2.7260	2.646	2.739	3.024
Std	MN/kg	0.1381	0.1228	0.1058	0.2514	0.1402	0.1072	0.5613	0.4974	0.1167

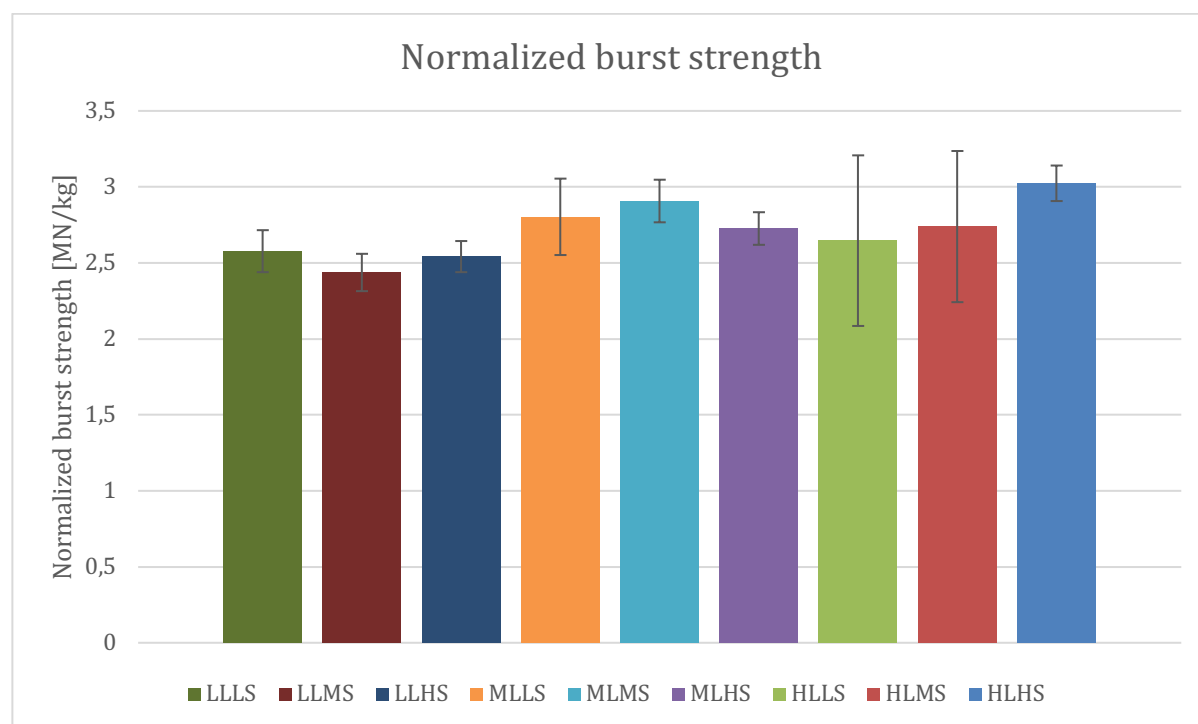


Figure 4.14. Normalized burst strength of the differently treated papers. LLLS = Low lignin, low sugar. LLMS = Low lignin, medium sugar. LLHS = Low lignin, high sugar. MLLS = Medium lignin, low sugar. MLMS = Medium lignin, medium sugar. MLHS = Medium lignin, high sugar. HLLS = High lignin, low sugar. HLMS = High lignin, medium sugar. HLHS = High lignin, high sugar.

The burst strength is higher for the papers which has been treated with lignin, which is fairly consistent with the porosity and thickness tests. The higher the porosity, the fibers are more tightly bound, which in turn means that a higher force is required in order to break them.

For these tests, the sugars do seem to have an effect. The HLMS treated paper required higher force to break than the HLLS treated paper, and the HLHS paper required more force than both. The same can be seen with the MLMS treated paper requiring a higher force than the MLLS. However, the MLHS treated paper does not follow this trend. Which of these results are coincidences is however difficult to say without further experiments.

4.3.5. Compression strength

The next destructive test was the compression strength tests. Once again, the results needed to be normalized with the surface weight in order to compare them. The results of the compression strength tests can be seen in table 4.6. and figure 4.14.

Table 4.6. Normalized results and standard deviations of the compression strength tests.

		LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Avg	kNm/kg	17.18	16.30	17.97	19.12	19.25	19.12	18.63	19.02	19.97
Std	kNm/kg	0.7385	1.179	1.130	0.8042	0.8968	0.8548	2.366	1.661	0.9950

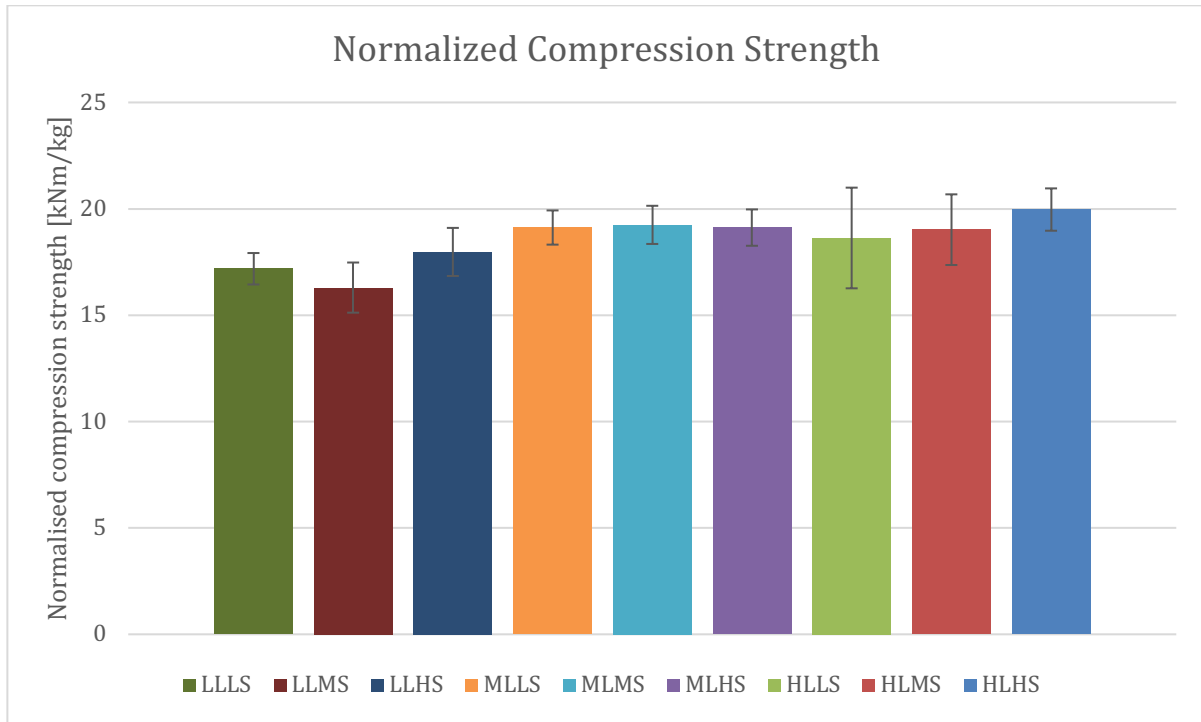


Figure 4.15. Normalized compression strength of the differently treated papers. LLLS = Low lignin, low sugar. LLMS = Low lignin, medium sugar. LLHS = Low lignin, high sugar. MLLS = Medium lignin, low sugar. MLMS = Medium lignin, medium sugar. MLHS = Medium lignin, high sugar. HLLS = High lignin, low sugar. HLMS = High lignin, medium sugar. HLHS = High lignin, high sugar.

Once again, the results follow the same trend as the other tests. The HLHS treated paper has the highest compression strength of the treated papers, which once again is consistent with the porosity and thickness tests. The papers treated with lignin all seem to have a higher compression strength than the ones without the lignin treatment.

In contrast to the burst strength, the addition of sugar does not seem to have a very large effect on the compression strength. The strength does increase with increasing sugar concentration for the high lignin mixtures, but there is virtually no change in strength with the increase of sugars for the medium lignin mixtures.

4.3.6. Cobb₆₀ test

Since one of the desired properties to improve was the moisture resistance, Cobb₆₀ tests to test the absorptivity of the papers were next tests in line. The results of the Cobb₆₀ tests can be seen in table 4.7. and figure 4.16.

Table 4.7. Average results and standard deviations of the Cobb₆₀ tests.

		LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Avg	g/m ²	283.7	267.7	305.4	257.9	257.1	263.1	240.7	238.1	251.78
Std	g/m ²	12.45	33.54	17.58	14.48	16.94	33.33	31.97	33.82	19.37

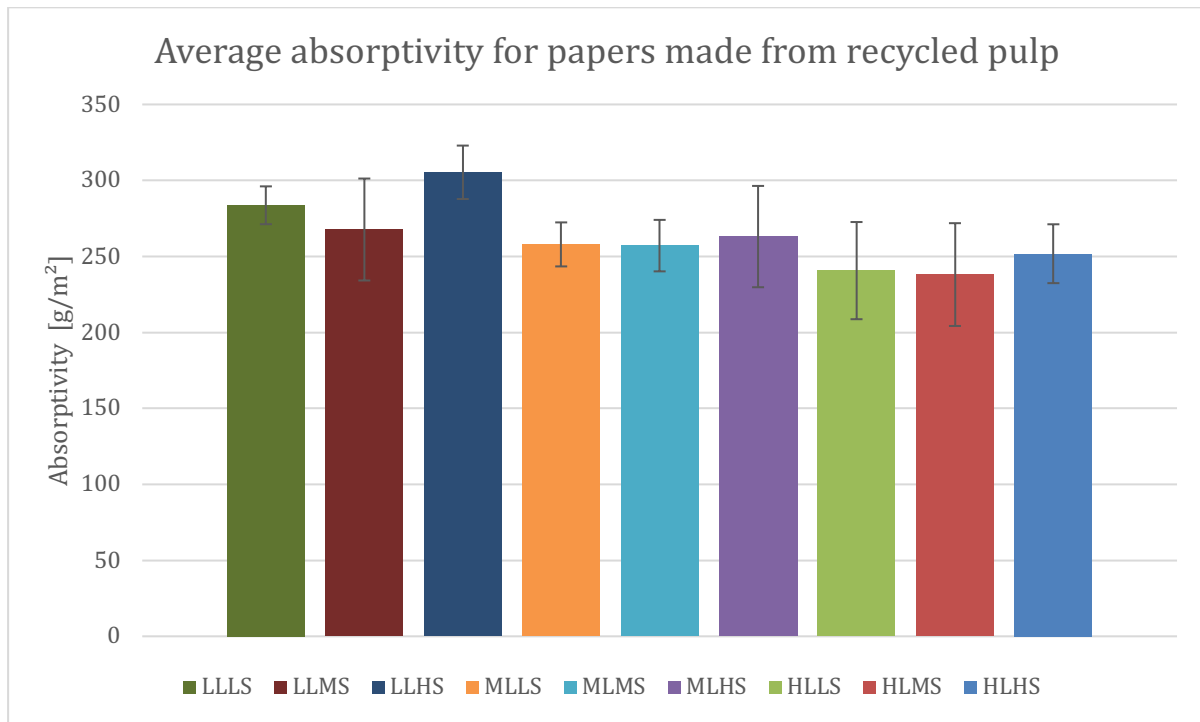


Figure 4.16. Average absorptivity of the differently treated papers. LLLS = Low lignin, low sugar. LLMS = Low lignin, medium sugar. LLHS = Low lignin, high sugar. MLLS = Medium lignin, low sugar. MLMS = Medium lignin, medium sugar. MLHS = Medium lignin, high sugar. HLLS = High lignin, low sugar. HLMS = High lignin, medium sugar. HLHS = High lignin, high sugar.

All the papers treated with lignin, i.e. the MLLS, MLMS, MLHS, HLLS, HLMS and HLHS mixtures absorb less water than the papers without any lignin added. This is consistent with the previous drop absorbance tests done on the treated papers and homemade pulp, described in sections 4.2.1 and 4.2.2.

These results show that the enzymatic treatment of the lignin in together with pulp does affect the papers ability to absorb water, making it more resistant to moisture. The difference between the treated papers and untreated papers are small however, which might indicate that the differences are not very significant.

However, one thing that is not consistent with the drop tests is that the addition of sugar did not have an effect on the absorptivity of the paper. The reason for this is not easy to figure out, but one explanation could be that the final concentration of sugars was lower for these experiments than the earlier ones. The final concentration for the paper treatment and homemade pulp experiments were around 12.5 g/l and 10 g/l, while it for these experiments was only around 5 g/l and 2 g/l. The ratio between the lignin, sugar and pulp was still the same, but it still might have been a too low concentration to give the same effect on absorptivity.

4.3.7. Moisture content

Since the aim of the project was to make recycled paper treated with enzymes and lignin more moisture resistant, the final, and perhaps most significant tests done on the paper made from recycled pulp from the papermill was the tests on the moisture content

The results of the moisture content tests can be seen in table 4.8. and figure 4.16.

Table 4.8. Average results and standard deviations of the moisture content tests.

		LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Avg	%	8.1	8.1	8.2	8.1	8	8.1	8	8.1	8.1
Std	%	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0	0

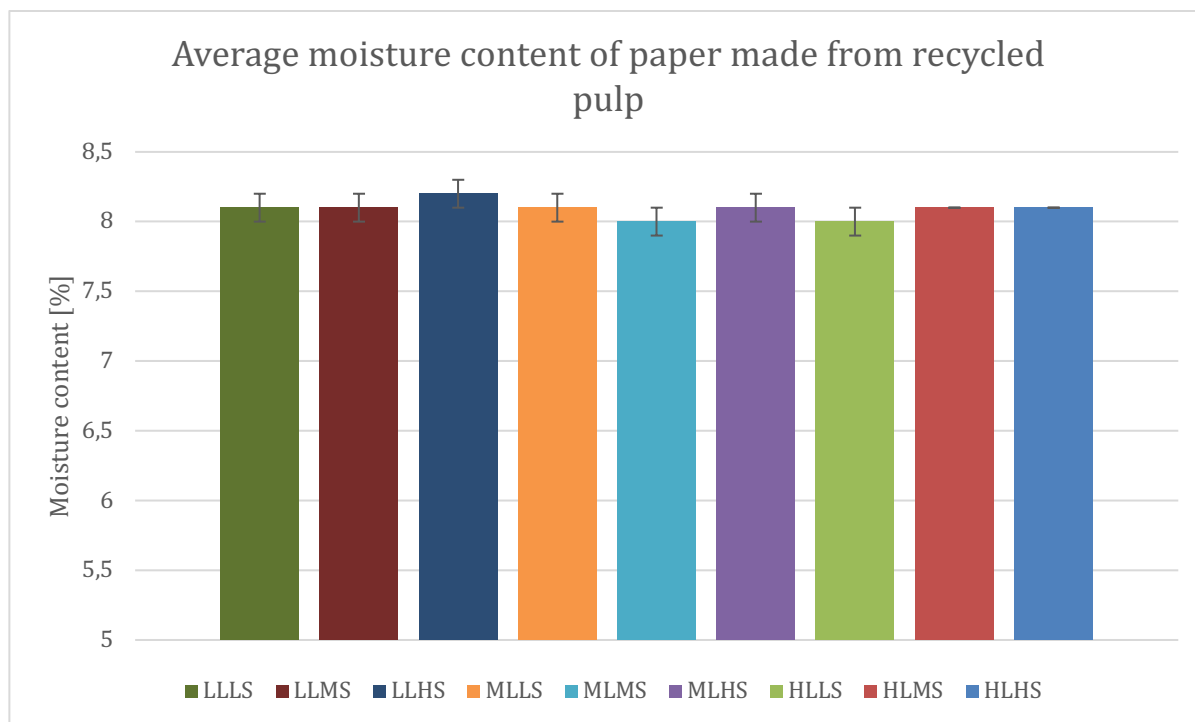


Figure 4.17. Average moisture content of the differently treated papers. LLLS = Low lignin, low sugar. LLMS = Low lignin, medium sugar. LLHS = Low lignin, high sugar. MLLS = Medium lignin, low sugar. MLMS = Medium lignin, medium sugar. MLHS = Medium lignin, high sugar. HLLS = High lignin, low sugar. HLMS = High lignin, medium sugar. HLHS = High lignin, high sugar.

The difference in moisture content between papers was miniscule, and virtually all the papers had the same moisture content regardless of treatment. This indicates that neither enzymes, lignin nor sugar have an effect on the paper's ability to absorb moisture from the surrounding air.

If the average moisture content of the three high lignin mixtures are taken together, they average at 8.133%. The medium lignin mixtures, as well as the low lignin mixtures, both average at 8.067%. This could mean that there is a small improvement when a high amount of lignin is used, but that is most likely not the case. It once again indicates that the addition of sugar does not have an effect.

5. Conclusion

In this project, the objective was to investigate if the interaction between lignin and cellulose fibers could be affected by being treated with laccases and different carbohydrates. To this end, investigations were made to see an effect on lignin molecular size could come from the laccase treatment. The results showed that the laccase and lignin react very efficiently and that even a low volume of enzymes could have a large effect on the molecular size of the lignin in under a couple of hours.

Knowing that the enzyme reacted with the lignin, the next step was to see if it also could make the lignin interact with different carbohydrates, such as hemicellulose. This proved difficult to prove since the hemicellulose could not be analyzed by the same UV detector as the lignin. When using a RI detector, the signal from the hemicellulose and the treated samples overlapped, meaning that it did not show if the hemicellulose had reacted or not.

What followed was to test the effects of the enzymatic treatment of the lignin and cellulose fibers needed to be tested. Both strips of paper as well as homemade pulp was treated with the enzyme and lignin solution, and different carbohydrates were added to see if they had an effect as well. The drop tests performed on the papers after the treatment showed that there was an improvement in water resistance of the treatment, and that the addition of sugars improved this effect.

Subsequently, the standardized paper tests that were performed show that the paper treated with enzyme, lignin and carbohydrates has become stronger and more resistant to breakage. However, the treatment does not show a clear improvement in moisture resistance of the paper.

Overall, this work has shown that there is a difference between the papers made from pulp that was treated with lignin and enzymes. This difference is not very large, but it is there. The possibility to enzymatically modify the interactions between technical lignin and recycled fibers exist, but more extensive studies needs to be done in order to optimize the procedures and to create a commercial product.

