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Extraction of hemicelluloses from softwood and hardwood cellulosic fibers by enzymatic treatments

Jahan Golestani

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THÈSE

Pour obtenir le grade de

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Procédés Papetiers**
dans l'**École Doctorale I-MEP2 - Ingénierie - Matériaux,
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Production**

**Extraction d'hémicelluloses de fibres
cellulosiques de résineux et de feuillus par
traitements enzymatiques**

**Extraction of hemicelluloses from softwood
and hardwood cellulosic fibers by enzymatic
treatments**

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devant le jury composé de :

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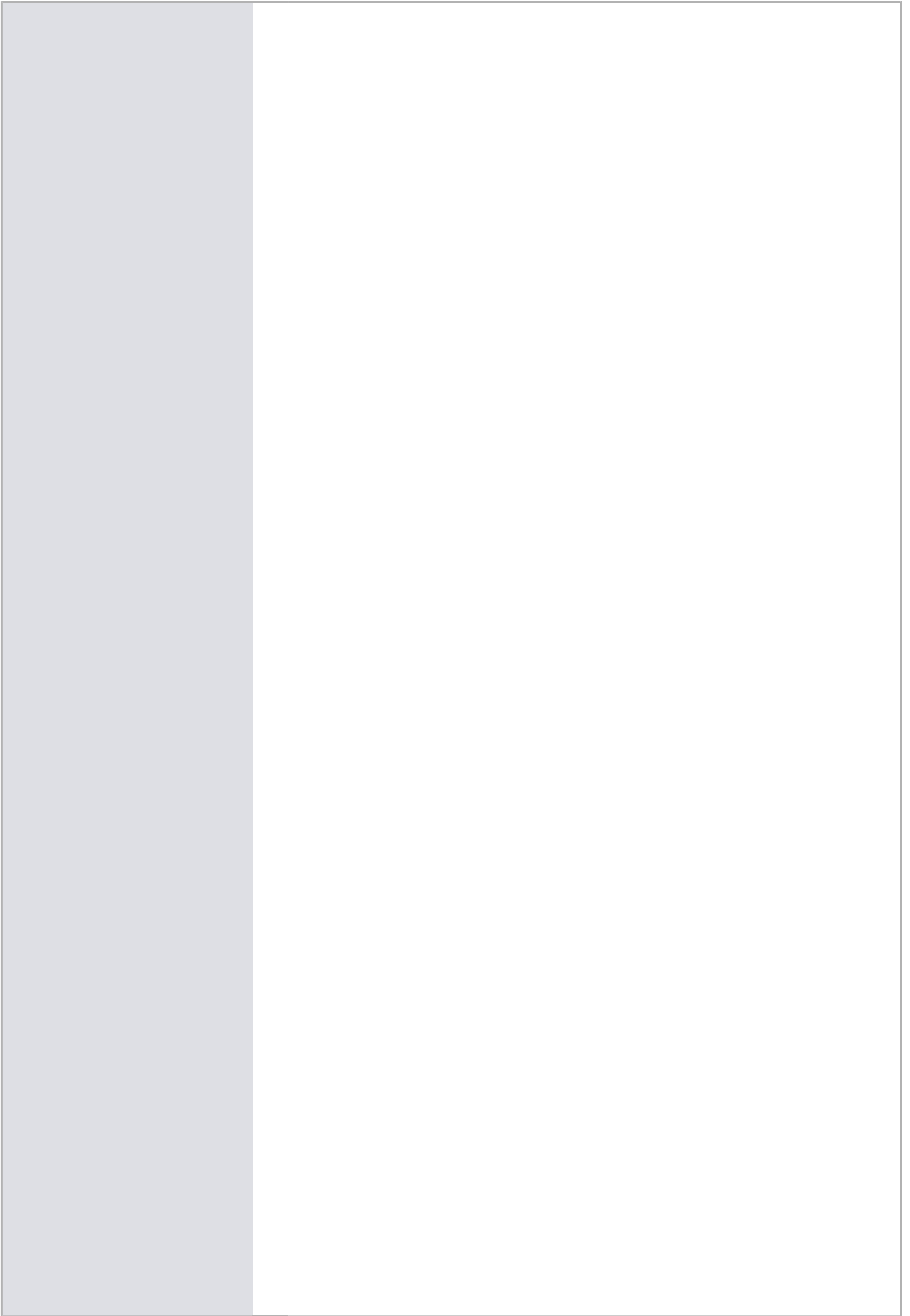
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List of abbreviations:

3-D: three Dimensional

Ac: Acetyl group

AOX: Adsorbable Organic Halogen

CCE: Cold Caustic Extraction

Da: Dalton

DMSO: DiMethylSulfOxide

DP: Degree of polymerization

dRI: differential Refractive-Index instrument

EC number: Enzyme Commission number

ECF: Elemental Chlorine Free

EG: EndoGlucanase

F: Furfural

FXU: Xylanase activity

GPC: Gel Permeation Chromatography

h: hour(s) (incubation time)

HCE: Hot Caustic Extraction

HexA (or Hex): Hexenuronic Acid

HMF: HydroxyMethylFurfural

HPAEC-PAD: High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection

HPLC: High Performance Liquid Chromatography

HW: HardWood pulp

IL: Ionic Liquid

LCC: Lignin-Carbohydrate Complexes

LS: Light Scattering detector

M_i: Molecular weight of a chain

M_n: Number average molecular weight

M_w: Weight average molecular weight

M_z: Higher average molecular weight

MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization - Time Of Flight

Mann: Mannanase

MeGlcA: 4-O-methylglucuronic acid

MFC: MicroFibrilated Cellulose

MIUM: Mannanase activity

ML: Middle Lamella

Mn: The number of mannose unit in the oligomer of mannan

MWD: Molecular Weight Distribution

N_i: Number of chains of the molecular weight of M_i

o.d.p: oven-dried pulp

P: Primary wall

PHK: Pre-Hydrolysis Kraft

pK_a: Dissociation constant

RI: Refractive Index

RLCC: Residual Lignin–Carbohydrate Complex

rpm: round per minute

S1, S2, S3: Secondary cell wall layers

SEM: Scanning Electron Microscopy

Stage D: Bleaching stage of chlorine Dioxide

Stage E: Stage of Extraction (in the sequence of bleaching)

Stage O: Bleaching stage of Oxygen

Stage P: Bleaching Stage of Peroxide hydrogen

Stage X: Stage of Xylanase (pre)treatment

Stage Z: Bleaching stage of Ozone

SW: SoftWood pulp

T: Temperature

TCF: Total Chlorine Free

WRV: Water Retention Value

XO: Xylo-Oligosaccharide

Xn: The number of xylose unit in the oligomer of xylan

Xyl: Xylanase

Introduction

Introduction

Hemicelluloses constitute 20-30% of wood. They are valuable sources of polysaccharides. During kraft pulping process, which is the dominant pulping process for the production of paper quality pulps, most of the hemicelluloses are degraded, solubilized and burned. Hemicelluloses are thus only valorized as a source of energy today. Wood hemicelluloses have degree of polymerization (DP) in the 100-200 range, much smaller than cellulose, and are composed of five main sugars (xylose, mannose, glucose, galactose arabinose). They can be partly acetylated and can contain lateral groups, like methyl glucuronic acid group that can be found in xylans. The nature and quantities of hemicelluloses vary in different tree species, giving a diversity of structure which, on the one hand opens the way to many different types of valorization (as sugar monomers or oligomers of different nature), but on the other hand makes the valorization challenging. One interesting ways of valorization, studied at LGP2, is the use of hemicelluloses oligomers as prebiotics.

There are two main ways to recover hemicelluloses from wood: either hemicelluloses are extracted from wood prior to wood cooking into pulp, by an autohydrolysis process, or they can be extracted from pulp after cooking as pulp after cooking still contains from 15 to 30% of hemicelluloses. Among different chemical methods for removing hemicelluloses from kraft pulps, the most applicable and well-known is cold caustic extraction (CCE) with sodium hydroxide, which is used industrially to purify kraft pulps. The major drawback of this method is that huge quantities of caustic soda are used, which necessitates very good washing of the pulp afterwards.

Another solution could be to use enzymes to extract hemicelluloses from pulp. The advantage of enzymes is that they are selective, which can be beneficial if a specific type of hemicellulose is looked for, for a further valorisation. Another advantage is that the use of enzymes can be considered as eco-processes. The most common enzymes used today in the pulp industry are xylanases, but they have been used so far as bleaching boosters. The use of enzyme to purify paper pulps and transform them into dissolving pulps has been tested in research and development so far. One of the big challenges in applying enzymes is the fact that they are proteins that possess a complex three-dimensional molecular structure which is sensitive to severe conditions. That is why modified enzymes are necessary to be applied in pulp and paper

Introduction

industry: for example, enzymes that have thermostability characteristic are preferred if they are to be applied in the pulp industry.

In the process of dissolving pulp production, the major points are the purity and the reactivity of the pulp. So, applying endoglucanase in order to increase the reactivity of the pulp and improve its morphology and xylanase in order to decrease the remained content of hemicelluloses in the pulp has been proposed by different research groups.

From the literature, another challenge in applying enzymes to remove hemicelluloses is the limitation in hemicelluloses' extraction. The ceiling of this limitation is different considering the type of pulp and the operational conditions. There are some hypotheses explaining the reason why not all hemicelluloses can be removed by enzymatic hydrolysis, even though the enzymes possess the characteristics with which theoretically it should be possible. The main declared reason so far is partial inaccessibility of the substrates to enzymes. Another reason that has been suggested as a limiting factor is the presence of lignin-hemicellulose complexes. This limitation is standstill even with elevated dosage of enzymes or longer incubation times. The modified structure of hemicelluloses after cooking, and their connection to other components of the fibers have been mentioned as the main reasons of inaccessibility.

The first chapter of the thesis presents a literature review on wood hemicelluloses, on different ways to extract hemicelluloses from lignocellulosic material and on the use and effects of enzymes on hemicelluloses. Chapter 2 gives a description on the material and methods used in this work. In the third chapter of the thesis, we will study the extraction of hemicelluloses from fully bleached kraft pulp, by using xylanase and mannanase and by using a combination of enzymes and CCE. CCE alone will be used as a control treatment. The treated fibres will be characterised by their hemicellulose content and nature, and the molecular weight distribution of their polysaccharides. The effect of enzymes on the morphology of the fibres will also be studied. The fourth chapter will deal with the characterisation of the hemicelluloses extracted by the enzymatic treatments, by the combination of enzymes and CCE treatments and by CCE treatment alone. Their osidic composition, molecular weight and structure will be determined using different analytical techniques.

Chapter 1 Bibliography Review

1- The Structure of Wood

1-1- Basic Structure

The major constituents of wood are cellulose (35 to 50%), hemicellulose (20 to 30%), lignin (20 to 30%), along with extractives (a few percent) [28, 170]. Cellulose and hemicelluloses are macromolecules from different sugars, whereas lignin is an aromatic polymer synthesized from phenylpropanoid precursors. The composition and percentages of these polymers vary from one plant species to another and even within a single plant with age, stage of growth, even between samples taken from different parts of the same tree [22, 170].

Cellulose is a linear polymer whose long thread-like molecules are composed of D-glucose subunits linked by β -1,4 glycosidic bonds forming cellobiose molecules. These subunits form long chains (called elemental fibrils) linked together by hydrogen bonds and van der Waals forces without any side branching. Figure 1 represents chemical structure of cellulose molecule. Hemicellulose and lignin cover microfibrils (which are formed by elemental fibrils). The orientation of microfibrils is different in different cell wall layers. Microfibrils group together to constitute the cellulose fiber. Cellulose can appear in crystalline form, called crystalline cellulose, although there is a small percentage of non-organized cellulose chains, which form amorphous cellulose [22, 5].

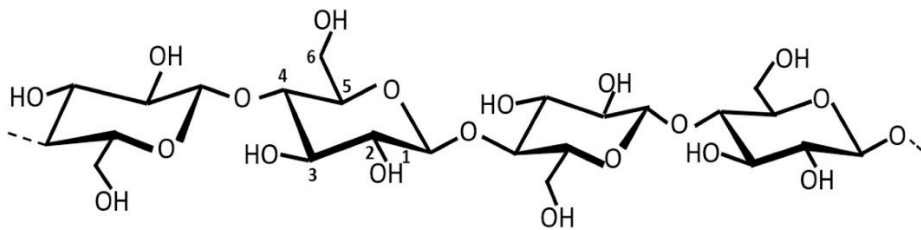


Figure 1- Chemical structure of a cellulose molecule [86].

Lignin is present in the cellular cell wall, conferring structural support, impermeability, and resistance against microbial attacks, which can occur by common cellulolytic microorganisms and oxidative stress. Structurally, lignin is an amorphous heteropolymer, non-water soluble and optically inactive [22, 6], a highly poly-disperse material whose chemical structure is hard to determine. The phenyl propanoid units in lignin consist of an aromatic ring and a 3-C side chain, which form a polymer of phenylpropane phenol units, namely, coniferyl alcohol and sinapyl alcohol, with a minor quantity of p-coumaryl alcohol [186]. Figure 2 represents lignin building blocks.

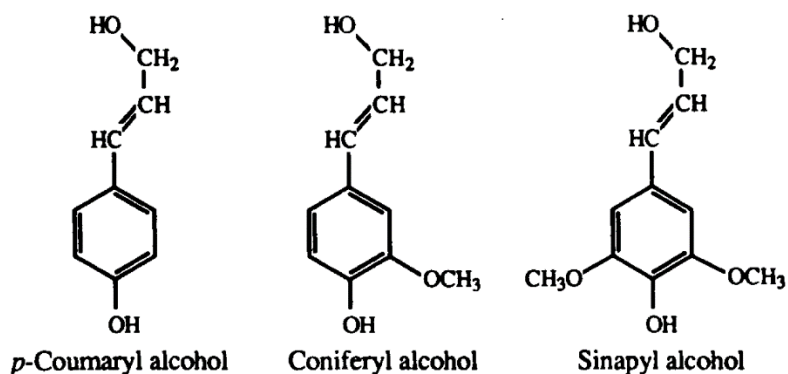


Figure 2- Lignin building blocks [23].

The random assembly of these phenylpropane units, cross-linked together through carbon–carbon, ester, and ether linkages [186], produces a hydrophobic assembly providing structural rigidity. The dense nature, hydrophobicity, and nonspecific structure of lignin make it difficult for enzymes to attack. Naturally, bacteria and fungi may attack parts of the lignin structure to gain access to the energy-rich cellulose and hemicellulose [278]. Figure 3 represents one of the most common models of native lignin structure.

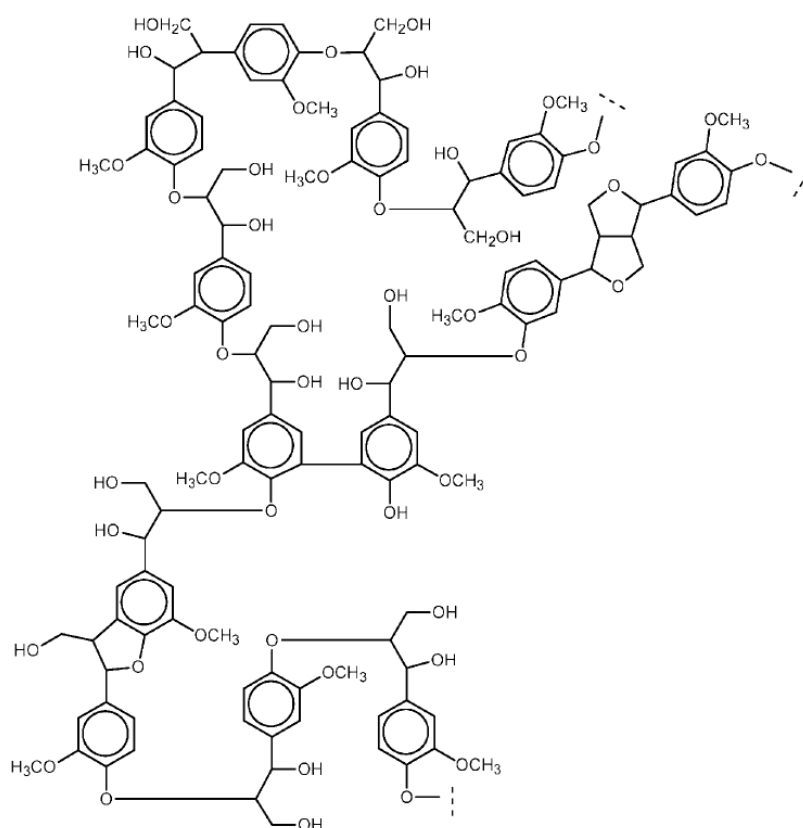


Figure 3- A schematic picture of lignin showing the different linkages between the phenylpropane units [22].

The structure of the fibers in wood is composed of a thin primary wall (P) and three layers of secondary walls (S1–S3) [56]. The middle lamella bonds the cells together. The S2 layer consists majorly of cellulose [3, 5] and while lignin is located mostly in the outer layers to bond

the components of cell together, the hemicelluloses are mostly located in the inner layers [6].

Figure 4 represents the configuration of wood tissues.

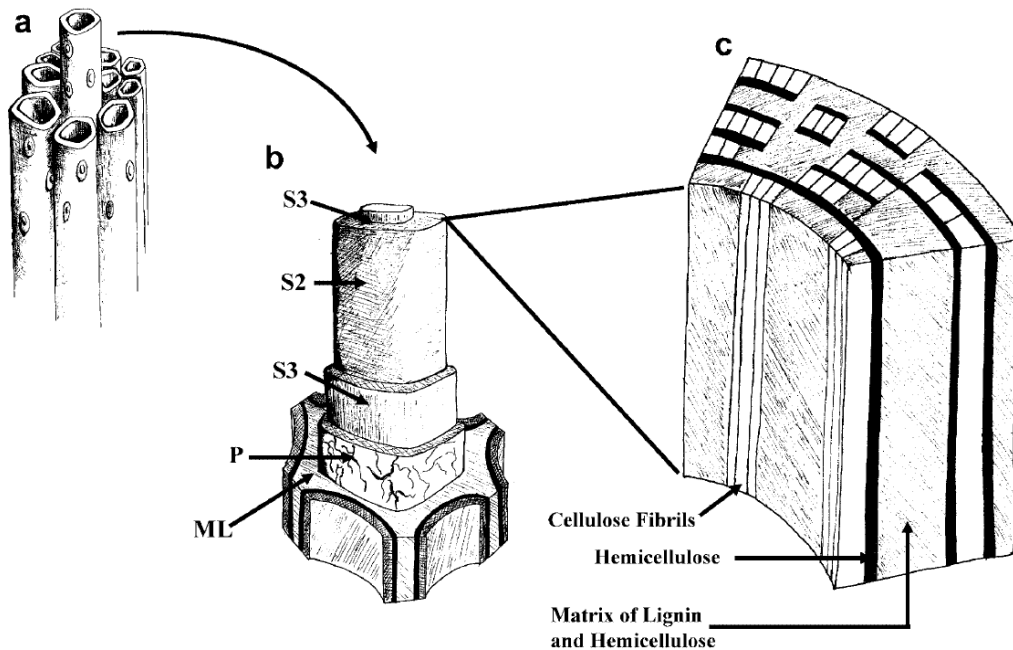


Figure 4- Configuration of wood tissues. a) Adjacent cells, b) cell wall layers. S1, S2, S3 Secondary cell wall layers, P primary wall, ML middle lamella. c) Distribution of lignin, hemicellulose and cellulose in the secondary wall [57, 22].

Various orientations and angles of cellulosic aggregated fibrils in different layers of the cell wall gives mechanical strength to the wood. Furthermore, the degree of polymerization (DP) of these fibrils are another critical parameter determining the strength of the wood [5]. The DP of the cellulose differs depending on the types of the wood, the stage of growth, but it can reach 10 000 in wood.

1-2- Types of Wood

There are two main types of wood: hardwood and softwood. Hardwoods possess leaves that are broad (like maple, beech, birch, eucalyptus). In contrast, softwoods possess needles and cones and principally are evergreen (like pines, firs, and spruces). The lignin content of softwoods is higher than that of hardwoods [5]. Furthermore, softwoods contain more guaiacyl lignin whereas hardwoods are made up of a mixture of guaiacyl and syringyl lignins [6, 56]. Extractives constitute 2–10 dry wt% of wood and are the nonstructural components of lignocellulose, including fats, phenolics, resin acids and waxes. Wood also contains inorganics [33].

The length and strength of fibers which differ in hardwood and softwood determine their applications. Generally, the length and strength of softwood fibers are higher than hardwood fibers, therefore they can be used for production of products that need to be harder and stronger like packages. On the other hand, hardwood fibers are used for production of finer and smoother products like writing and printing papers, in combination with softwood fibers [17].

2- Hemicelluloses in Wood

2-1- Basic Structure

Schulze (1891) first introduced the term of 'hemicellulose' for the fractions isolated or extracted from plant materials with dilute alkali [77, 167]. Hemicelluloses, which exist closely associated with cellulose and lignin [104, 170], are complex carbohydrate copolymers of hexoses (mannose, glucose, galactose) and pentoses (xylose, arabinose) sugars and make up 25–30% of total wood dry weight [22, 60, 68]. Figure 5 represents the main monomers of hemicelluloses.

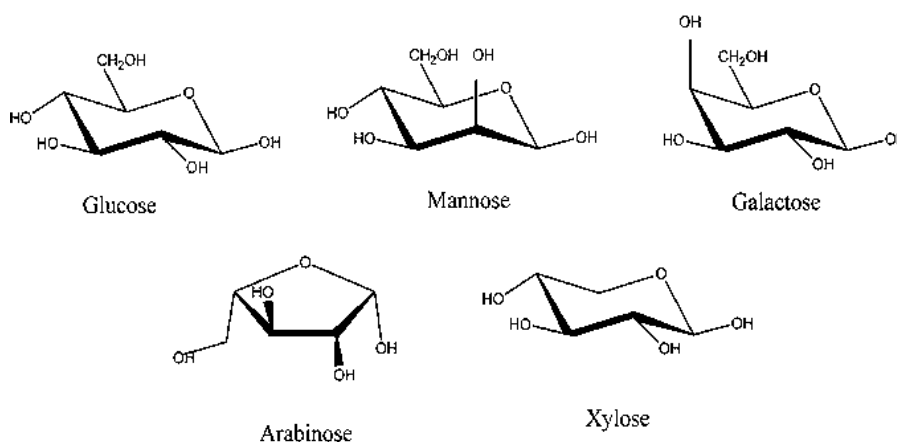


Figure 5- Monomers of hemicelluloses [35].

The hemicelluloses are further associated with pectins and proteins in primary plant cell walls and with lignin in secondary walls, the exact composition of which varies between organism and with cell differentiation [28]. These hetero-polysaccharides, which possess lower molecular weight than cellulose, consist of D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acids. Figure 6 represents the molecular structure of the last three organic compounds.

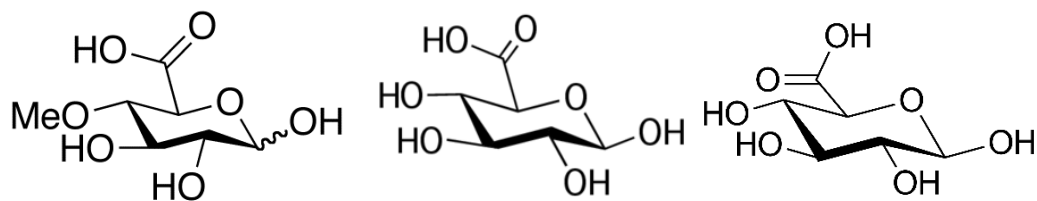


Figure 6- 4-O-methyl-glucuronic (left), D-galacturonic (center) and D-glucuronic (right) acids [36].

Sugars are linked together by β -1,4- and occasionally α -1,3-, α -1,2- or α -1,6- glycosidic bonds [22]. Hemicelluloses form a complex of polymeric carbohydrates including xylan, xyloglucan (hetero-polymer of D-xylose and D-glucose), glucomannan (hetero-polymer of D-glucose and D-mannose), galactoglucomannan (hetero-polymer of D-galactose, D-glucose and D-mannose) and arabinogalactan (hetero-polymer of D-galactose and arabinose), the quantities of which depending on the wood species [51].

2-2- Differences between hemicelluloses and cellulose

There are many differences between wood hemicelluloses and cellulose. Hemicelluloses have side groups with short lateral chains consisting of different sugars, they can contain acetyl groups, they have much lower molecular masses. Cellulose can have a DP of 10 000 or more, while wood hemicelluloses usually have a DP around 200 [22, 170]. The branched structure allows hemicellulose to exist in an amorphous form that is more susceptible to hydrolysis [60]. In contrast to cellulose, they are easily hydrolysable polymers. They do not form aggregates, even when they are co-crystallized with cellulose chains [22].

2-3- Hemicelluloses in hardwood and softwood species

Xylan and glucomannan form the basic backbone polymers of wood hemicelluloses [18]. The principal component of hardwood hemicellulose is xylan, whereas glucomannan is predominant in softwood [22, 68]. In hardwoods, the main hemicellulose is O-acetyl-O-methylglucuronoxylan, with, on average, one α -(1-2)-linked 4-O-methyl glucuronic acid substituent per 10 to 20 of β -(1-4)-D-xylopyranose units, whereas in softwoods, arabino-4-O-methylglucuronoxylan comprises about one third of the total hemicelluloses [18, 28]. The most important hemicelluloses in softwoods are galactoglucomannans and arabinoglucuronoxylans. Furthermore, softwoods contain arabinogalactan, xyloglucans, and other glucans. The amounts of different hemicelluloses in wood are listed in Table 1 [104].

Table 1- Average distribution of different hemicelluloses in wood in wt% [210].

Hemicellulose	Hardwood	Softwood
Methylglucuronoxylans	80-90	5-15
Arabinomethylglucuronoxylans	0.1-1	15-30
Glucomannans	1-5	1-5
Galactoglucomannans	0.1-1	60-70
Arabinogalactans	0.1-1	1-15
Other galactans	0.1-1	0.1-1
Pectins	1-5	1-5

2-4- Xylan

2-4-1- Basic Structure

The most abundant hemicellulose present on the earth surface is xylan [167]. Xylan is the second most abundant polysaccharide next to cellulose and accounts for approximately one-third of all renewable organic carbon on earth [28, 51]. Xylan is the most important hardwood hemicellulose, especially *O*-acetyl-4-*O*-methylglucuronoxylan, amounting to about 80-90% of the hardwood hemicelluloses [104]. In fact, the plant cell wall of hardwoods is a composite material in which cellulose, xylan, and lignin are closely linked. Similar to most of the other polysaccharides of plant origin, xylan displays a large poly-diversity and poly-molecularity [28]. Therefore, the type and frequency of side chains on the xylan backbone vary between plant species and within each species [170]. Xylans can be categorized as linear homo-xylan, arabinoxylan, glucuronoxylan, and glucuronoarabinoxylan [68, 185] in which the main chain is composed of β -xylopyranose residues [77, 115]. This hetero-polysaccharide contains substituent groups of acetyl, 4-*O*-methyl-D-glucuronosyl and α -arabinofuranosyl, feruloyl and/or *p*-coumaroyl side-chain units linked to the backbone of β -1, 4,-linked xylopyranose units and accounts for 15-30% of hardwood and 7-10% of softwoods and has binding and adhesive properties mediated by covalent and noncovalent interactions with lignin, cellulose, and other polymers, essential to the integrity of the cell wall [28, 51, 68, 115, 51].

The degree of polymerization in xylans is also variable. Hardwood and softwood xylans generally consist of 150–200 and 70–130 β -xylopyranose units, respectively [60, 68, 77].

2-4-2- Xylan in hardwoods and softwoods

The frequency and composition of branches are dependent on the source of xylan [68]. Hardwoods contain acetyl-glucuronoxylan in about 15-30 wt% of the dry weight, without any arabinose side chains opposite to softwood xylans [104]. Hardwood xylan contains 4-*O*-methylglucuronic acid and acetyl side-groups. Figure 7 represents *O*-Acetyl 4-*O*-methyl- D-

glucuronoxylan in hardwoods. Methylglucuronic acid is linked to the backbone by $\alpha(1\rightarrow2)$ glycosidic bonds. Acetyl groups are present at carbon 2 and/or 3: 60–70% (70–80% [115]) of the xylose units are acetylated [68, 77]. The presence of these acetyl groups and of the ethyl glucuronic groups is responsible for the partial solubility of xylan in water [77, 115, 167]. These acetyl groups are readily removed when xylan is subjected to alkali extraction [77]. They are, to a lesser extent, ester-linked with feruloyl or *p*-coumaroyl residues [18, 66, 28].

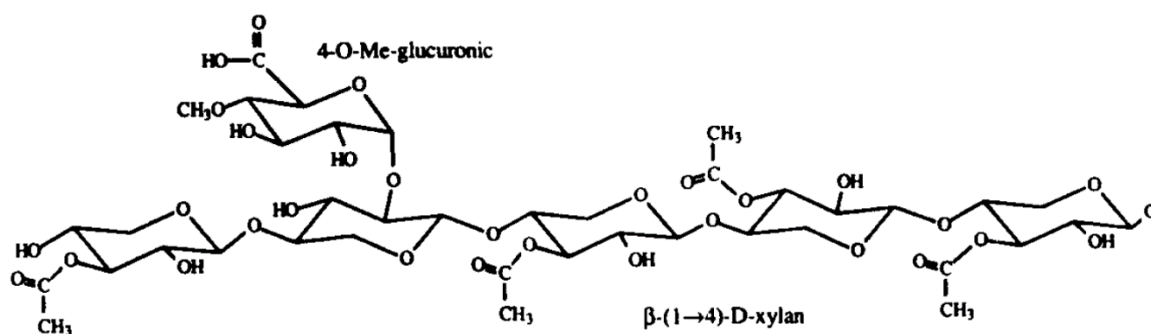


Figure 7- O-Acetyl 4-O-methyl- D-glucuronoxylan in hardwoods [23].

Softwood xylan contains 4-*O*-methylglucuronic acid and L-arabinofuranoside side groups linked to the backbone by $\alpha(1\rightarrow2)$ - and $\alpha(1\rightarrow3)$ -glycosidic bonds, respectively [18, 28]. Figure 8 represents arabino-4-*O*-methylglucuronoxylan in softwoods. The arabinosyl substituents occur on almost 12% of the xylose units. They have a higher 4-*O*-methylglucuronic acid content than hardwood xylans [77]. These xylans are not acetylated [115, 167, 185] and are freely soluble in water [167] and, because of their furanoside structure, the arabinose side-groups are readily hydrolyzed by acid [115] and also possess a lower degree of branching [167].

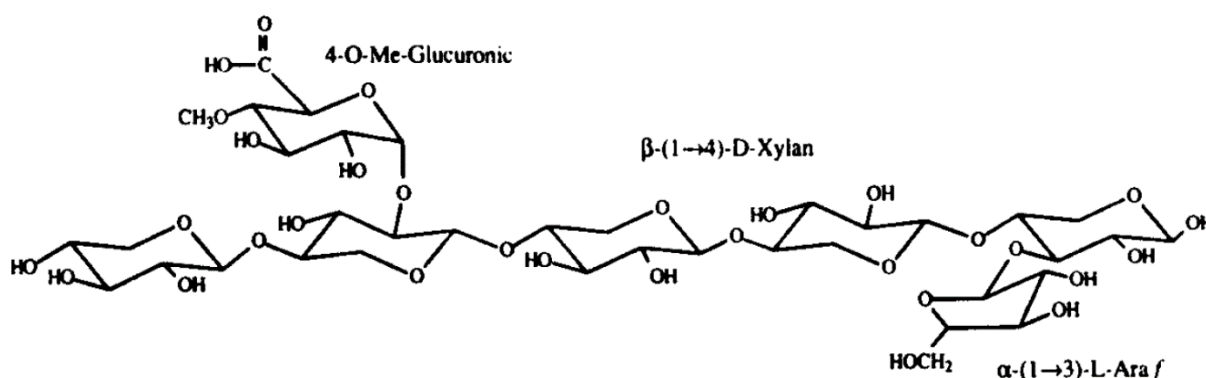


Figure 8- Arabino-4-*O*-methylglucuronoxylan in softwoods [23].