6. Future work

There are still many different methods to try in order to improve the results.

In order to see if the enzyme reacts with both the lignin and the carbohydrates, more extensive analyses could be performed. For example, the pH of the solution could be lowered, making all the lignin precipitate. The remaining solution could then be analyzed in different ways. An analysis of the carbohydrates in the mixture could be performed after the reaction has taken place. If the carbohydrates have been bound to the lignin, there should be a lower concentration of free sugars after the reaction is finished. The solution could also be analyzed by SEC and UV absorption. If the lignin and carbohydrates have reacted, the lignin could be more soluble at lower pH, which then could be detected.

Since the carbohydrates used don't contain any aromatic substances, the laccase should in theory not react with them. In order to increase the chance of a reaction occurring, mediator substances such as ABTS could be added to the carbohydrate mixtures or the pulp. Other carbohydrates could also be tested to see if they have a better effect on the recycled pulp than the sucrose. For example, fructose, galactose or even starch could be tested.

When it comes to the creation of paper sheets, there was a rather large difference between the surface weight of the sheets made from recycled pulp. To minimize the risk of this happening, only enough pulp to produce one sheet of paper should be added to each reaction vessel. This will minimize the risk of one sheets of paper getting a higher surface weight than the other and will thereby also decrease differences between the tests. Fresh pulp from the mill should also be tried in order to guarantee that the bacteriocidic material added to keep the pulp from spoiling did not affect the enzyme.

Since part of the aim of the project was to improve the strength of the papers under humid environments, tests on the paper made from recycled pulp should be done at for example 90 % RH. If the enzymatic treatment makes the paper more moisture resistant, it would show more prominently under humid conditions.

Finally, other types of enzymes and different types of lignin could be used. MetGen provides a variety of laccases that work under different conditions and pH, and some of these could work better. Other types of oxidizing enzymes, such as peroxidases could also be tried. SunCarbon have at least two more different fractions of lignin consisting of different molecular weights. Another composition of these could maybe prove more efficient and give a better and more concrete result.

7. References

- Abdelaziz, O.Y., Brink, D.P., Prothmann, J., Ravi, K., Sun, M., García-Hidalgo, J., Sandahl, M., Hulteberg, C.P., Turner, C., Lidén, G. & Gorwa-Grauslund, M.F. 2016, 'Biological valorization of low molecular weight lignin.', *Biotechnology advances*, vol. 34, no. 8, pp. 1318–46.
- Antonsson, S. 2007, *The use of lignin derivatives to improve selected paper properties*, Fiber- och polymerteknologi, Stockholm.
- Antonsson, S. 2008, *Strategies for improving kraftliner pulp properties*, Kemivetenskap, Kungliga Tekniska högskolan, Stockholm.
- Aracri, E., Fillat, A., Colom, J.F., Gutiérrez, A., del Río, J.C., Martínez, Á.T. & Vidal, T. 2010, 'Enzymatic grafting of simple phenols on flax and sisal pulp fibres using laccases', *Bioresource Technology*, vol. 101, no. 21, pp. 8211–6.
- Arkell, A. 2018, *Method and system for treating spent pulping liquor*, Patent och Registreingsverket, Sweden.
- Bajpai, P. 2015, *Chemistry and Sustainability in Pulp and Paper Industry*, Springer International Publishing, New York.
- Biricik, Y., Sonmez, S. & Ozden, O. 2011, 'Effects of surface sizing with starch on physical strength properties of paper', *Asian Journal of Chemistry*, vol. 23, no. 7, pp. 3151–4.
- Borg, O.F. 1989, *Papper och Pappersmassa - en grundbok*, SSIF Förlag, Markaryd.
- CEPI 2017, *Key Statistics European Pulp and Paper Industry*, pp. 1–32.
- Dence, C.W. 1992, 'The Determination of Lignin', *Methods in Lignin Chemistry*, pp. 33–61.
- Elegir, G., Kindl, A., Sadocco, P. & Orlandi, M. 2008, 'Development of antimicrobial cellulose packaging through laccase-mediated grafting of phenolic compounds', *Enzyme and Microbial Technology*, vol. 43, no. 2, pp. 84–92.
- Gellerstedt, G. 2015, 'Softwood kraft lignin: Raw material for the future', *Industrial Crops and Products*, vol. 77, pp. 845–54.
- Gellerstedt, G. & Henriksson, G. 2008, 'Monomers, Polymers and Composites from Renewable Resources', *Monomers, Polymers and Composites from Renewable Resources*, pp. 201–24.
- Hämäläinen, V., Grönroos, T., Suonpää, A., Heikkilä, M.W., Romein, B., Ihalainen, P., Malandra, S. & Birikh, K.R. 2018, 'Enzymatic Processes to Unlock the Lignin Value', *Frontiers in Bioengineering and Biotechnology*, vol. 6, no. March.
- Hamzeh, Y., Najafi, S.M.H., Hubbe, M.A., Salehi, K. & Firouzabadi, M.R.D. 2012, 'Recycling potential of unbleached and bleached chemical pulps from juvenile and mature wood of *Populus deltoides*', *Holzforschung*, vol. 66, no. 2, pp. 155–61.
- Holladay, J.E., White, J.F., Bozell, J.J. & Johnson, D. 2007, *Top value added chemicals from Biomass_lignin_Vol2.pdf*, vol. II, no. October.

- Kaczmarek, M.B., Kwiatos, N., Szczesna-Antczak, M. & Bielecki, S. 2017, 'Laccases – enzymes with an unlimited potential', *Biotechnol Food Sci*, vol. 81, no. 1, pp. 41–70.
- Kudanga, T., Prasetyo, E.N., Sipilä, J., Nyanhongo, G.S. & Guebitz, G.M. 2010, 'Enzymatic grafting of functional molecules to the lignin model dibenzodioxocin and lignocellulose material', *Enzyme and Microbial Technology*, vol. 46, no. 3–4, pp. 272–80.
- G. Liden, O. Sterner and M.F. Gorwa-Grauslund: "Post-paper forestry?" in "Forests and Mankind – The relationship must be sustainable", Eds. G. Jönson and T. Johannesson, Pufendorf Institute for Advanced studies, Lund University, 2015, ISBN 978-91-979893-7-4
- Mate, D.M. & Alcalde, M. 2017, 'Laccase: a multi-purpose biocatalyst at the forefront of biotechnology', *Microbial Biotechnology*, vol. 10, no. 6, pp. 1457–67.
- Maximova, N. 2004, 'Adsorption of Lignin and Lignin / Cationic Polymer Complexes on Cellulose Fibres and Their Effect Complexes on Cellulose Fibres and Their Effect', *Helsinki University of Technology, Laboratory of Forest Products Technology Reports, Series A 22*.
- Morozova, O. V., Shumakovich, G.P., Shleev, S. V. & Yaropolov, Y.I. 2007, 'Laccase-mediator systems and their applications: A review', *Applied Biochemistry and Microbiology*.
- Oinonen, P., Zhang, L., Lawoko, M. & Henriksson, G. 2015, 'On the formation of lignin polysaccharide networks in Norway spruce', *Phytochemistry*, vol. 111, pp. 177–84.
- Palmqvist, B. 2014, *Processing Lignocellulosic Biomass into Ethanol - Implications of High Solid Loadings*, University of Lund.
- Ragnar, M., Henriksson, G., Lindström, M.E., Wimby, M., Blechschmidt, J. & Heinemann, S. 2014, 'Pulp', *Ullmann's Encyclopedia of Industrial Chemistry*, pp. 1–92.
- Rodríguez-Couto, S. 2018, 'Solid-State Fermentation for Laccases Production and Their Applications', *Current Developments in Biotechnology and Bioengineering*, pp. 211–34.
- Saake, B. & Lehnen, R. 2007, 'Lignin', *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
- Schmitt, U., Koch, G. & Lehnen, R. 2014, 'Wood', *Ullmann's Encyclopedia of Industrial Chemistry*, vol. 147, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp. 1–18.
- Sixta, H. 2006, *Handbook of Pulp*, Wiley-VCH, Weinheim.
- Tarasov, D., Leitch, M. & Fatehi, P. 2018, 'Lignin-carbohydrate complexes: Properties, applications, analyses, and methods of extraction: A review', *Biotechnology for Biofuels*.
- Yuan, T.Q., Sun, S.N., Xu, F. & Sun, R.C. 2011, 'Characterization of lignin structures and lignin-carbohydrate complex (LCC) linkages by quantitative¹³C and 2D HSQC NMR spectroscopy', *Journal of Agricultural and Food Chemistry*, vol. 59, no. 19, pp. 10604–14.

Online references

Data.worldbank.org. (2019). Forest area (% of land area) | Data. [online] Available at: <https://data.worldbank.org/indicator/ag.lnd.frst.zs> [Accessed 25 Apr. 2019].

En.wikipedia.org. (2019). Cellulose. [online] Available at: <https://en.wikipedia.org/wiki/Cellulose> [Accessed 3 Jun. 2019].

En.wikipedia.org. (2019). Paper recycling. [online] Available at: https://en.wikipedia.org/wiki/Paper_recycling [Accessed 26 Apr. 2019].

Pkvarel.de. (2019). [online] Available at: <https://www.pkvarel.de/bilder/unternehmen/produktionslinien/PM5ENG.png?m=1510583530> [Accessed 3 Jun. 2019].