The average molar ratio of xylose:4-*O*-methylglucuronic acid:acetic acid in hardwood xylan is 10:1:7, and that of xylose:4-*O*-methyl-glucuronic acid:arabinose sugar units in softwood xylans

is 8:1.6:1 [18, 28, 77]. In softwood heteroxylans, arabinofuranosyl residues are esterified with *p*-coumaric acids and ferulic acids.

Similar to other biopolymers, xylan is also capable of forming intrachain hydrogen bonding, which supports a twofold extended ribbon-like structure. The β -(1-4) D-xylan chain is reported to be more flexible than the twofold helix of β -(1-4) cellulose because there is only one hydrogen bond between adjacent xylose units in contrast with two hydrogen bonds between adjacent glucose units in cellulose. The absence of a primary alcohol functional group external to the pyranoside ring as in cellulose and mannan has a strong effect on the intra- and inter-chain hydrogen bonding interactions. Intrachain hydrogen bonding is occurring in unsubstituted xylan through the O-3 position, which results in the helical twist to the structure. Nevertheless, the acetylation and substitution disrupt and complicate this structure [28].

2-5- Mannan

2-5-1- Basic Structure

Mannans and hetero-mannans are widely distributed in nature as part of the hemicellulose fraction in hardwoods and softwoods. Mannan is the predominant hemicellulosic polysaccharide in softwoods, but is the minor hemicellulose in hardwood.

2-5-2- Mannan in hardwoods and softwoods

Hardwood mannans are composed of β -1,4-linked mannopyranose and glucopyranose units, whereas softwood contains two different types of acetylated galactoglucomannans. They consist of glucose, mannose and galactose in the ratio 1:3:1 and 1:4:0.1 respectively in hardwood and softwood. The high degree of galactose substitution of the (1-4) β -D-mannan in galactomannans is clearly sufficient to prevent the chain aggregation that leads to insolubility and crystalline order in the mannans and glucomannans. The mannose:galactose ratio (M:G) is key in determining the amount of intermolecular association called hyperentanglements. Without any galactose side chains, mannan backbone will aggregate due to intermolecular interaction between the unbranched parts of mannans [60].

Figures 9 and 10 represent the typical structure of glucomannans in hardwoods and softwoods.

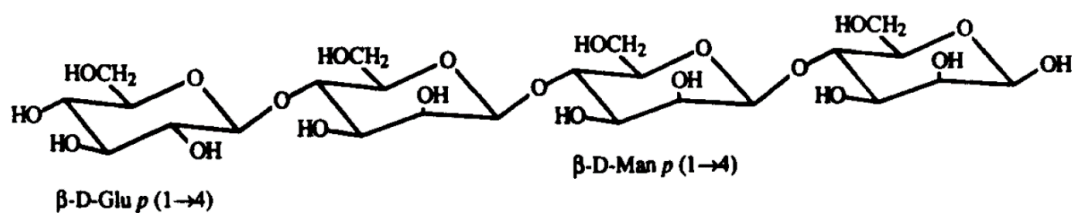


Figure 9- Glucomannan in hardwoods [23].

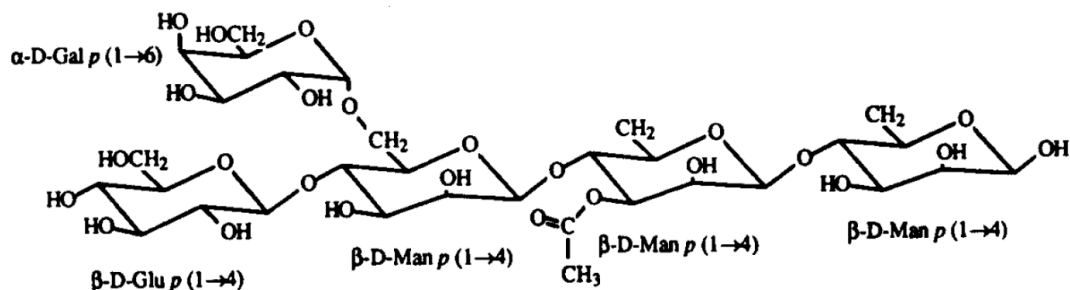


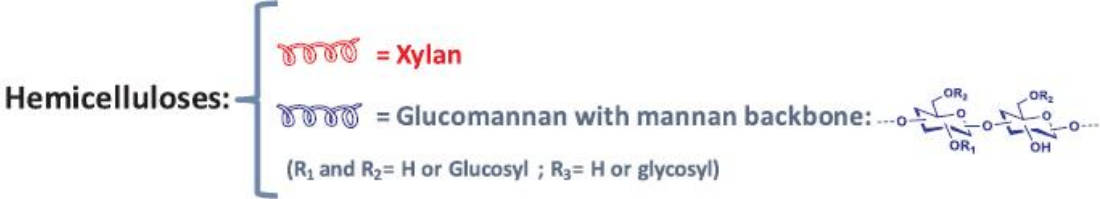
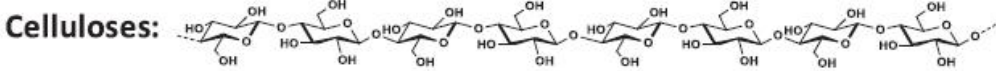
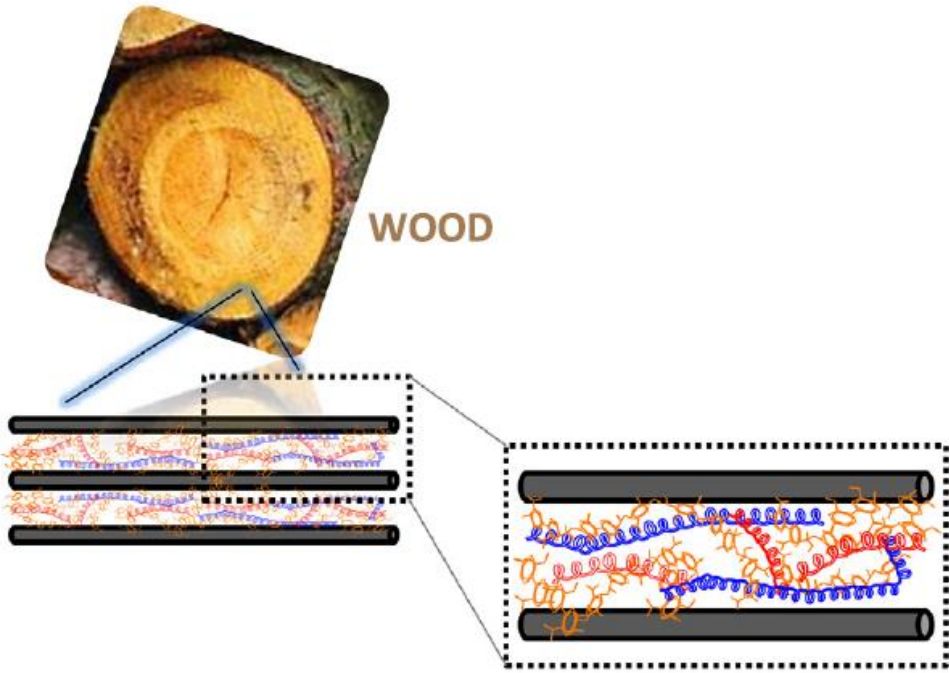
Figure 10- O-Acetylgalactoglucomannan in softwoods [23].

3- Lignin-Hemicellulose Bonds

Existence of chemical bonds between lignin and carbohydrate, known as Lignin-Carbohydrate Complexes (LCC), has been questioned because of the intimate physical integration between the lignin and carbohydrate constituents, the possibility of entrapment or adsorption, and the lability of many linkages. The proposed Lignin-Carbohydrate bonds include bonds to xylan, glucomannan, cellulose, various other hemicellulosic sugars, and pectin. Xylan is the predominant carbohydrate polymer associated with lignin, and about 90% of it can be removed by mild alkaline treatment [23]. However, some studies show that about 90% of the lignin in a spruce Kraft pulp was covalently linked to carbohydrates, the glucomannan–lignin complex being predominant [58, 4]. Thus, it has been suggested two types of lignin; one surrounded by xylan and another by glucomannan [59, 4].

Accordingly, it has been proposed that mannans and xylans are covalently linked with lignin at various points producing a coat around underlying cellulose strands via hydrogen bonds, but as few H-bonds are involved they are much more easily broken down than cellulose. The mannan and xylan layer with its covalent linkage to lignin and its non-covalent interaction with cellulose may be important in maintaining the integrity of the cellulose in situ and in helping protect the fibers against degradation by cellulases [60, 77].

Figure 11 represents a schematic illustration of lignocellulosic matrix.



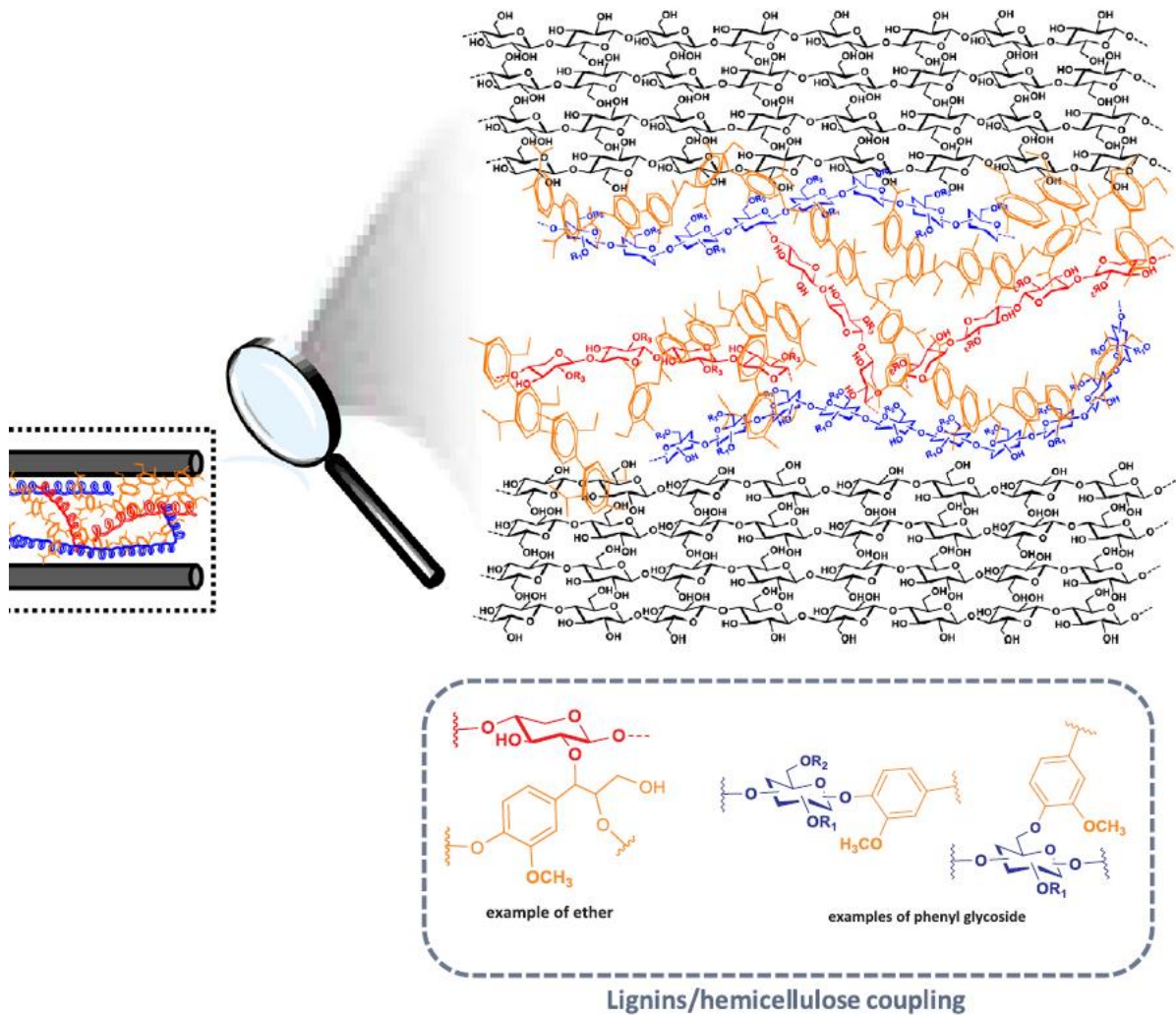


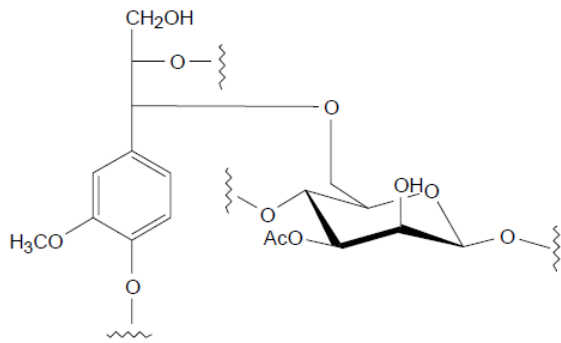
Figure 11- Schematic illustration of lignocellulosic matrix [4].

Benzyl ester, benzyl ether, glycosidic and acetal bonds are the four types of lignin-carbohydrates covalent linkages [4, 212]. Figure 12 represents these 4 types of bonds.

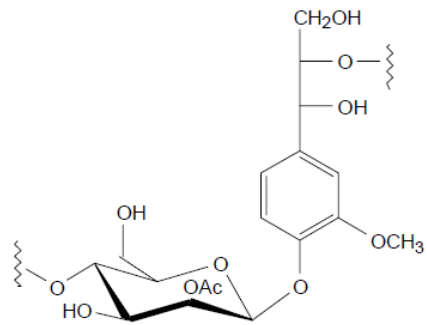
The most labile chemical bonds are ester linkages. Ester linkages occur between the free carboxyl group of a carbohydrate in hemicellulose and the benzyl groups in lignin [23, 212]. Many of these linkages in wood are broken by alkali [23]. An ester linkage between 4-O-methylglucuronic acid residue in arabinoglucuronoxylan and lignin in pine wood has been proposed [23]. Figure 13 represents this linkage.

It is likely that pectins have ester linkage with lignin, although in practice, it has not been proved yet [61, 63, 4]. Pectin thus seems to have a role in controlling/regulating the shape of lignin in the middle lamella [4].

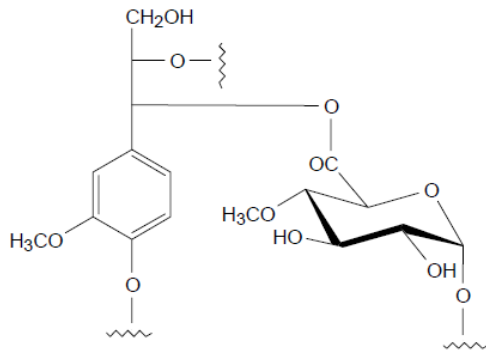
Benzyl ether type



Glycosidic type



Benzyl ester type



Acetal type

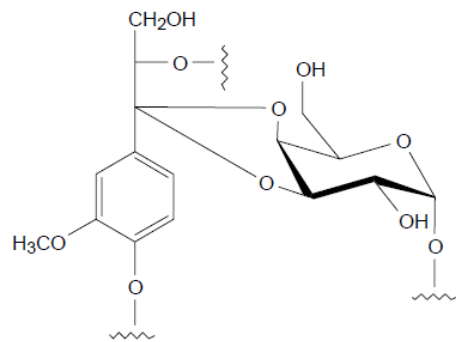


Figure 12- Proposed structures of the different bonds between lignin and hemicelluloses [64, 212].

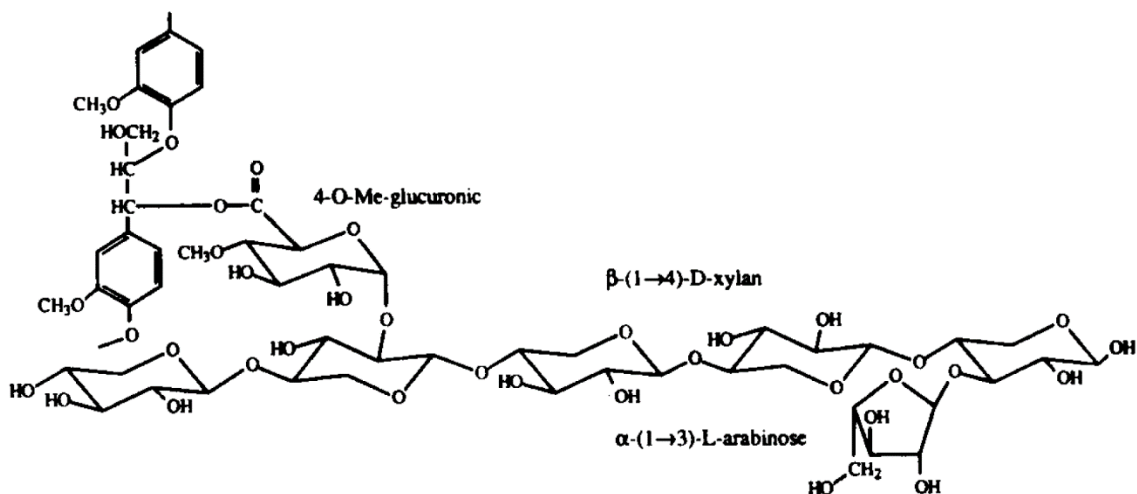


Figure 13- Proposed structure of ester linkage between lignin and arabino-4-O-methylglucuronoxylan in pine [65, 23].

It was found that mannose, galactose, and glucose are O-6 ether linked and xylose is O-2 or O-3 ether linked to the alpha-benzyl hydroxyl in a neutral fraction of pine LCC [23]. Figure 14 represents the proposed structure for ether linkages between lignin and glucomannan of pine.

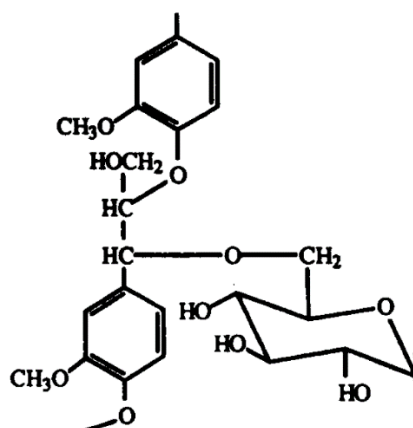


Figure 14- Proposed structure for ether linkages between lignin and glucomannan of pine [67, 23].

4- Kraft pulping processes and its effects on hemicelluloses

The objective of chemical pulping processes is to extract cellulose fibers from wood, by removing the lignin while preserving cellulose quality as well as fibers mechanical properties depending on the final applications. Part of the hemicelluloses is removed during pulping although certain amount is needed to fulfil the needs of some products, for example in papermaking the presence of hemicelluloses is needed to give strength to the papers by making fiber-to-fiber bonding [17].

Alkaline kraft (sulfate) pulping and acidic sulfite pulping are two main industrial chemical pulping processes [8, 12]. Selection of each type depends on the desired product, the wood species available, and economic considerations [9]. Kraft pulping is the dominant process and since more than 90% of chemical pulps are produced by this process, we will thus concentrate only on that process.

4-1- Description of the kraft process

The kraft process was first developed in Germany in 1879 and was applied industrially in a Swedish mill in 1885. The resulting paper was much stronger than any paper previously made, and therefore the process was named “Kraft”, (German and Swedish for “strength”) [69].

Kraft pulping has several advantages. It represents 91% of chemical pulping and 75% of all pulp and produces a variety of pulps used mainly for packaging and high-strength papers and boards [13]. Kraft pulping uses sodium hydroxide and sodium sulfide (named as white liquor) added to the wood chips in the cooking process at high temperature (160-170°C) which leads to lignin solubilization and extraction of cellulosic fibers. The effluent of this process, called

black liquor, is then concentrated and burnt, and the cooking chemicals are recovered, which makes the kraft process autonomous in energy and chemicals. Figure 15 shows a typical kraft mill.

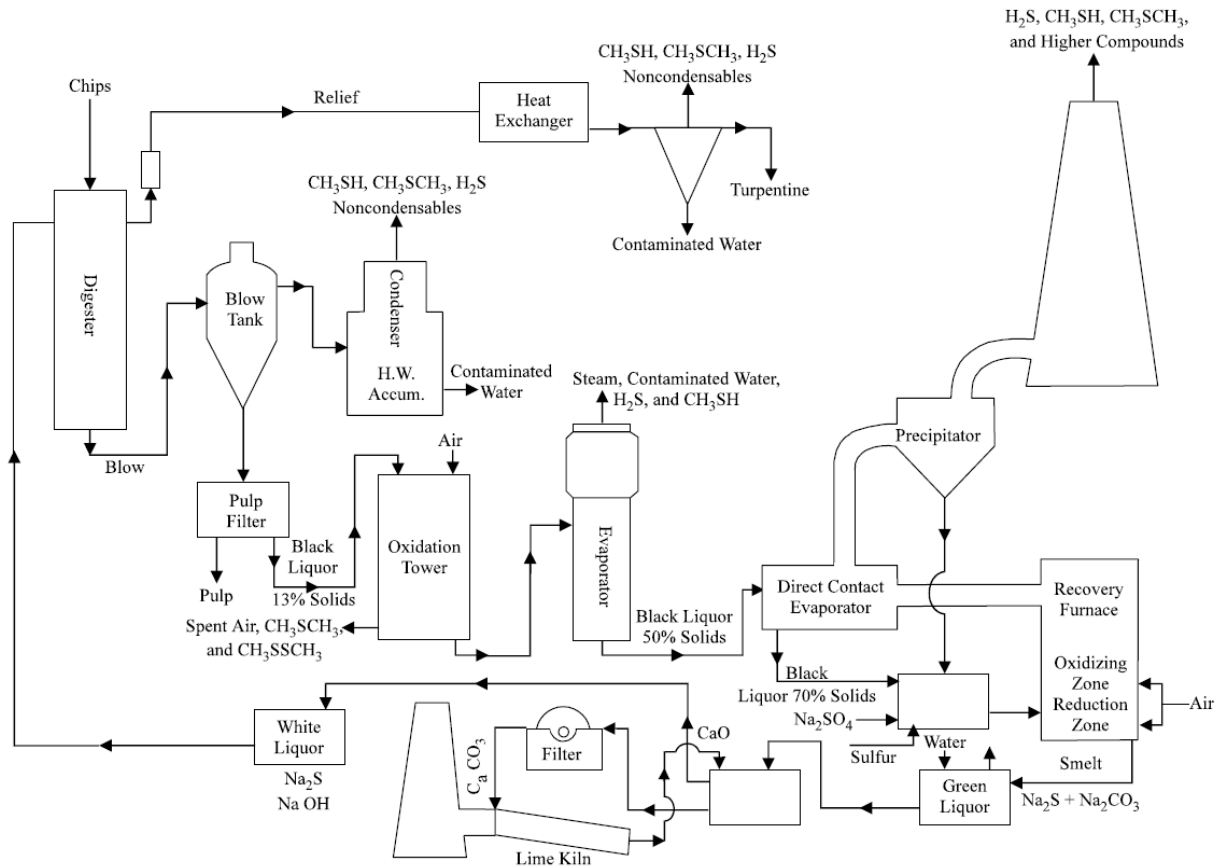


Figure 15- Typical kraft sulfate pulping and recovery process [9].

Kraft pulping is efficient enough for almost all wood species [15, 7].

The kraft process consists of three principal operations:

- 1) Cooking and washing,
- 2) Evaporation and sodium sulfide recovery in the recovery boiler,
- 3) Causticizing and lime recovery.

Following debarking and chipping, the chipped wood is "cooked" in the digester with a water solution of sodium hydroxide (NaOH) and sodium sulfide (Na₂S) known as white liquor under high temperature (~160°C) and pressure in order to dissolve chemically the lignin that binds the cellulose fibers of the wood together. There are two types of digester systems: batch and continuous. After cooking for about four to six hours [17], lignin is dissolved and the cellulose fibers are separated from the spent cooking liquor (black liquor) in the pulp washers. The wood pulp is washed, screened and dried or further delignified in an oxygen stage and

bleached in a bleach plant. Most of the lignin and some of the hemicelluloses are dissolved, leaving the remaining cellulose fibers separated. Remainder of the kraft process is designed to recover chemicals and heat. The spent cooking liquor, containing process chemicals and water and spent chemicals from the wood, is combined with pulp wash water to form what is called weak black liquor. This liquor is concentrated through evaporation and then combusted in a recovery furnace, where heat from the combustion of organics is recovered for process use and for the generation of electrical power; inorganic chemicals are recovered as molten smelt (Na_2S and Na_2CO_3). Water and quicklime are used to convert sodium carbonate to caustic soda. The lime mud (CaCO_3) that precipitates from the tank is calcined in a lime kiln to regenerate quicklime (CaO) [10, 8]. Figure 16 shows a general principal picture of kraft pulping.

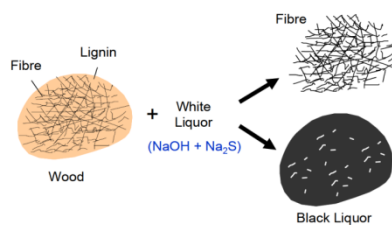


Figure 16- The principal of kraft pulping process in which the chipped wood is cooked with white liquor and as a consequence, lignin is dissolved and the cellulose fibers are separated from the spent cooking liquor (black liquor) [15].

Black liquor is the fifth most important fuel in the world, next to coal, oil, natural gas and gasoline [70]. Since black liquor is derived from wood, it is the most important renewable bio-fuel, particularly in Sweden and Finland [15].

Valuable extractives (e.g., turpentine and tall oil) are separated for sale as commodity chemicals. Process chemicals are recovered with only a relatively small loss in volume, and after replenishment with sodium salts (addition of sodium sulfate), they are returned to the digester for reuse. Pulp yield is largely a function of the wood species used, the time and temperature of cooking, the degree of bleaching, and the paper strength required. Generally, kraft pulp yields from softwoods are approximately 47 percent for unbleached pulp and 44 percent for bleached pulps. Hardwood pulp yields range from 50 to 52 percent for unbleached kraft pulps to 50 percent for bleached pulps [17, 15].

4-2- Effect of kraft process on hemicelluloses

Extensive modification of hemicelluloses takes place during kraft pulp production process. In fact, polysaccharides suffer from two main reactions during kraft cooking: primary peeling,

and the chain cleavage through alkaline hydrolysis with subsequent secondary peeling. Apart from these two degradation reactions carbohydrate losses also originates from an initial dissolution of soluble carbohydrates and the hydrolysis of substituents, mainly acetyl groups [72, 37]. The Peeling reaction that takes place under alkaline conditions already at temperatures of 80°C and higher, starts at the reducing end groups contained in hemicelluloses and cellulose, and leads to the peeling of the saccharidic units one by one. The reaction product is an organic acid, like saccharinic acid, which contributes to the consumption of alkali during kraft cooking. It is admitted that an average of 50 saccharidic units are removed, as a competing reaction takes place which removes the reducing end groups and thus stops the peeling reaction [73, 37]. While the peeling proceeds at low alkali levels [74], the stopping reaction requires sufficiently alkaline conditions [75, 37]. Figure 17 and 18 represent reaction mechanisms of peeling and stopping reactions.

The peeling reaction does not affect cellulose significantly as its DP is about 10 000 in wood, however it leads to important yield losses for hemicelluloses, particularly glucomannans. Xylans are much more resistant to peeling as they contain methyl-glucuronic acid groups attached to carbon 2 of some xylose units in the backbone chain, which makes peeling not possible [76, 72, 79, 37]. Arabinose substituents on C-3 in xylan are however better leaving groups than hydroxide ions and thus promote the stabilizing stopping reaction and reduce the effect of peeling greatly [80, 81, 84, 37]. This effect is decreased with increasing cooking temperatures as the arabinose units are removed through alkaline hydrolysis [85, 37].

The second reaction, called alkaline hydrolysis, takes place for temperatures higher than 130°C, at which alcohol groups are ionized, which leads to glycosidic bond breakage. Figure 19 represents the reaction mechanism for the alkaline hydrolysis. This leads to severe DP loss and the creation of new reducing end groups [88, 37]. Peeling can thus restart, which is why it is called “secondary peeling” [76, 37]. This contributes to the dissolution of the major part of glucomannans. The glucomannan remaining in the fibers is, however, stable against dissolution and degradation [37].

During kraft cooking, xylans are modified. The alkaline and high temperature conditions transform some methyl-glucuronic groups into hexenuronic groups, which have a negative effect on bleaching further on in the process, as they reduce the brightness stability of the

pulp and increase the consumption of bleaching chemicals [40]. Figure 20 represents the formation of hexenuronic acid from 4-O-methylglucuronic acid in xylans.

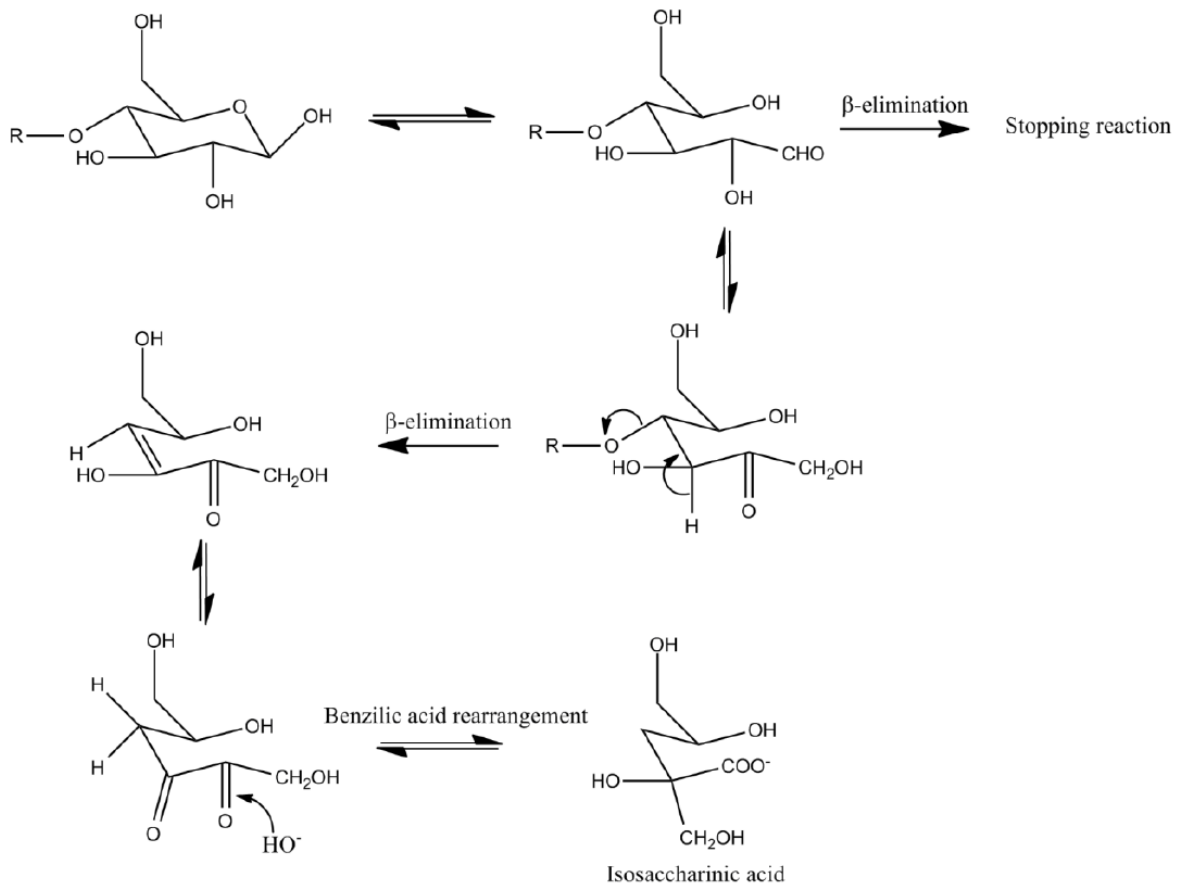


Figure 17- Reaction mechanism for the peeling reaction on a cellulose or glucomannan chain [47, 37].