Smurfitkappa.com. (2018). Smurfit Kappa celebrates 40 years of powering Pitea. [online] Available at: <https://www.smurfitkappa.com/newsroom/2018/smurfit-kappa-celebrates-40-years-of-powering-pitea> [Accessed 12 Mar. 2019].

Appendix I

Calculations and supplemental data

Table AI.1. Calculations for the loss of lignin after the first preparation.

	Filter papers before filtration [g]	Filter paper + Lignin after drying [g]	Lignin [g]		Total weight of lost lignin [g]	Mean loss from lowering of pH [g]
Pellets	0.9274	2.8815	1.9541	Pellets	1.9541	
Sample 11.1	1.5451	1.5678	0.0227	pH 11	0.0433	0.014433333
Sample 11.2	1.542	1.5469	0.0049	pH 10	0.045	0.015
Sample 11.3	1.4089	1.4246	0.0157	pH 9	5.2854	1.7618
Sample 10.1	1.5334	1.5361	0.0027	pH 8	6.7179	2.2393
Sample 10.2	1.5139	1.533	0.0191			
Sample 10.3	1.5225	1.5457	0.0232	Total	12.0916	4.030533333
Sample 9.1	1.5451	2.8767	1.3316			
Sample 9.2	6.0454	7.8197	1.7743			
Sample 9.3	2.9549	5.1344	2.1795			
Sample 8.1	3.068	5.4007	2.3327			
Sample 8.2	3.0529	5.3199	2.267			
Sample 8.3	2.9695	5.0877	2.1182			

Table AI.2. Results and calculations from the drop tests made on homemade pulp. L = Lignin, H = Hemicellulose and E = Enzyme.

	Paper [s]	Pulp [s]	L [s]	L+H [s]	L+E [s]	L+H+E [s]
1	168	1,86	3050	3381	8.09	35.91
2	160	2	3486	3437	10.85	34.41
3	230	1.47	3538	3534	11.05	31.51
Avg	186	1.77666667	3358	3450.66667	9.99666667	33.9433333
Std	38.3144881	0.27465129	268	77.410163	1.65424706	2.23681321

Table AI.3. Results and calculations from the drop tests made on homemade pulp with different carbohydrates. Glu = Glucose, Suc = Sucrose, Xyl = Xylose, Man = Mannose and Arab = Arabinose.

	Glu [s]	Suc [s]	Xyl [s]	Man [s]	Arab [s]
1	10.68	22.99	23.73	36.46	28.63
2	17.66	22.94	29.23	35.97	34.63
3	18.25	21.86	28.83	22.53	24.03
Avg	15.53	22.5966667	27.2633333	31.6533333	29.0966667
Std	4.21057003	0.63846169	3.0664855	7.90483607	5.31538647

Table AI.4. Results and calculations from the drop tests made on paper, part 1. L = Lignin, H = Hemicellulose, E = Enzyme, T = Treated and U = Untreated.

	L, T [s]	L, U [s]	L+E, T [s]	L+E, U [s]	L+H, T [s]	L+H, U [s]
1	82	54	230	48	59	65
2	117	73	147	93	190	90
3	56	70	300	48	129	63
4	94	82	155	46	214	61
Avg	87,25	69,75	208	58,75	148	69,75
Std	25,3952095	11,6726175	71,8284995	22,8527898	69,2868434	13,5984068

Table AI.5. Results and calculations from the drop tests made on paper, part 2. L = Lignin, H = Hemicellulose, E = Enzyme, T = Treated and U = Untreated.

	L+H+E, T [s]	L+H+E, U [s]	H+E, T [s]	H+E, U [s]
1	128	93	18	74
2	195	214	46	95
3	188	55	33	70
4	241	300	30	44
Avg	188	165,5	31,75	70,75
Std	46,396839	112,411447	11,5	20,9344214

Table AI.6. Results and calculations for the weight tests on paper made from recycled pulp.

		LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Area	cm ²	1	31400	31400	31400	31400	31400	31400	31400	31400
Weight	g	1	3.085	3.185	3.08	2.755	2.875	2.595	2.98	2.88
Weight	g	2	2.875	2.94	2.88	3.055	2.645	2.88	2.47	2.665
Weight	g	3	3.015	2.72	2.95	2.845	2.805	3.105	3.02	3.05
Weight	g	4	2.69	2.975	2.9	3.155	3.015	3.105	3.1	2.78
Avg	g/m ²		92.85	94.05	94	94	90.25	92.95	92.05	90.55
Std	g/m ²		5.2025	4.4625	2.105	4.8675	3.8025	1.2975	2.39	4.48

Table AI.7. Results and calculations for the thickness tests on paper made from recycled pulp.

			LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Value	µm	1	173.1	178.8	176.4	151.9	158.9	144	166.6	156.9	182.1
Value	µm	2	172.2	183.2	179.5	147.4	159.9	141.6	166	156.8	181.3
Value	µm	3	170.9	184.3	168.9	153.8	155.2	145.5	165.9	163.3	166.5
Value	µm	4	172.3	185.6	172.1	152.1	161.4	142.9	169	155.1	170.8
Value	µm	5	174.1	181.7	174.1	153.9	157.3	145.7	162.1	155.1	181.2
Value	µm	6	162.6	171.6	162.9	166.2	150.2	162.1	165.8	152.1	164.6
Value	µm	7	164.7	169.9	164.8	173.9	149	158.3	160.3	134.7	155.2
Value	µm	8	159.4	174.4	164.5	172.8	146.6	157.5	153.3	153.7	164.2
Value	µm	9	161.9	172	161.3	163.5	147	161.2	159.1	141.2	158.9
Value	µm	10	163.4	164.6	166.2	173.2	149.1	160.5	158.3	159.3	159.8
Value	µm	11	175	152	164.6	159	148.8	170.3	161.4	168.3	168.1
Value	µm	12	171.5	155.1	164.1	159.7	158.2	170.8	166.6	172.9	166.9
Value	µm	13	174.5	152.8	164.1	158.7	157.3	172.6	158.9	167.5	178.5
Value	µm	14	169.2	158.3	165.4	164.1	154	170.3	162	165.5	184.2
Value	µm	15	171.3	160.2	165.8	161.8	156.6	170.2	164.1	166.6	181.3
Value	µm	16	155.4	168.6	166.2	175.9	167.9	171.2	160.1	159.1	163.9
Value	µm	17	153.7	172.5	167.3	168.5	164	178.1	148	152.2	149.6
Value	µm	18	152.1	166.1	161.9	167.3	167.7	172.1	154.9	155	162.5
Value	µm	19	147	179.2	174.1	173.5	172.8	173	149.1	156.5	166
Value	µm	20	149	172.1	158.1	173.9	169.1	170.5	132.4	155.7	160.6
Avg	µm		164.7	170.2	167.1	163.6	157.6	161.9	158.9	157.4	168.3
Std	µm		9.2	10.5	5.5	8.8	7.8	12	8.4	8.9	10

Table AI.8. Results and calculations of the Bendtsen porosity tests on paper made from recycled pulp.

		LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS	
Value	ml/min	1	1320	2020	1810	1810	1610	2010	1730	1510	1280
Value	ml/min	2	1320	1990	1790	1800	1610	2050	1680	1470	1240
Value	ml/min	3	1630	1800	1850	1640	2110	2080	1790	2090	1270
Value	ml/min	4	1610	1790	1810	1610	2120	2070	1770	2060	1270
Value	ml/min	5	2000	2060	1630	1700	1810	1580	1590	1280	1260
Value	ml/min	6	1970	2030	1590	1670	1800	1570	1610	1240	1220
Value	ml/min	7	2240	1980	1950	1750	1660	1580	2160	1640	1030
Value	ml/min	8	2240	2010	1970	1750	1660	1550	2160	1600	1010
Avg	ml/min		1791	1960	1800	1716	1798	1811	1811	1611	1198
Std	ml/min		374.4	104.7	134.8	73.3	210.4	258.9	226.4	318.3	111.3

Table AI.9. Results and calculations from burst strength on paper made from recycled pulp.

		LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS	
TS val	kPa	1	261	230	253	214	267	219	249	259	298
TS val	kPa	2	269	236	250	247	287	223	261	268	311
TS val	kPa	3	226	217	216	270	240	243	263	246	280
TS val	kPa	4	238	231	224	267	249	238	248	239	272
TS val	kPa	5	245	204	242	265	256	277	271	267	309
TS val	kPa	6	246	215	248	253	260	278	276	278	298
TS val	kPa	7	200	233	222	214	271	260	212	268	281
TS val	kPa	8	202	229	227	257	254	267	186	220	285
TS avg	kPa		236	224	235	248	261	251	246	256	292
BS val	kPa	1	279	260	265	243	244	234	287	269	309
BS val	kPa	2	257	247	259	241	293	225	275	264	326
BS val	kPa	3	255	252	241	288	249	247	155	108	301
BS val	kPa	4	228	222	239	258	245	257	275	173	295
BS val	kPa	5	253	218	229	226	254	287	261	277	316
BS val	kPa	6	242	229	232	278	265	269	279	304	290
BS val	kPa	7	221	216	236	300	278	286	140	262	306
BS val	kPa	8	214	227	246	286	286	249	230	264	299
BS avg	kPa		244	234	243	265	264	257	238	240	305
TS std	kPa		25.2	11.1	14.5	22.5	14.5	23.2	31.2	19.2	14.3
BS std	kPa		21.7	16.8	12.7	26.7	19.3	22.7	58.4	65.3	11.7
TS&BS avg	kPa		240	229	239	257	262	254	242	248	299
TS&BS std	kPa		23.1	14.6	13.9	25.4	16.6	22.4	45.4	47.2	14.4

Table AI.10. Calculations for the normalization of burst strength results.

			LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
TS	MN/kg	1	2.65653	2.26750	2.57928	2.43905	2.91610	2.6499422	2.62369	2.82381	2.86152
TS	MN/kg	2	2.73796	2.32665	2.54870	2.81517	3.13453	2.6983429	2.75013	2.92194	2.98636
BS	MN/kg	1	2.83974	2.56326	2.70162	2.76958	2.66494	2.8314450	3.02409	2.93284	2.96715
BS	MN/kg	2	2.61582	2.43510	2.64045	2.74678	3.20007	2.7225433	2.89765	2.88333	3.13039
TS	MN/kg	3	2.46831	2.31761	2.355	2.77512	2.84914	2.649375	3.34340	2.92037	2.95529
TS	MN/kg	4	2.59937	2.46714	2.44222	2.74428	2.95599	2.5948611	3.15271	2.83727	2.87085
BS	MN/kg	3	2.78504	2.69142	2.62756	2.96013	2.95599	2.6929861	1.97044	1.28211	3.17694
BS	MN/kg	4	2.49016	2.37102	2.56217	2.651784	2.90850	2.8020138	3.49595	2.05376	3.11361
TS	MN/kg	5	2.55158	2.355	2.57586	2.92478	2.86574	2.8012238	2.81768	2.98887	3.05113
TS	MN/kg	6	2.56199	2.48198	2.63979	2.79233	2.91051	2.8113365	2.86966	3.11201	2.94251
BS	MN/kg	5	2.63489	2.51661	2.43749	2.49434	2.84335	2.9023510	2.71370	3.10082	3.12025
BS	MN/kg	6	2.52033	2.64360	2.46942	3.06826	2.96648	2.7203220	2.90086	3.40306	2.86352
TS	MN/kg	7	2.33457	2.45922	2.40372	2.36189	2.82235	2.6293075	2.14735	2.79111	2.96087
TS	MN/kg	8	2.35792	2.41700	2.45786	2.83648	2.64530	2.7000966	1.884	2.29121	3.00302
BS	MN/kg	7	2.57970	2.27979	2.55531	3.31107	2.89525	2.8922383	1.41806	2.72862	3.22429
BS	MN/kg	8	2.49799	2.39589	2.66358	3.15655	2.97857	2.5180676	2.32967	2.74945	3.15053
avg	MN/kg		2.57699	2.43680	2.53985	2.80297	2.90705	2.7260283	2.64619	2.73879	3.02364
std	%		0.13817	0.12279	0.10584	0.25140	0.14017	0.1072633	0.56129	0.49736	0.11687

Table AI.11. Results and calculations from the compression strength tests on paper made from recycled pulp.

			LLLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
CD val	kN/m	1	1.524	1.675	1.626	1.724	1.783	1.617	1.949	1.641	2.066
CD val	kN/m	2	1.69	1.411	1.954	1.587	1.714	1.607	1.788	1.392	2.105
CD val	kN/m	3	1.685	1.651	1.788	1.592	1.841	1.592	1.656	1.646	1.929
CD val	kN/m	4	1.617	1.421	1.607	1.734	1.792	1.519	1.714	1.734	2.012
CD val	kN/m	5	1.675	1.709	1.626	1.675	1.768	1.441	1.792	1.724	1.9
CD val	kN/m	6	1.538	1.509	1.695	1.973	1.548	1.788	1.201	1.641	1.802
CD val	kN/m	7	1.607	1.392	1.524	1.836	1.597	1.636	1.651	1.827	1.802
CD val	kN/m	8	1.641	1.568	1.661	1.895	1.548	1.695	1.7	1.348	1.91
CD val	kN/m	9	1.548	1.538	1.788	2.046	1.451	1.7	1.836	1.665	1.949
CD val	kN/m	10	1.582	1.534	1.729	1.841	1.582	1.617	1.753	1.841	2.056
CD val	kN/m	11	1.778	1.431	1.67	1.719	1.763	1.988	1.944	1.783	2.076
CD val	kN/m	12	1.534	1.524	1.827	1.788	1.773	2.002	1.866	1.817	2.012
CD val	kN/m	13	1.68	1.426	1.534	1.709	1.797	1.841	1.812	1.705	2.134
CD val	kN/m	14	1.748	1.26	1.578	1.7	1.924	1.88	1.773	1.778	1.861
CD val	kN/m	15	1.719	1.343	1.753	1.714	1.788	1.954	1.783	1.968	1.949
CD val	kN/m	16	1.416	1.631	1.7	1.934	1.827	1.983	1.548	1.714	1.998
CD val	kN/m	17	1.426	1.617	1.582	1.973	1.763	1.998	1.646	1.792	1.944
CD val	kN/m	18	1.524	1.621	1.695	1.841	1.861	1.954	1.631	1.832	2.007
CD val	kN/m	19	1.504	1.69	1.719	1.929	1.812	1.91	1.529	1.792	1.968
CD val	kN/m	20	1.475	1.705	1.729	1.753	1.836	1.9	1.534	1.763	1.919
CD avg	kN/m		1.6	1.53	1.69	1.8	1.74	1.78	1.71	1.72	1.97
CD std	kN/m		0.1	0.13	0.1	0.13	0.13	0.18	0.17	0.14	0.09

Table AI.12. Calculations for the normalization of the compression strength results.

			LLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
CD	kNm/kg	1	15.5117	16.5133	16.5767	19.6492	19.4734	19.566011	20.5364	17.8914	19.8386
val											
CD	kNm/kg	2	17.2013	13.9106	19.9206	18.0877	18.7198	19.445009	18.84	15.1766	20.2131
val											
CD	kNm/kg	3	17.1504	16.2767	18.2283	18.1447	20.1069	19.263506	17.4491	17.9459	18.5231
val											
CD	kNm/kg	4	16.4582	14.0092	16.3830	19.7631	19.5717	18.380192	18.0602	18.9054	19.3201
val											
CD	kNm/kg	5	17.0486	16.8485	16.5767	19.0907	19.3096	17.436377	18.8821	18.7963	18.2446
val											
CD	kNm/kg	6	16.7976	16.1165	18.4802	20.2789	18.3770	19.494166	15.2677	19.3348	19.0194
val											
CD	kNm/kg	7	17.5512	14.8669	16.6158	18.8708	18.9587	17.836944	20.9884	21.5263	19.0194
val											
CD	kNm/kg	8	17.9225	16.7466	18.1095	19.4772	18.3770	18.480208	21.6113	15.8826	20.1593
val											
CD	kNm/kg	9	16.9068	16.4262	19.4941	21.0292	17.2254	18.534722	23.3402	19.6176	20.5709
val											
CD	kNm/kg	10	17.2781	16.3835	18.8509	18.9222	18.7806	17.629791	22.2851	21.6913	21.7003
val											
CD	kNm/kg	12	15.9759	17.5932	19.4467	19.7339	19.8474	20.245668	19.4014	18.7061	19.8669
val											
CD	kNm/kg	13	17.4965	16.4619	16.328	18.8620	20.1161	18.617520	18.84	17.5531	21.0715
val											
CD	kNm/kg	14	18.2047	14.5455	16.7963	18.7627	21.5378	19.011916	18.4345	18.3046	18.3759
val											
CD	kNm/kg	15	17.9026	15.5037	18.6590	18.9172	20.0154	19.760257	18.5384	20.2607	19.2448
val											
CD	kNm/kg	16	16.5287	17.2145	18.4069	19.2480	19.0274	20.053526	15.6797	19.3595	21.0527
val											
CD	kNm/kg	17	16.6455	17.0668	17.1292	19.6362	18.3609	20.205217	16.6723	20.2405	20.4837
val											
CD	kNm/kg	18	17.7894	17.1090	18.3527	18.3224	19.3815	19.760257	16.5204	20.6923	21.1475
val											
CD	kNm/kg	19	17.5559	17.8373	18.6126	19.1982	18.8712	19.315297	15.4872	20.2405	20.7366
val											
CD	kNm/kg	20	17.2174	17.9956	18.7209	17.4466	19.1211	19.214170	15.5379	19.9130	20.2203
val											
CD	kNm/kg		17.1830	16.2973	17.9732	19.1207	19.2457	19.117742	18.6292	19.0197	19.9654
avg											
CD	kNm/kg		0.73899	1.17852	1.13027	0.80418	0.89677	0.8547561	2.36617	1.66085	0.99501
std											

Table AI.13. Results and calculations for the Cobb₆₀ tests on paper made from recycled pulp.

			LLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Weight dry	g	1	0.6226	0.6463	0.614	0.5492	0.5741	0.5167	0.6236	0.5832	0.6749
Weight dry	g	2	0.5886	0.5868	0.5881	0.6109	0.5201	0.5904	0.5868	0.5395	0.6141
Weight dry	g	3	0.6084	0.5465	0.5968	0.5655	0.5686	0.6443	0.604	0.6272	0.6524
Weight dry	g	4	0.5402	0.5888	0.5937	0.6229	0.6084	0.6416	0.4908	0.5621	0.6084
Weight wet	g	1	1.3424	1.429	1.4354	1.1421	1.2061	1.0711	1.2276	1.2224	1.3157
Weight wet	g	2	1.3114	1.2685	1.3064	1.2697	1.108	1.2234	1.2352	1.0354	1.3022
Weight wet	g	3	1.3393	1.145	1.3415	1.2159	1.62364	1.3925	1.2697	1.3136	1.2696
Weight wet	g	4	1.2032	1.2027	1.3626	1.2996	1.2917	1.3364	0.9791	1.1209	1.1801
Value	g/m ²	1	287.9	313.1	328.6	237.2	252.8	221.8	241.6	255.7	256.3
Value	g/m ²	2	289.1	272.7	287.3	263.5	235.2	253.2	259.4	198.4	275.2
Value	g/m ²	3	292.4	239.4	297.9	260.2	267.1	299.3	266.3	274.6	246.9
Value	g/m ²	4	265.2	245.6	307.6	270.7	273.3	277.9	195.3	223.5	228.7
Avg	g/m ²		283.65	267.7	305.35	257.9	257.1	263.05	240.65	238.05	251.775
Std	g/m ²		12.45	33.54	17.58	14.48	16.94	33.33	31.97	33.82	19.37

Table AI.14. Results and calculations from the moisture content tests on paper made from recycled pulp.

			LLS	LLMS	LLHS	MLLS	MLMS	MLHS	HLLS	HLMS	HLHS
Glass No	g	1	12	19	22	32	35	37	39	41	43
Glass No	g	2	13	21	23	33	36	38	40	42	45
Glass weight empty	g	1	29.6158	33.1413	32.8893	27.5126	27.7879	27.6638	27.6192	29.4067	27.4292
Glass weight empty	g	2	32.4435	32.7377	33.5199	27.3975	27.0914	27.4731	27.4229	27.8446	27.7663
Glass weight before drying	g	1	30.1838	33.7259	33.457	28.0734	28.3134	28.1868	28.2368	29.9421	28.0205
Glass weight before drying	g	2	32.9894	33.2825	34.0752	27.9677	27.6466	28.0666	27.9514	28.4009	28.3529
Glass weight after drying	g	1	30.1377	33.6791	33.4112	28.028	28.2714	28.1444	28.187	29.899	27.9729
Glass weight after drying	g	2	32.9456	33.2383	34.0294	27.922	27.6028	28.0192	27.9094	28.3556	28.3053
Moisture content	%	1	8.1	8	8.1	8.1	8	8.1	8.1	8.1	8.1
Moisture content	%	2	8	8.1	8.2	8	7.9	8	7.9	8.1	8.1
Avg	%	0	8.1	8.1	8.2	8.1	8	8.1	8	8.1	8.1
Std	%	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0	0

Appendix II

Supplemental graphs

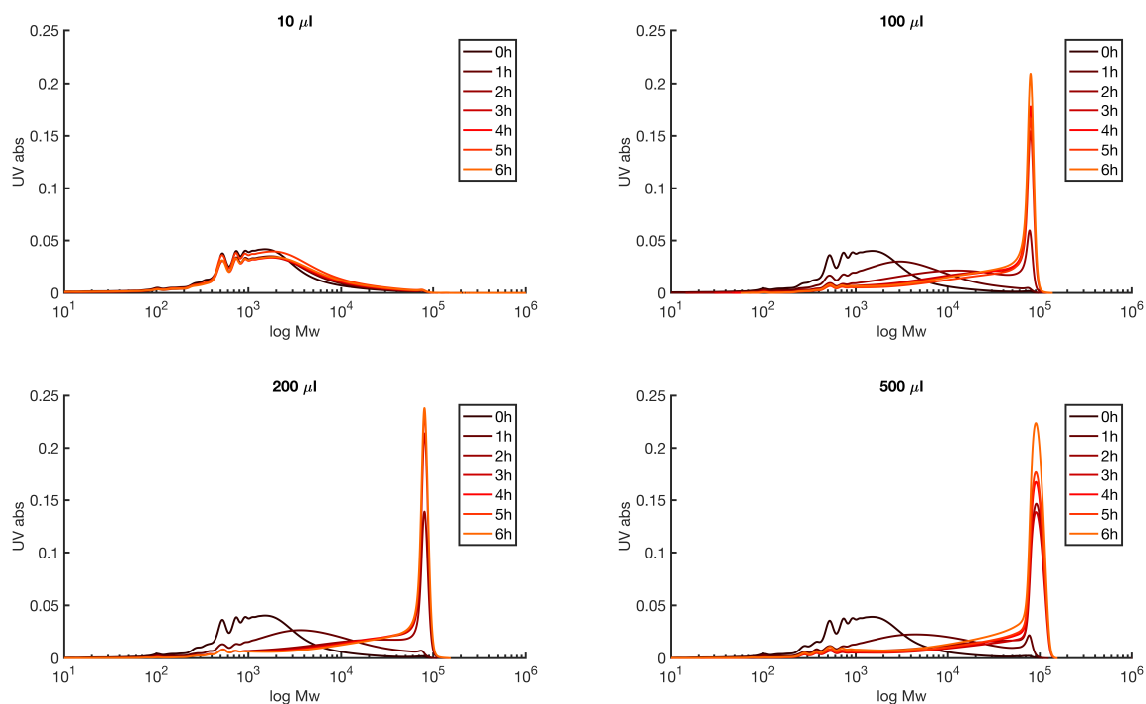


Figure AII.1. Chromatogram results of the enzymatic treatment of lignin with different enzyme concentrations and pH 10. UV absorbance vs. logarithmized molecular weight.

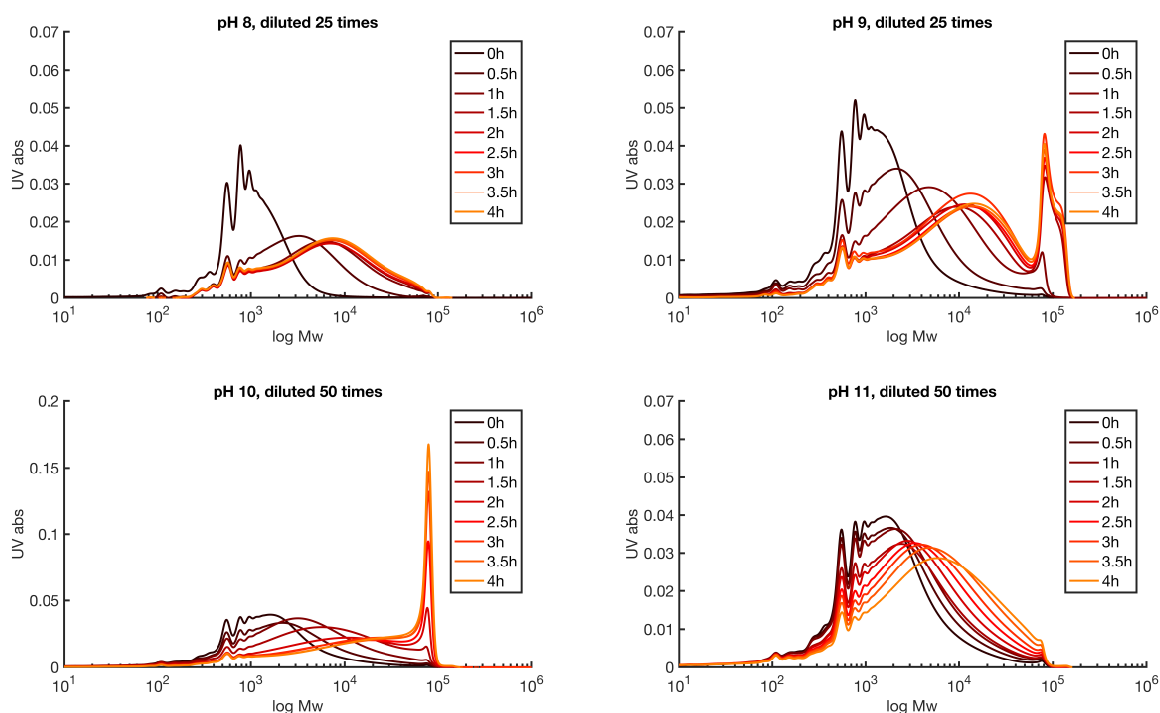


Figure AII.2. Chromatogram results of the enzymatic treatment of lignin with different pH. UV absorbance vs. logarithmized molecular weight.