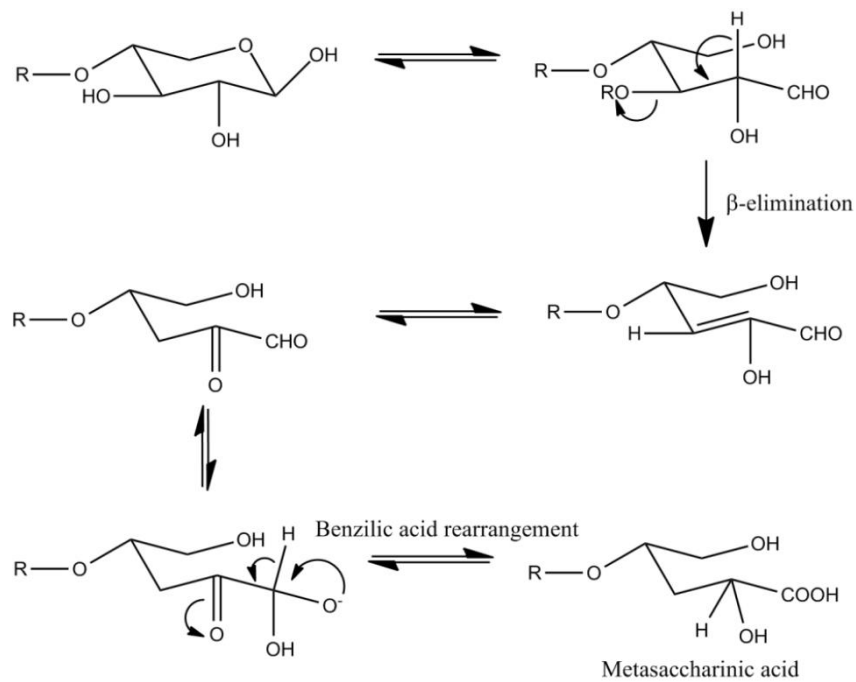


Figure 18- Reaction mechanism for the stopping reaction on a xylan chain [47, 37].

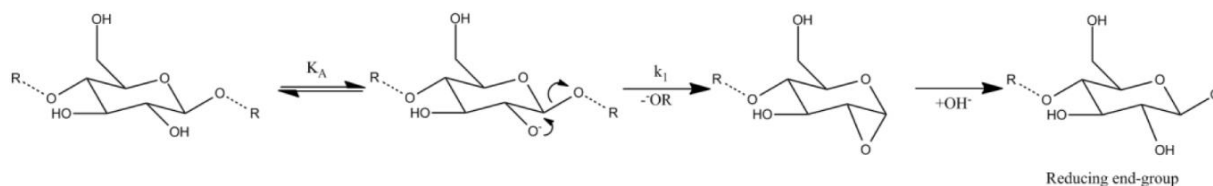


Figure 19- Reaction mechanism for the alkaline hydrolysis [37].

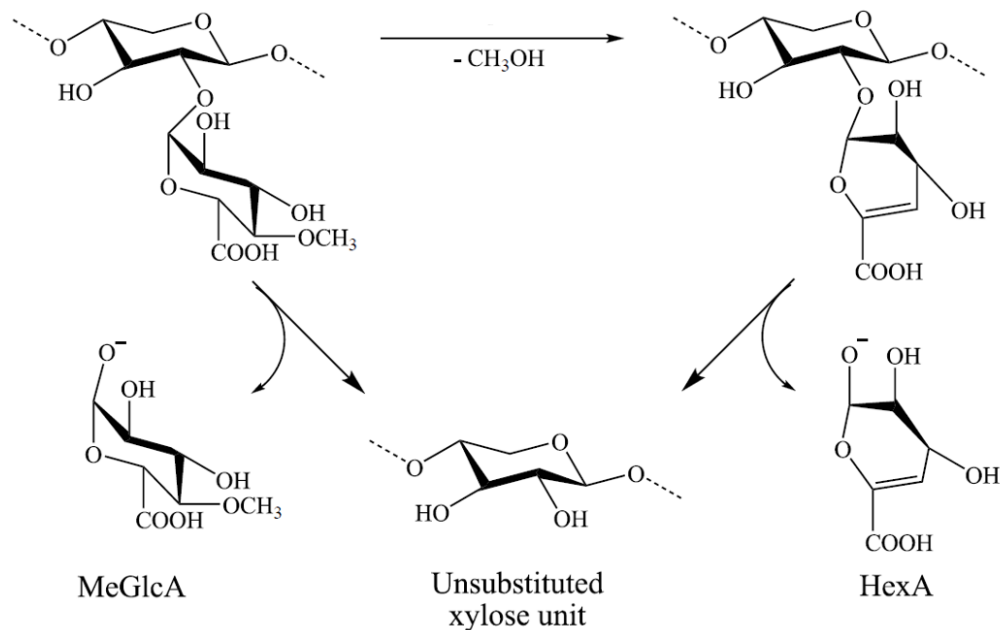


Figure 20- The formation of hexenuronic acid from 4-O-methylglucuronic acid and the cleavage of the two substituents [39].

If the degree of polymerization of xylans becomes small enough, they become soluble which is facilitated by the fact that they contain carboxyl groups, and by the high alkalinity and high temperature. As the cooking proceeds the alkali concentration decreases, and degraded short-chain xylans are precipitated in a more or less crystalline form on the surface of cellulose micro fibrils [89, 92, 18]. Apparently, the arabinose and glucuronic acid units, protruding from the linear 1,4-xylopyranose chains, prevent crystallization and their removal during the kraft cook increases the tendency of xylan to crystallize. The configuration of the xylose units allows close contact of the xylan chains with the cellulose and it is likely that part of the xylan, after removal of its substituents, tends to co-crystallize with, or become adsorbed on, the cellulose of the pulp. A considerable part of the xylan is re-adsorbed or re-precipitated onto the cellulose, although a part remains undissolved at its original location in the fiber [90, 91, 18]. The re-precipitation of xylan is preceded by the re-precipitation of dissolved lignin during kraft pulping. These redeposited polymers have also been suggested to be chemically linked to each

other [93, 94, 95, 18]. Furthermore, hemicelluloses seem to physically restrict the passage of high molecular mass lignin out of the pulp fiber [96, 97, 18, 116].

In addition, hemicelluloses undergo deacetylation during kraft cooking. Although there is a huge removal of acetyl groups during the initial phase of pulping, some studies show there are still some acetyl groups remaining after kraft pulping. The value of about 20% of the acetyl groups has been reported [42].

5- Bleaching processes and its effect on hemicelluloses

Removing the remaining lignin in the pulp after pulping processes is the main objective of bleaching processes in order to produce fiber with high brightness and brightness stability. Parallel to removing the lignin, the cellulose fiber should be preserved in length and strength depending on their final application, and the environmental regulations should be respected as well [260].

Various chemical treatments in bleaching exist. Oxidants like chlorine dioxide, oxygen, hydrogen peroxide, peracetic acid and ozone react with lignin [260], leading to its oxidation and depolymerization. Alkaline extraction stages are usually applied after acidic oxidizing stages to enhance the removal of oxidized lignin [260]. Chlorine and chlorine dioxide produce chlorinated organic compounds, called AOX, that are detrimental to environment which has led to the development of more environmentally friendly processes based on oxygen, hydrogen peroxide, ozone and peracetic acid [260].

Hemicelluloses are influenced by bleaching agents as well, but certainly not as extensively as lignin. The hexenuronic acid (HexA) formed from 4-O-methylglucuronic acid groups of hemicelluloses during kraft pulping, has an effect of bi-functionality due to the presence of enol ether and unsaturated carboxylic acid group which makes it an easy target for both electrophilic and nucleophilic attacks during bleaching [1]. It is able to react with electrophilic bleaching agents such as elemental chlorine, chlorine dioxide, ozone and peracetic acid which will result in consumption of bleaching agents [99, 43, 1], as well as its inverse influence on the properties of bleached pulps [101] by increasing brightness reversion [102, 1]. It can bind heavy metal ions which will result in indirect consumption of other bleaching chemicals, such as hydrogen peroxide [103, 1]. Its oxidative degradation (for example, during ozonation) produces calcium oxalate deposits in the surface of bleaching equipment [105, 1]. It consumes

permanganate during the standard procedure of kappa number determination, commonly used for lignin quantification in pulps [106, 1]. HexA is not degraded during oxygen or alkaline peroxide bleaching [107, 43, 54].

Some research suggested [204] that hydroxyl radicals are responsible for degradation of carbohydrates during oxygen delignification or hydrogen peroxide bleaching [109, 46]. All residual kraft pulp lignin after the O-stage has been found to be linked to polysaccharides compared to the unbleached pulp: at least 50% of the residual lignin after the cook is linked to both xylan and glucomannan and after a subsequent O-stage, this value increases to 80–90% [47]. Residual Lignin–Carbohydrate Complexes (RLCC) appear to be degraded into simpler structures during delignification and oxygen delignification shortens the oligosaccharide chains present in RLCC [46].

During oxidative conditions, any reducing end group present in the polysaccharides will rapidly become oxidized to the corresponding aldonic acid group thereby preventing the endwise degradation from taking place [47], thus hemicelluloses do not suffer from the peeling reaction during oxidative bleaching carried out under alkaline conditions at high temperature.

Figure 21 represents an outline of the reaction.

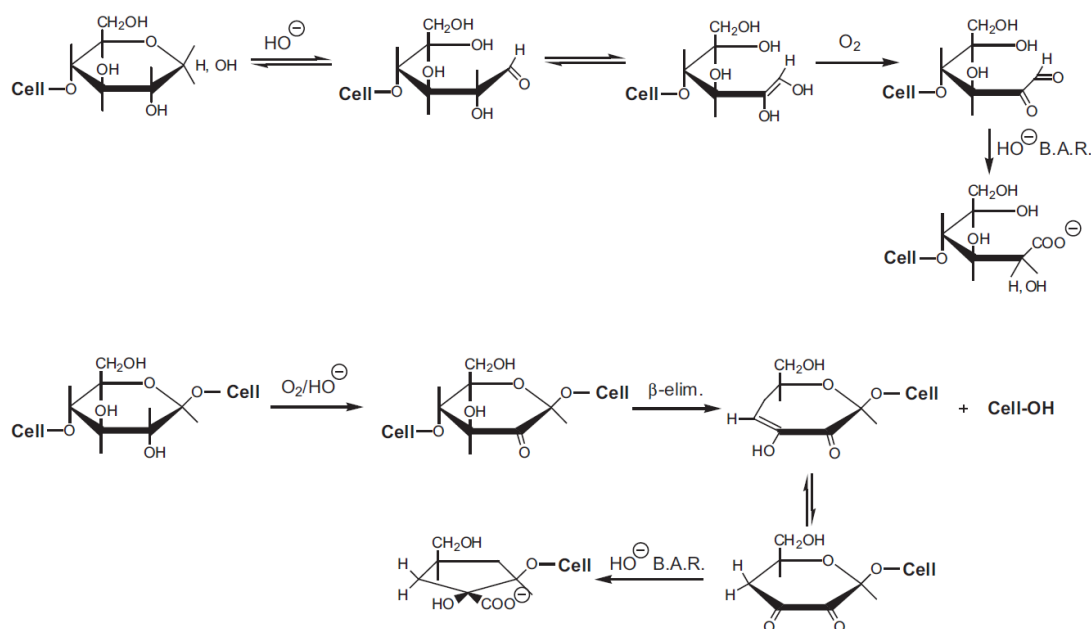


Figure 21- Major carbohydrate reactions in the O-stage. Oxidative stabilization of a reducing end group (upper reaction), oxidative cleavage of a polysaccharide chain (lower reaction). B.A.R. = benzilic acid rearrangement [47].

It is generally acknowledged that an O- or OO-stage can remove 35–50% of the residual lignin in hardwood kraft pulp and 40–65% in softwood kraft pulp, without significantly impairing the

selectivity of delignification and the physical pulp properties [46]. The total dissolution of carbohydrates, on the other hand, is rather low resulting in a high overall yield of pulp [47]. In another study, it was shown that the amount of polysaccharides in the bleaching effluent after oxygen delignification was reported to be 4.5 kg per ton of pulp [110, 47]. The same type of result was found for hydrogen peroxide bleaching [47].

In ozone delignification, the attack of hydroxyl radicals begins with a hydrogen abstraction, followed by an oxygenation of the resultant carbon-centered radical, which leads to the introduction of carbonyl groups [111, 46]. Figure 22 represents hydrogen abstraction from carbohydrates. Glycosidic bonds, during ozonation, are cleaved by ozone and the radical species during ozonation in water [112] that lead to decomposition of carbohydrates depending on pH, the temperature, and transition metal ions concentration [114, 46]. Thus both ozone and radical species are responsible for glycosidic bond cleavage of carbohydrates [46]. Ozonation of unbleached pulp leads to the formation of hydrogen peroxide resulting in an additional degradation due to the hydroxyl radicals generated by the peroxide formed during ozonation [46]. In addition, ozone is known to be very effective and selective in removing HexA, without simultaneously impairing pulp properties [46].

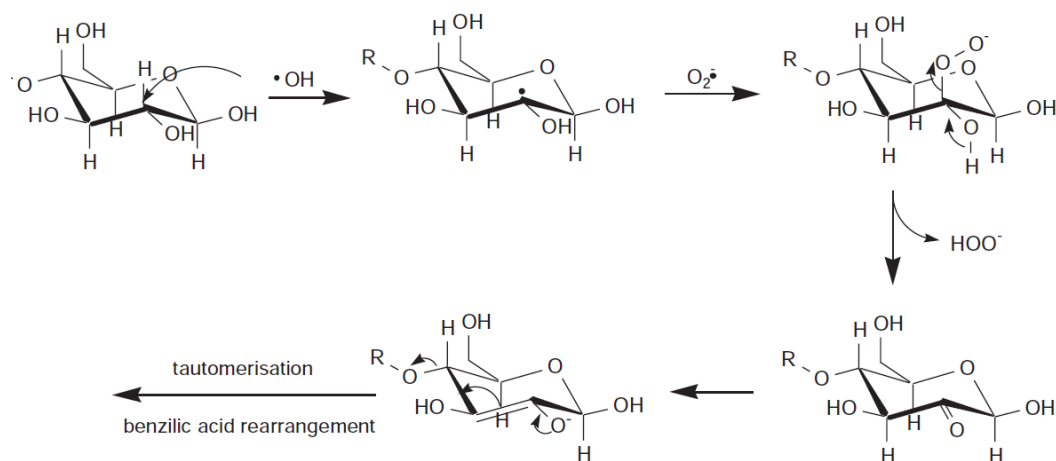


Figure 22- Hydrogen abstraction from carbohydrates [46].

However, it seems that low molecular weight hemicelluloses are preferentially degraded firstly and defer the degradation of cellulose chains, acting like a barrier for cellulose attack by ozone. The concentrations of xylan and/or mannan, beside other parameters, affect the pattern of carbohydrate degradation. In two kraft pulps with different initial kappa number, ozone bleaching of the one with the kappa number of 3.4 led to the 7.1% degradation of xylan and 6.5% degradation of mannan, while these values for kappa number of 17.5 were 7.3% and

6.8%, respectively, suggesting the effect of initial purity of pulp on the pattern of attack by ozone [46].

In hydrogen peroxide bleaching, the radical species such as hydroxyl radicals generated from the hydrogen peroxide alkaline decomposition, are responsible for some solubilization of hemicelluloses [117, 44].

In another study, a treatment of oxygen delignified hardwood kraft pulp by hydrogen peroxide resulted in almost no effect on pentosan content (changing from 23 $\mu\text{mol/g}$ to 22 $\mu\text{mol/g}$) [54].

The fact that hexenuronic acid is sensitive to acidic conditions has promoted some mills to introduce a specific hydrolysis stage which can be done either as an isolated stage or as part of a chlorine dioxide stage. In either case, the objective is to achieve a selective hydrolytic degradation of hexenuronic acid groups [47]. Thereby, the hexenuronic acid groups attached to the xylan can be removed. Figure 23 represents acidic hydrolysis of hexenuronic acid (HexA).

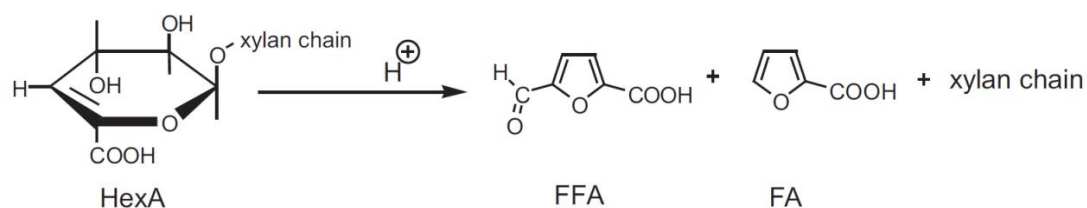


Figure 23- Acidic hydrolysis of hexenuronic acid (HexA) resulting in the formation of 5-formylfuroic acid (FFA) and furoic acid (FA) [47].

To conclude, the main impact of a bleaching sequence on hemicelluloses is the removal of hexenuronic acid groups during acidic oxidative treatments. Although decomposition of hemicelluloses happens during some bleaching sequences, it seems that their content is hardly lowered.

6- Ways to remove hemicelluloses from pulp

6-1- Chemical Extraction of hemicelluloses

6-1-1- Alkaline Extraction

Alkaline extraction can dissolve hemicelluloses: dissolving pulps are frequently purified by cold caustic extractions [118, 48] in the case of kraft pulps, and by hot and cold caustic extraction in the case of sulphite pulps.

Alkaline extraction, either cold (Cold Caustic Extraction (CCE)) or hot (Hot Caustic Extraction (HCE)), have been studied extensively [119, 120, 121, 122, 123, 232], between them CCE is more popular and effective with a typical alkali concentration of 8–10% [233]. In CCE, the mechanism of removal of hemicelluloses is based on physical swelling/solubilisation, while in HCE it is based on chemical reactions (peeling reaction, glycosidic bond cleavage) in which the latter results in higher yield loss [124] and so it seems that CCE is much more selective in removing hemicelluloses than HCE [237]. Furthermore, it is claimed that Cold Caustic Extraction has the potential to extract high molecular weight hemicelluloses [240] and CCE obtains better results, compared to Hot Caustic Extraction (HCE), in removal of hemicelluloses from long-fiber fractions [237].

The mechanism of removal of hemicelluloses from pulp fibers through CCE can be described in two steps: 1) swelling through physical interaction between fiber and aqueous sodium hydroxide, 2) solubilisation in which the hemicelluloses diffuses through the pores of the fiber wall, from interior to exterior and then into bulk [46, 233]. In practice, CCE is a very selective process in removing hemicelluloses from pulp fibers [46, 120], due to the excellent swelling of fibers and solubility of hemicelluloses [233]. But there are some challenges with this method. The main drawback of CCE is the conversion of cellulose I (native cellulose) to cellulose II (regenerated cellulose) [125, 126, 233] whose reactivity is relatively low due to the very compact fiber structure with an extensive inter-molecule hydrogen bonding. The other drawback is separating alkali from highly swollen pulp after a CCE process, which demands high capital investment [120, 233]. Furthermore, CCE acts more efficiently on xylan extraction than on galactoglucomannan's [120, 227].

In another study and more precisely, the hemicellulose removal through CCE has been outlined in a three-step process: 1) alkaline penetration and diffusion, 2) cellulosic fiber

swelling, and 3) hemicelluloses removal. Figure 24 represents a schematic of these processes. It has been claimed that the factors that could complicate such a process include: 1) non-uniformity of the hemicelluloses distribution across the fiber cell wall [127, 128] and 2) differences in the hemicelluloses' molecular weights [129, 130, 281].

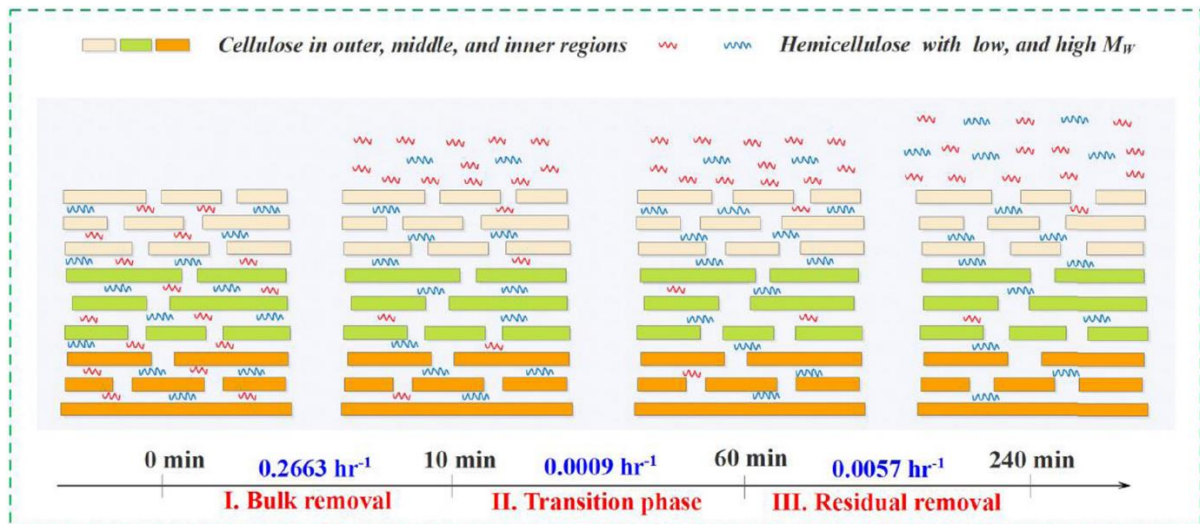


Figure 24- Mechanism of hemicelluloses removal during the CCE treatment. (In the bulk phase, due to alkali-induced fiber swelling, low-M_w fraction of hemicelluloses is dissolved and diffused to the bulk phase; in the transition phase, the relatively high-M_w hemicelluloses in the inner layer of fiber wall is transferred to the outer layer due to the hemicelluloses concentration gradient; in the residual removal phase, the relatively high-M_w hemicelluloses continue to be removed) [32].

The swelling effect of cellulose fibers under the alkali conditions makes the pores larger and therefore, during the initial phase of hemicelluloses bulk removal, the removal of hemicellulose is significant, particularly the low-molecular weight hemicelluloses which are located in the outer region of fiber wall. The negligible removal of hemicellulose during transition phase can be due to the high molecular weight hemicelluloses remained in the fibers. The concentration gradient makes the hemicelluloses to diffuse from the inner to outer region. In the residual removal phase, some hemicelluloses with relatively high-molecular weight continue to be removed, although the process is a slow one [32].

Many studies have investigated the effect of CCE in removal of hemicelluloses in order to produce dissolving pulp with the desired qualities [233, 120]. It has been shown that most of the pulp hemicelluloses can be solubilized under the following conditions: 9% NaOH at -5°C [49].

In another study, it has been shown that CCE was more efficient to remove hemicellulose from the long fibers fraction of an unbleached softwood sulphite pulp than the short fiber fraction

[237]. Figure 25 represents process concept consisting of pulp fractionation and caustic treatment for enhancing the hemicelluloses removal.

It has been shown that long-fiber fraction, in contrary to short-fiber fraction, had a lower content of hemicelluloses, as well as larger average pore diameter, but a smaller specific surface area demonstrating the positive effects on the removal of hemicelluloses in the followed caustic extraction [237].

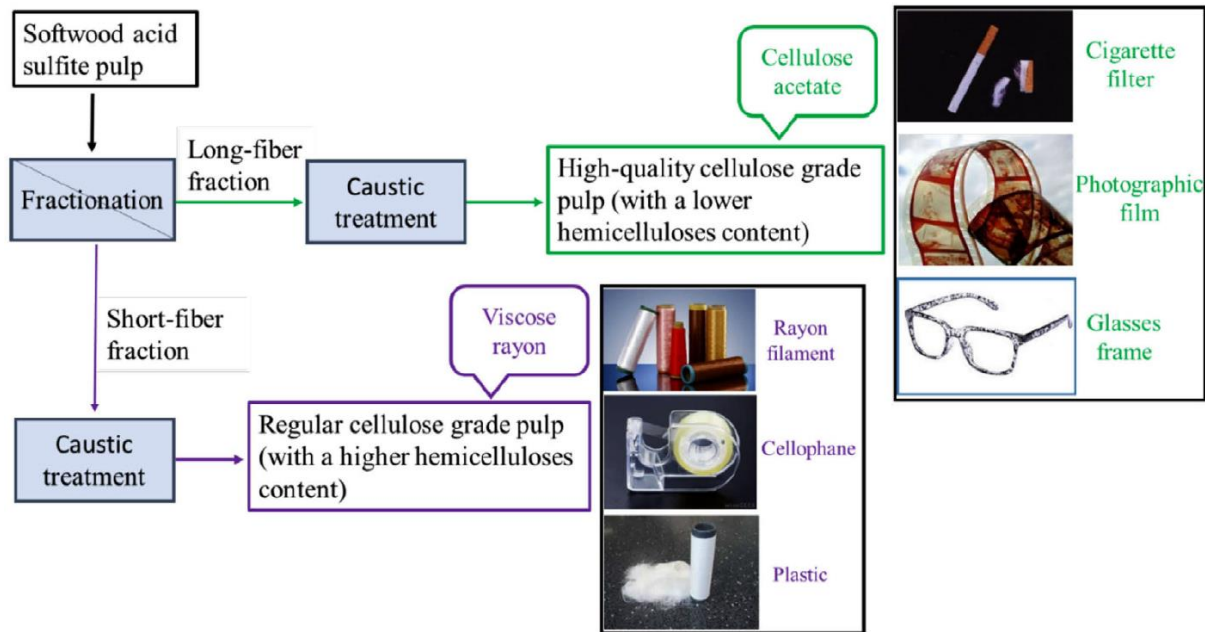


Figure 25- Process concept consisting of pulp fractionation and caustic treatment for enhancing the hemicelluloses removal [237].

As a summary, in spite of some drawbacks rising from applying CCE, this cold alkali extraction is still practically a major selective method used for treatment of pulp in order to solubilize the hemicelluloses.

6-1-2- Acid Extraction

Applying hot acid hydrolysis step has been developed to remove HexA from kraft pulp prior to bleaching. It has been shown that it can remove selectively 90% of HexA in birch kraft pulp [99, 43]. In a study, diluted acid treatment of a poplar pulp, under the conditions of 0.5% w/w sulphuric acid at 170°C in 30 min, decreased the hemicelluloses molecular weight by almost 85% [232, 131]. In addition, it has been found that LCCs are cleaved during sulphuric acid treatment of kraft pulp [132, 43].

Theoretically, acid hydrolysis hydrolyses the hemicelluloses chains producing shorter chained oligomers [38] and if the conditions are strong enough (pH, T) it can produce monomers and

sugar degradation products and in the same time it has a negative effect on cellulose with lowering its viscosity [46]. In a study, the acid pretreatment of an eucalyptus kraft pulp resulted in a 11.2% loss of viscosity, in spite of the fact that this treatment was performed using the method of Vuorinen to minimize the loss of viscosity and physical strength [133, 43]. The cellulose degradation will increase with increased reaction time. In practice hot acid treatment is not applied for more than 2 hours in order to preserve the pulp yield and viscosity and in general it is rarely used in the mills [46].

Regarding release of monomer sugars due to the hot acid treatment, hardly no sugar was solubilised (2.53 mg/g o.d.p), which indicated that the carbohydrate degradation caused by hot acid pretreatment was dominated by hemicellulose or cellulose depolymerisation rather than sugar solubilisation [43].

In addition, applying hot acid has undesired effect regarding corrosion of equipment [46].

6-1-3- Solvent Extraction

Ionic Liquids (ILs) have attracted attention as solvents for clean technology in biorefinery concepts during the last two decades [134, 226]. They are salts consisting entirely of ions, have melting points below 100°C, a very low vapour pressure and distinct solvent properties which enable them to dissolve various biopolymers [135, 136, 137, 138, 139, 140, 226].

ILs can be used to fractionate hemicelluloses from paper-grade pulp, when the solvent is mixed with a defined share of water at moderate temperatures. This method, called IONCELL-P(ulp) process, extracts hemicelluloses without degrading them [141, 142, 226], without converting cellulose I to cellulose II [143, 226] and without touching the molar mass distribution of the individual polymers [141, 227]. In this method the pulp is treated at a pulp/solvent ratio of 1:20 for 3 h at 60°C. But in general, the best solvent mixture consisting of an IL with its optimum water content can be defined as a compromise between the highest degree of hemicellulose extraction and the lowest amount of possible cellulose dissolution, dependent on pulp species [226].

The performance of IONCELL-P on different paper-grade pulps using two 1-ethyl-3-methylimidazolium ([emim])-based ILs containing the anions acetate or dimethyl phosphate has been studied. The pine pulps based on their hemicellulose contents and compositions were subjected to hemicellulase pretreatment (combination of xylanase and mannanase)

prior to the IONCELL process, which has been found effective [226]. This pretreatment on birch decreased the residual contents of xylan and mannan from 0.9% to 0.76% and from 2.2% to 1.89%, respectively, corresponding to a 15% decrease in total residual hemicellulose content. Some other studies have investigated this process and similar results were obtained with eucalyptus pulp [142, 227].

Hemicellulose removal from dissolving pulp through nitren and cuen has been studied in different researches [144, 145, 48, 146, 227]. It has been claimed that nitren, a metal complex of tris(2-aminoethyl)amine and nickel(II)-hydroxide, in the form of 3% solution, removes selectively the hemicelluloses from kraft pulp and high purity xylans are precipitated by lowering the pH to 4 [48]. Figure 26 represents the formation of complex between xylan and nitren. However, this is not a good solvent for glucomannans, so softwoods are not a good raw material for nitren [48].

Dimethylsulfoxide (DMSO) is another solvent that can dissolve efficiently most xylan and mannan grades [50]. While aqueous alkali dissolves most (acetylated) hemicelluloses, DMSO is a good solvent for hemicellulose – also the non-acetylated ones. A yield of about 65% was obtained from DMSO treatment of MFC (DMSO - 5% LiCl) [191] based on the method introduced in an original study in which the birch and spruce holocellulose were extracted with DMSO and then with water and with 18% sodium hydroxide solution. Even though the recovery of extracted material was not quantitative; it was reported that a considerable part of the hemicelluloses was extracted. From birch holocellulose the water and sodium hydroxide extracts were 87% and 48% of the original holocellulose, while these values for spruce holocellulose were 88% and 76%, respectively [191]. In another study heteroxylans of sugarcane bagasse and straw were isolated using mild delignification (with PAA – peracetic acid) followed by DMSO extraction with the yield of 19-22% (base on the initial content of xylose) [192]. This yield was 46% w/w (based on initial pentosans content in *E. globulus* wood) for eucalyptus wood [55].

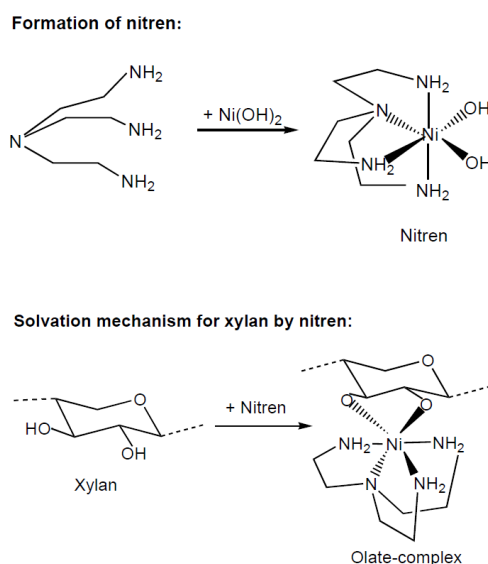


Figure 26- Constitution of nitren and its complexation with xylan in aqueous solution [48].