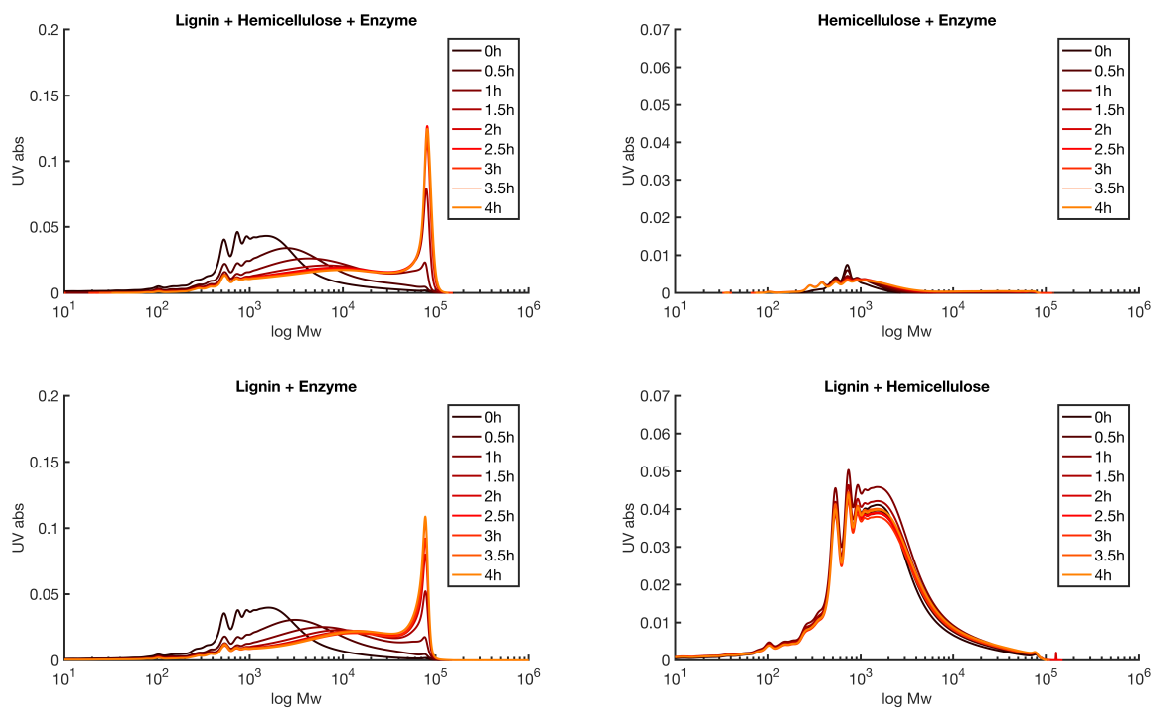


Figure AII.3. Chromatogram results of the enzymatic treatment of lignin and hemicellulose. UV absorbance vs. logarithmized molecular weight.

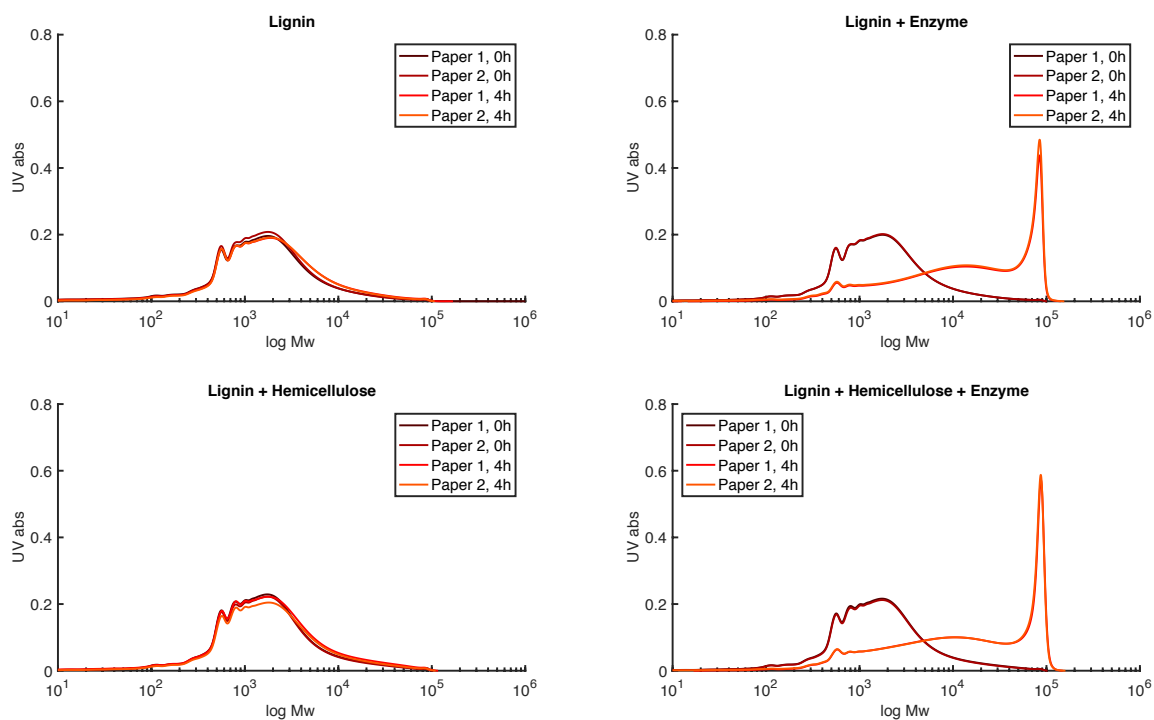


Figure AII.4. Chromatogram results of the paper treatment experiments. UV absorbance vs. logarithmized molecular weight.

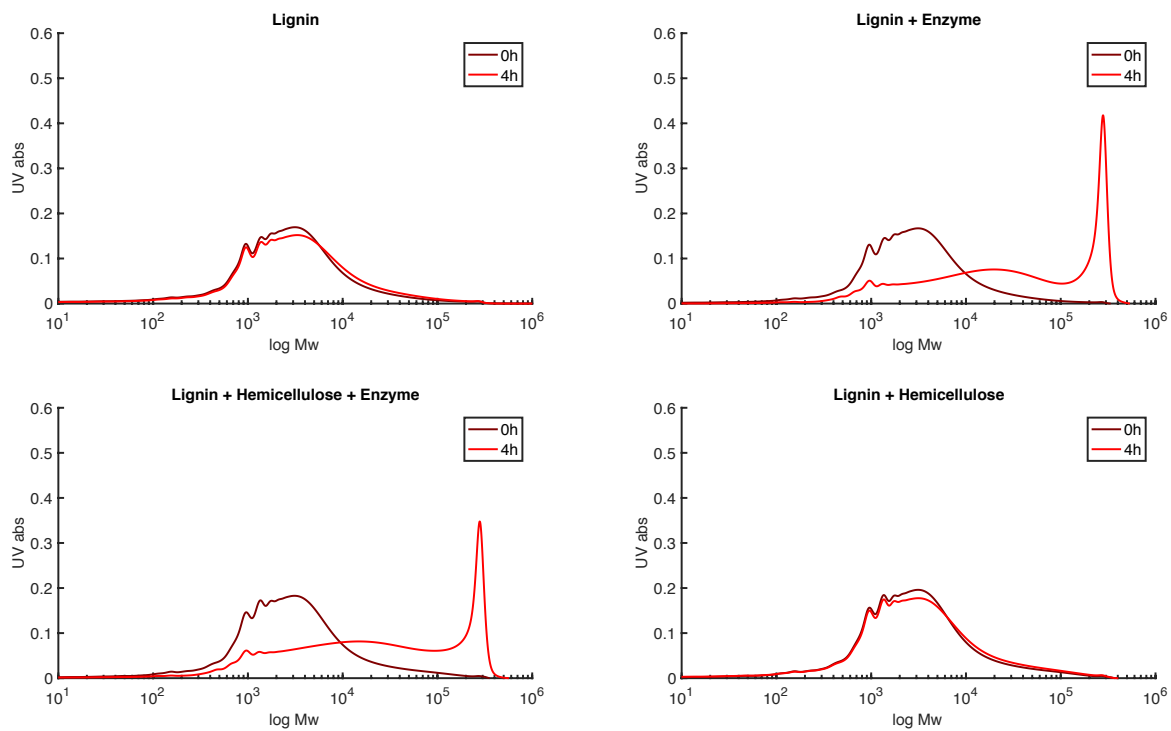


Figure AII.5. Chromatogram results of the homemade pulp experiments. UV absorbance vs. logarithmized molecular weight.

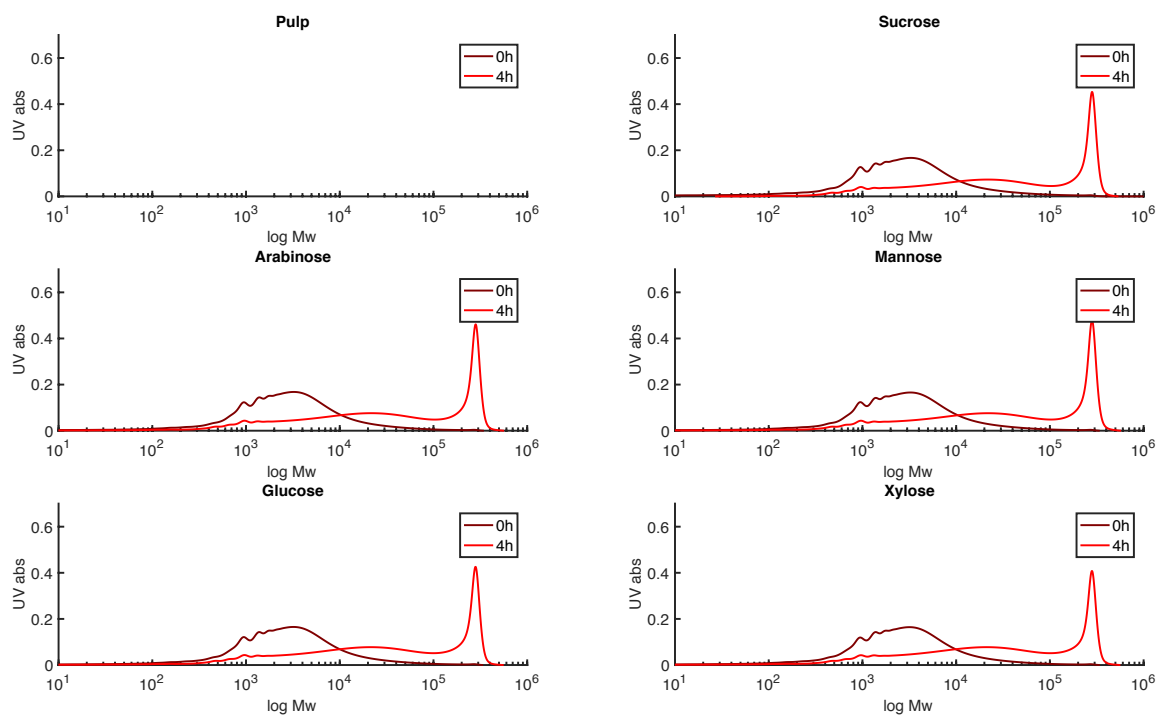


Figure AII.6. SEC analysis of the homemade pulp experiments with different carbohydrates. UV absorbance vs. logarithmized molecular weight.