6-2- Combination of Chemical and Mechanical Extractions

The mechanism of hemicellulose removal through CCE is mainly based on fiber morphology/structure and mechanical treatments such as pulp refining can modify that effectively [233].

The mechanical refining causes remarkable changes on fiber morphologies by increasing the fiber pore size, pore volume and specific surface area. This happens through loosening fibril aggregation by destroying the fiber amorphous zone contributing to the formation of new pores and expansion of original pores [147, 233] and so promoting the fibrillation which leads to improved alkali-induced swelling of fibers and hemicellulose diffusion during CCE [233]. Improving the porosity of the fiber wall [147], opening/enlarging of pores and voids in the fiber wall, disruption of the cellulose amorphous region, slit fibril aggregation and creation of fines [147] through mechanical treatment have been reported on different studies [233].

The mechanical refining can also collapse and/or break the fiber structure, resulting in the dislocation of matrix and formation of cracks in fibers, which can additionally contribute to the improvement of WRV [148, 233]. It produces more secondary fines, as well, which results in an increase in the specific surface area [149, 150, 233].

Figure 27 represents the concept of combined mechanical refining and CCE for enhancing the hemicelluloses removal. It has been shown that the addition of mechanical refining in the treatment process can facilitate the hemicelluloses removal during the subsequent CCE process, which can decrease the NaOH concentration to obtain a similar degree of

hemicelluloses removal. The advantage of applying low concentration solution of NaOH is that the resulting products can maintain a good pulp reactivity since the conversion of cellulose I to cellulose II occurs only at a high NaOH concentration [126, 233].

The results have shown that the yield for the CCE alone was higher than that for the combined mechanical refining and CCE process which was due to a higher removal of hemicelluloses [233].

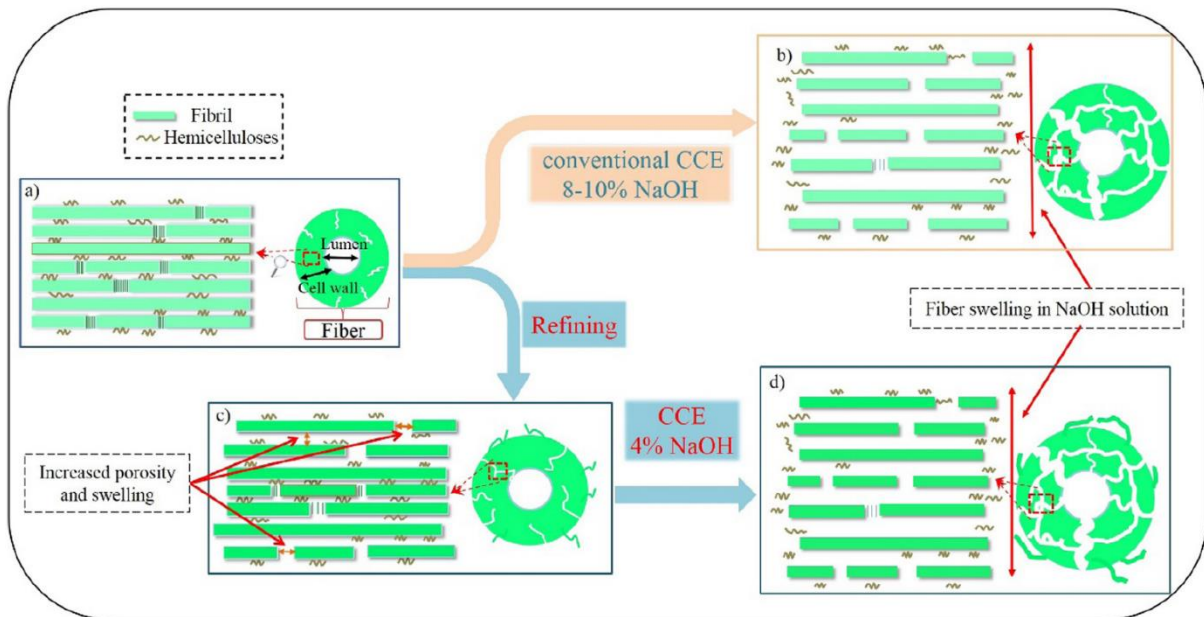


Figure 27- Schematic of the combined mechanical refining and CCE concept for enhancing the hemicelluloses removal from a softwood sulfite pulp. (a) Original pulp fiber sample, (b) sample after conventional CCE (8–10% NaOH) treatment, (c) sample after mechanical refining, which can open up the fiber wall, thus facilitating the subsequent alkali-induced swelling, (d) sample after combined mechanical refining and CCE (4% NaOH), the refining pretreatment allows a lower NaOH concentration in the CCE stage to have a similar hemicelluloses removal to that in a conventional CCE process, which, otherwise, would not be possible [233].

6-3- Enzymatic Extractions

Enzyme technologies using xylanase and mannanase is another method for selectively removing hemicelluloses after kraft pulping either during or after bleaching. The use of xylanase has been reported in different studies [151, 152, 153, 43]. It has been reported that up to 50% of the bleached pulp xylan could be removed by different xylanases from different origins [98, 154, 155, 156, 53]. Repeated enzymatic treatment [98, 156] and applying endoglucanase treatment with the aim of partial cellulose hydrolysis has been shown to improve the removal of hemicelluloses [53].

Besides, applying xylanase reduces the HexA content of the pulps [158, 159, 163, 175, 1, 43] for example by 10-20% in eucalyptus kraft pulp [158, 159, 163, 43]. In a comparison, it has

been shown that xylanase and acid treatments can reduce the HexA content of a eucalyptus kraft pulp by 15% and 61%, respectively [43].

Enzymatic extraction is often used as an improvement procedure in removal of hemicelluloses for softwood and hardwood dissolving pulp [164], for example in combination with cold caustic extraction [171, 237, 130, 233, 172, 173, 174, 227].

A modified Kraft cooking has been suggested as a combination of Kraft cooking and subsequent hemicellulose by various enzymatic treatments (endoglucanase and xylanase) in order to produce dissolving pulp [11] which resulted in narrower molecular mass distribution of final pulp compared to sulfite and PHK pulp [11].

6-3-1- Generalities about enzymes

Enzymes are very interesting for their selectivity in doing reaction. Indeed, they are catalyst that act on a certain bond and accelerate the reaction rate [261]. They have an active site in which the substrate enters, producing a complex along with the change of active site and then the products are released [52]. Figure 28 represents this theory of binding substrate to enzyme named as induced fit theory. Although the enzymes present advantages like selectivity of reactions, biodegradability, being environmentally friendly, they have some disadvantages over the classic chemical reactions. For instance, the reaction in which they can participate should be done under specific conditions like at mild temperature and pH [262] and this was and still is a big challenge for modern biotechnology to overcome, because many industrial processes are carried out under severe conditions.

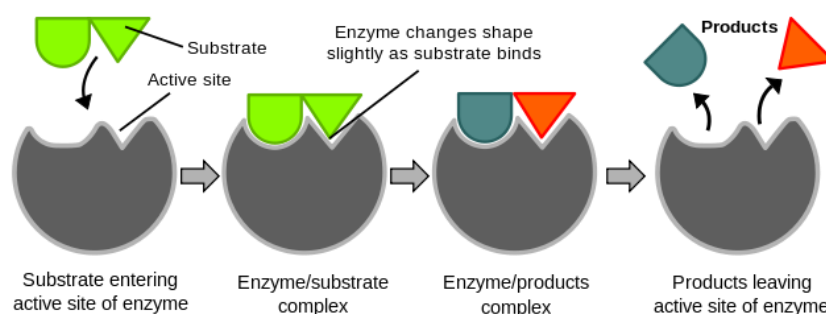


Figure 28- The mechanism of enzyme's function on substrate (induced fit theory).

The enzymes are protein and the proteins possess a complex three-dimensional molecular structure which is sensitive to severe conditions and if it is not respected they lose their three-dimensional structure and consequently their performance. Originally, they come from

nature and the nature often does not afford tough conditions like what is going on in the bodies of living creatures or various natural environments outside. Furthermore, enzymes can be hindered in the presence of chemicals like organic solvent [26] that makes them denatured [177, 31]. Considering these facts among many others, modern biotechnology has advanced extensively to overcome the main obstacles of applying enzymes industrially: low activity, selectivity and poor stability under the process conditions [25]; the factors that make the enzymes economically undesirable.

The initial factor in order to modify the properties of an enzyme is its thermostability characteristic which often is directly proportional to its tolerance against organic solvents [178, 179, 183], that is why improving the thermostability characteristics of an enzymes is in the center of attention, wanted by industries [187]. Current trend in engineering thermostability is a combination procedure with computational methods [20, 188, 190, 192, 193, 194, 195, 26]. For instance, thermostability of xylanase was significantly improved through these combined methods [196, 197, 198, 26].

Table 2- Enzymes used in textile and pulp and paper industries [20, 26].

Industry	Enzyme class	Application
Textile	Cellulase	Denim finishing, cotton softening
	Amylase	De-sizing
	Pectate lyase	Scouring
	Catalase	Bleach termination
	Laccase	Bleaching
	Peroxidase	Excess dye removal
Pulp and paper	Lipase	Pitch control, contaminant control
	Protease	Biofilm removal
	Amylase	Starch-coating, de-inking, drainage improvement
	Xylanase	Bleach boosting
	Ligninase	Bleach boosting
	Laccase	Bleaching
	Cellulase	De-inking, drainage improvement, fiber modification

Many industries are using enzymes like the food industry for the production of functional foods such as prebiotics, low calorie sweeteners, and rare sugars [26, 199], like the chemical industry for the production of detergent, the fuel and alcohol industries [27], agriculture and leathers industry [21], and also the pulp and paper industry. In the textile industry enzymes are used in the refining, bleaching, dyeing, and polishing [200, 26], staining of jeans [201, 26], drainage improvement [100], fiber modification [20], color improvement and surface

vividness, and resistance to wrinkles [202, 203, 26]. In the pulp and paper industry, they are used for degrading the pitch and contaminant control [20, 26], recycling of printed papers such as newspaper [205, 26], starch coating, de-inking, drainage improvement [20] or bleaching. But among all, proteases are the most used enzymes [20]. Table 2 represents some enzymes and some of their applications in textile and pulp and paper industries.

6-3-2- Enzymes in pulp and paper processes

Three types of enzymes, namely, cellulases, hemicellulases and ligninases, have been tested in the lignocellulosic biomass treatment process [206, 207]. Cellulases can be used to improve the performance of dissolving pulp [208] and reduce the energy consumption of pulp refining [209]. Hemicellulases are primarily used to assist pulp bleaching for the reduction of environmental pollution load [211, 213]. The major ligninases, such as laccase, can be used to treat the lignocellulosic biomass, to reduce the pollution due to the use of some bleaching chemicals [214, 215, 216, 2], but they are not used industrially.

Tables 3 and 4 present the effects of enzymatic treatments on the quality of dissolving pulp and on the pulp refining, respectively.

Table 3- Effects of enzymatic treatment on the quality of dissolving pulp [2].

Impacts of dissolving pulp quality	Enzymatic treatment	Effect of treatment
Reactivity of cellulose	Cellulase combined with mechanical, pulp fractionation and alkaline hydrogen peroxide treatment	Reactivity improved
Purity of cellulose	Xylanase	Hemicellulose removed
	Laccase	Lignin removed
Viscosity of cellulose	Cellulase	Viscosity reduced

Table 4- Effect of enzymatic treatments on the pulp refining [2].

Enzyme	Types of pulp	Effects
Cellulases	Combed cotton pulp	Energy consumption reduced by 26%, tearing index, tensile index, bursting strength and folding strength improved by 8.2, 11.2, 9 and 8.9%, respectively.
	Bleached pine kraft pulp	Energy consumption reduced by 28%.
	Wheat straw soda-AQ pulp	Beating speed reduced, folding strength and tearing index increased by 8.63% and 0.73%, respectively.
	Bleached softwood kraft pulp	Beating and tensile index degree increased, energy consumption decreased by 13%- 24%.

	Waste paper pulp	Water filtration improved, energy consumption reduced.
	Bleached softwood kraft pulp	Refining intensity reduced by 33%, fiber swelling significantly improved.
	Softwood sulfite pulp	Beating speed decreased by 50%.
Endoglucanase	Softwood kraft pulp	Beating time shortened by 20%, and tensile index, tearing index, bursting strength not changed greatly.
	Bleached hardwood kraft pulp	Beating time greatly shortened, energy consumption reduced, pulp physical properties slightly improved.
Endoglucanases, cellobiohydrolase and β-glucanase	Hardwood kraft pulp	Energy consumption reduced by 29%, tensile strength and bursting index increased by 21% and 19%, respectively.
Cellulases and pentosan enzymes	Bleached Eucalyptus sulfate pulp	Viscosity slightly decreased, tensile strength almost unaffected, filtration performance increased to 80%.
Endoglucanases, β-glucanase, xylanase and other hydrolase	Recycled waste paper	Energy consumption reduced by 8.5–14.8%, mechanical properties virtually unaffected.
Xylanase	Poplar alkaline hydrogen peroxide mechanical pulp	Energy consumption reduced by 12.5%-22%, breaking length, tearing index, bursting strength and folding resistance increased by 16.8%, 8.8%, 8.9% and 25%, respectively.
	Bleached birch kraft pulp	Beating degree and filtration performance improved, filtration time shortened by 15%.
	Conventional KP pulp	Beating property increased, tensile and tearing index increased by 20–25% and 8–10%, respectively.
Laccase	Waste corrugated pulp	Tensile strength increased by 15%, energy consumption saved.
	Eucalyptus sawdust pulp	Kappa number and mechanical properties significantly improved, refining energy reduced.

The objectives of applying hemicellulases in bleaching are the reduction of chlorinated bleaching agents and consequently of the adsorbable organic halogen (AOX) content of the effluents. Apart from environmental issues, another advantage of applying enzymes in the bleaching processes is that they are easily biodegradable. Enzymes treatments have been successfully used in conventional chlorine-containing sequences, such as (C/D) E D E D, in ECF, in TCF sequences, and also in sequences where oxygen, peroxide, and ozone are applied [261].

In 1987, the idea of applying endo-beta-xylanases (xylanases) for treating unbleached kraft pulps was claimed. Previous non-bleaching uses of endo-beta-xylanases had been reported in

the early 1970s and included the removal of hemicelluloses from chemical pulps to produce dissolving pulps. The first mill trials were conducted in 1988 [261].

There are two main hypotheses for the mechanisms of xylanase performance in bleaching. One of the predominant hypotheses is that these enzymes catalyze the hydrolysis of reprecipitated xylan on the surface of the pulp fibers making the lignin fragments in and on the fiber easier to remove in the following bleaching stages. Another hypothesis is that xylanases, by catalyzing the depolymerization of xylan in the cell walls, allow entrapped lignin to diffuse more easily out of the fibers [261].

Most of the mills activities has been focused on softwood use because of its higher chemical requirements in bleaching. In general, hardwood hemicelluloses are more responsive to xylanase enzymes than those in softwood. The percentage of savings in bleaching chemicals is therefore greater for hardwood than for softwood but the overall economic benefit is less [261]. It should be mentioned that different kinds of tree of a certain type like hardwood or softwood response rather differently to the same enzyme. For example, aspen shows 30% more response to enzymes than many other hardwood pulp species. But among softwood pulp species, less variation in response to enzyme treatment is observed. Furthermore, mill experience has shown that day-to-day variation in the extent of brown stock washing has little impact on enzyme performance [261].

No all xylanase mill trials have been fully successful, though such findings are not often reported in the literature. If the enzyme is not well-mixed with the pulp or if the pH and temperature fall outside of the optimum range for the enzyme during a considerable part of its residence time on pulp, the bleaching effect will be diminished [261].