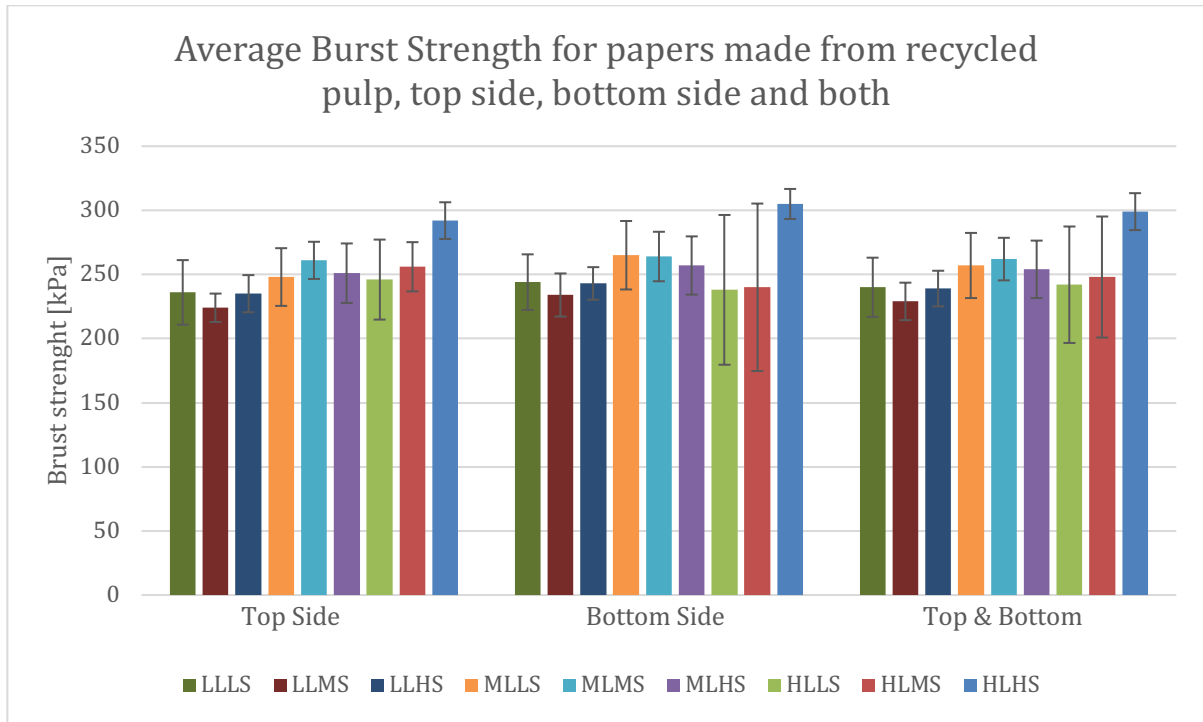


Figure AII.7. Non normalized results of the average burst strength of the papers made form recycled pulp.

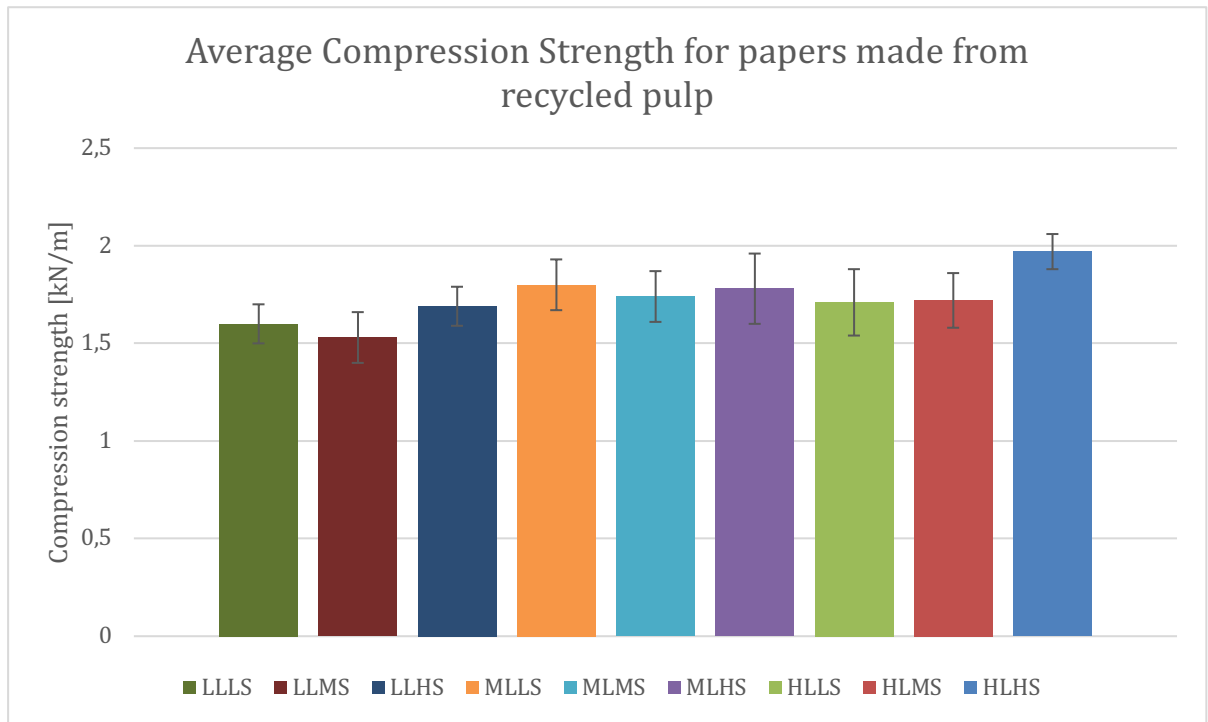


Figure AII.8. Non normalized results of the average compression strength of the papers made from recycled pulp.

Appendix III

Populärvetenskaplig sammanfattning

Papper och kartong är ett av det moderna samhällets viktigaste material. Det tillverkas av trä som bokstavligt talat växer på träd, och kan till skillnad från de flesta typer av plast göras om till nya produkter och återanvänds. Problemet med återvunnet papper är dock att det får sämre egenskaper och förlorar styrka ju fler gånger det återvinns.

Detta projekt har undersökt möjlighet att med hjälp av enzymer behandla återvunnet papper med lignin och olika sockerarter för att förbättra dess tidigare förlorade styrka under fuktiga förhållanden. Resultatet tyder på att det finns någon form av interaktion mellan pappret och ligninet, och att detta högst troligt beror på enzymets närvaro. Fler experiment behöver dock göras för att kunna dra några konkreta slutsatser.

Under en halvårsperiod gjordes ett flertal experiment där lignin behandlades med enzym och olika typer av kolhydrater, så som hemicellulosa, glukos, sackaros med flera. Dessa enzym-, lignin- och sockerblandningar användes sedan för att behandla pappersmassa för att se om man kunde se skillnad i styrka och fuktuptionsförmåga på det papper som blivit behandlat jämfört med det som inte blivit behandlat. Först tillverkades egen pappersmassa genom att mixa vatten och pappersark i en köksmixer. Denna massa behandlades sedan med de olika blandningarna och tiden det tog för en vattendroppe att absorberas av pappret mättes.

Utöver den egentillverkade pappersmassan gjordes även tester på pappersmassa taget direkt från ett pappersmassabruk. Sagda massa behandlades på samma vis som den hemmagjorda massan, men utvärderades på annat sätt. Under kontrollerade klimatförhållanden med en temperatur på 23 °C och 50% relativ luftfuktighet gjordes tester på bland annat hur mycket kraft som krävdes för att göra hål på pappret, hur mycket vatten som absorberades under en viss tid, samt hur mycket fukt pappret drog åt sig från omgivningen.

Att pappret visade sig ha blivit någorlunda starkare av behandlingen tyder på att detta är ett område som är värt att forska vidare i. Om man kan förbättra kvalitén på returpapper kommer man kunna dra ner på behovet av att tillverka sprillans nytt papper, vilket kommer dra ner på påfrestningar av miljön.