6-3-3- Hemicellulases: types and structures

Due to the complexity of the original structure of hemicelluloses several hemicellulases are needed for their enzymatic degradation and modification. The classification of all these hemicellulases are based on the substrate they act upon, the bonds they attack and the products they release [23, 24] (Table 6). The two main enzymes in order to depolymerize the hemicellulose backbone are endo-1,4- β -D-xylanase (EC 3.2.1.8) and endo-1,4- β -D-mannanase (EC 3.2.1.78). Small oligomers which have been released by these two enzymes can be further depolymerized by 1,4- β -D-xylosidase, 1,4- β -D-mannosidase and 1,4- β -D-glucosidase to

produce monomer sugars. Removing the side groups needs the use of other enzymes like α -L-arabinosidase, α -D-glucuronidase and α -D-galactosidase. Besides, acetyl xylan esterase, ferulic esterase, *p*-coumaric esterase and acetyl galactoglucomannan esterase hydrolyze the esterified side-groups. These accessory enzymes should work synergistically in order to be able to hydrolyze totally the crude wood xylans and mannans to xylose and mannose [116, 24, 66, 28, 68, 167, 185, 22].

Table 5- Different hemicellulases [23].

EC Number	Recommended name	Systematic name
3.2.1.8	Endo-1,4- β -xylanase	1,4- β -D-Xylan xylanohydrolase
3.2.1.37	Xylan 1,4- β -xylosidase	1,4- β -D-Xylan xylohydrolase
3.2.1.32	Xylan endo-1,3- β -xylosidase	1,3- β -D-Xylan xylanohydrolase
3.2.1.72	Xylan 1,3- β -xylosidase	1,3- β -D-Xylan xylohydrolase
3.2.1.55	α -L-Arabinofuranosidase	α -L-Arabinofuranoside arabinofuranosidase
3.2.1.99	Arabinan endo-1,5- α -L-Arabinosidase	1,5- α -L-Arabinan 1,5- α -L-arabinohydrolase
3.2.1.78	Mannan endo-1,4- β -mannosidase	1,4- β -D-Mannan mannanohydrolase
3.2.1.100	Mannan 1,4- β -mannobiosidase	1,4- β -D-Mannan mannobiohydrolase
3.2.1.101	Mannan endo-1,6- β -mannosidase	1,6- β -D-Mannan mannanhydrolase

6-3-3-1- Xylanase

Two main enzymes are needed to degrade xylans' backbone: endo-1,4- β -xylanase which transforms the xylan chain to xylan oligosaccharides [22, 28, 68, 18, 167] and 1,4- β -xylosidase which acts on the released oligomers to produce xylose, from the non-reducing end [22, 28, 68]. In the case of xylanase, the rate of hydrolysis reaction becomes smaller with the decrease of the chain length of oligomeric substrates. The main produced oligomers are xylobiose, xylotriose and substituted oligomers of two to four xylosyl units in which at least xylotriose has been reported to inhibit the action of xylanases [24]. The action of enzymes on specific bonds depends on the overall structure of the molecules as well. For example, some endo-xylanases act better upon linear molecules and some others act better with molecules containing side chains [23, 24]. Consequently, the chain length, the degree of branching and the nature of the side groups are influential. Therefore, applying different types of xylanases increases the chance of better performance of the enzyme solution [18]. The production of a multi-enzyme system of xylanases is one strategy for a microorganism to achieve effective hydrolysis of xylan and this multiplicity goes back to the heterogeneous nature of the xylan polymer. For example, *Aspergillus niger* releases five endo-xylanases and plant pathogen *Cochliobolus carbonum* has three different endo-xylanases [77, 78]. The hydrolysis of xylan by xylanase may be written as follows [115]:

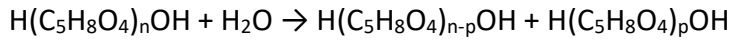


Figure 29 represents how different hemicellulases act upon xylan.

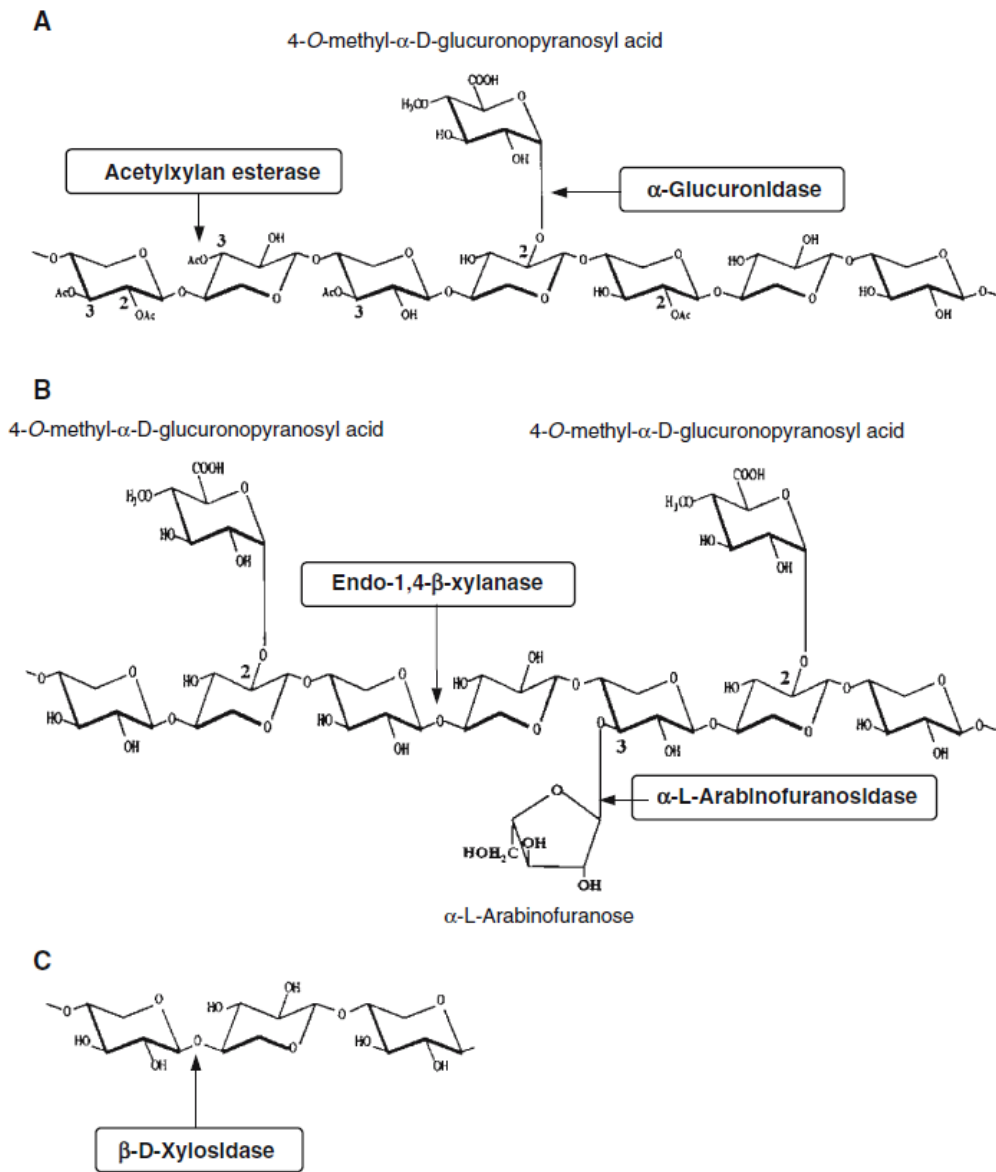


Figure 29- Structure of the O-acetyl-4-O-methylglucuronoxylan (a), of hardwood and of the arabino-4-O-methylglucuronoxylan (b), of softwood. Xylanolytic enzymes involved in the degradation of the xylan: acetylxytan esterase, α -glucuronidase, endoxylanase and α -L-arabinofuranosidase. Hydrolysis realized by β -xylosidase (c). The numbers indicate carbon atoms to which group substitutions are bound. Ac Acetyl group [115].

Importantly, it is very essential that the enzyme preparation is completely free of cellulase side activity. Any cellulase activity will degrade the cellulose chain and that means loss of fiber strength and loss of finished products' quality.

A variety of microorganisms are able to produce xylanases. The strains reported to be used for commercial production of xylanases include *Trichoderma reesei*, *Thermomyces*

lanuginosus, *Aureobasidium pullulans* and *Streptomyces lividans* [24]. Several purified xylanases are rather small (molecular mass around 20 kDa), whereas other xylanases have higher molecular masses [18, 78, 28, 51, 185]. The size of the enzyme is a factor showing whether the enzymes can pass through the pores to be able to access their desired substrates or not [78]. In contrary to xylanase, xylosidase have high molecular weight which is generally between 60 and 360 kDa [115].

Acetylation makes the xylans hydrophobic and prevents the formation of intrachain hydrogen bonds. Deacetylation in the kraft process results in formation of hydrogen bonding which leads to xylan precipitation and although it is less soluble in water, this precipitation increases the availability of xylans to enzymes [23].

Although the structure of xylan is more complex than cellulose and requires several different enzymes with different specificities for complete hydrolysis, the polysaccharide does not form tightly packed crystalline structures like cellulose and is, thus, more accessible to enzymatic hydrolysis [68].

Many of the studied xylanases are of fungal or bacterial origin and they are mostly active at, or near, mesophilic temperatures (approximately 40–60°C) [51, 22] and neutral (in particular for bacterial xylanases) or slightly acidic (in particular for fungal xylanases) pH values. However, scientists have found some xylanases which are active under extreme conditions regarding temperature and pH. Indeed, xylanases active at temperatures ranging from 5 to 105°C, pH from 2 to 11 and NaCl concentrations as high as 30% have been reported [51]. The scientists all over the world are working on discovering and isolating thermophilic and even extremophilic microorganisms: indeed, this type of enzyme could be interesting to be used in industrial processes using harsh pH or temperature conditions. A number of thermophilic (optimal growth at 50–80°C) and hyperthermophilic (optimal growth at >80°C) xylanases producing micro-organisms have been isolated from a variety of sources, including terrestrial and marine solfataric fields, thermal springs, hot pools and self-heating decaying organic debris [51]. Fungal xylanases are generally less thermostable than bacterial xylanases [22].

Apart from temperature, the optimum pH condition of enzymes should be used. Alkaline xylanases are important due to their applications in pulp and kraft bleaching [185]. While the majority of natural environments on earth are essentially neutral, with pH values between 5 and 9, habitats with extreme pHs are also common, in particular in geothermal regions,

carbonate laden soils, soda deserts and soda lakes such as found in Egypt (Wadi Natrun), the African Rift valley (Lakes Magadi and Nakuru in Kenya), Central Asia, Western USA (Yellowstone National Park) and Southern Europe (Vulcano Island, Italy). Indeed, xylanase produced alkaliphilic micro-organisms, which typically grow optimally at pH values above 9, have been isolated from these environments and also from mediums like pulp and paper industry wastes, decomposing organic matter, faeces, plant sources, soils and even from neutral environments where they are found coexisting with neutrophilic micro-organisms [51]. The optimum pH for xylan hydrolysis is around five for most fungal xylanases, and they are normally stable between pH values of two and nine [18]. The pH optima of bacterial xylanases are generally slightly higher than the pH optima of fungal xylanases [18, 24].

6-3-3-2- Mannanase

Like xylans, the complete hydrolysis of mannans needs the synergistic action of endo-1,4- β -mannanases (E.C 3.2.1.78) and β -mannosidases (E.C 3.2.1.25) and other enzymes such as β -glucosidases (EC 3.2.1.21), α -galactosidases (EC 3.2.1.22) and acetyl mannan esterases [60]. The main products obtained during the hydrolysis of mannans by β -mannanases are mannobiose and mannotriose. The fundamental theories and general facts are almost the same as with the use of xylanase and xylosidase on xylans and mannans. However, reprecipitation of mannan has not been reported to take place to the same extent as xylans [116]. Mannanases have been described less than xylanases [22]. Figure 30 represents how different mannanases act upon a typical mannan.

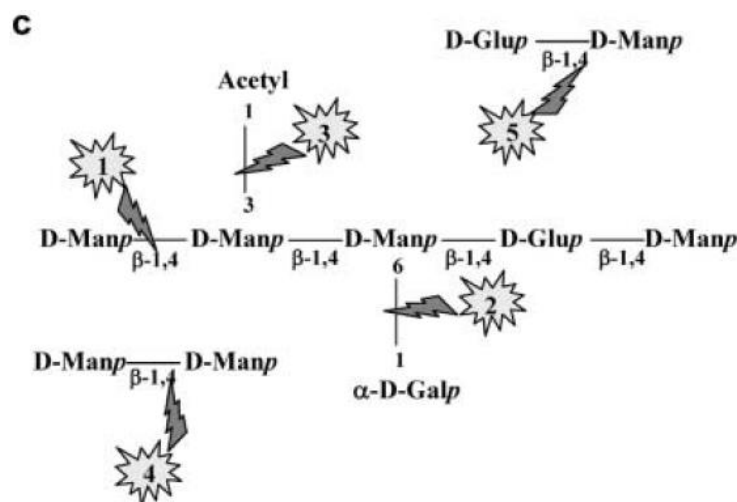


Figure 30- Enzymatic hydrolysis of glucomannan. 1) Endo-mannanase, 2) α -galactosidase, 3) acetylglucosidase, 4) β -mannosidase, 5) β -glucosidase [22].

Hydrolysis of galactoglucomannans by mannanases, depends on their molecular structures and action patterns of the enzymes [274, 275, 276], and can release a various range of oligomers including the oligomers of β -D-mannans and a mixture of oligomers containing D-mannose, D-glucose and D-galactose.

Although a number of mannanase-producing bacterial sources are available, only a few are commercially exploited as wild or recombinant strains: of these, the important ones are *Bacillus sp.*, *Streptomyces*, *Caldibacillus cellulovorans*, *Caldicellulosiruptor Rt8B*, *Caldocellum saccharolyticum* [60].

6-3-4- Different uses of hemicelluloses on pulps

Hemicellulases have been shown to be interesting as bleaching boosting agent, and could potentially help in the production of dissolving pulp by removing the residual hemicelluloses or in the modification of the fiber and their properties. The first one has been industrialized although many researches are being done to increase the performance and operational parameters and the latter two are mostly in the phase of research and development and the results show a promising future.

6-3-4-1- Hemicellulases in bio-bleaching

Different research groups tested different xylanases to compare their performance. A synthesis is given in table 6. Rafael Vicuna et al. used four commercially xylanases namely Cartazyme from Sandoz, Ecopulp from Alko-ICI, Irgazyme from Ciba-Genencor and Pulpzyme HB from Novo Nordisk prior various bleaching sequences: they could save 3.5-3.8 kg per ADT (air-dried tons) of chlorine dioxide without affecting the target brightness of the pulps, and they observed a similar performance for all enzymes [19]. The same result was obtained when they tested almost the same set of enzymes, namely Ecopulp, Cartazyme NS-10 and Pulpzyme HC from Alko-ICI, Clariant and Novo Nordisk respectively, and they observed the same ability for all enzymes. They could save 12 to 40% chlorine dioxide without affecting the target brightness of 90% ISO [19].

Among the processes using xylanase in the bleaching sequence, there is a process named as EnZone process which is a TCF bleaching sequence and had been successfully tested on kraft pulps by Yang et al., 1993, 1994 [30]. Rafael Vicuna et al [30] used of this process in their kraft pulp while comparing four enzymes, namely Cartazyme from Sandoz, Ecopulp from Alko-ICI,

Irgazyme 40 from Ciba-Genencor and Pulpzyme HB from Novo Nordisk, all in various TCF bleaching sequences but they could not obtain the same results as Yang. However, they could reach a brightness of 90% ISO in one of the bleaching sequences, although in the other bleaching sequence no effect of enzymes was observed.

Corina Popovici et al [82] observed a reduction of 10.9% in Total Kappa Factor (TKf), 8% for the first xylanase period and 16% for the second xylanase operation, as a result of xylanase treatment, while maintaining the final product properties and an increase in the feed kappa number of 0.8 units, a decrease in steam consumption of 0.21 GJ/adt and a boost in maximum bleach production of 10 adt/day without any measurable impact on effluent biological oxygen demand or aliphatic organic compounds [82].

Digvijay Verma et al [87] successfully cloned a metagenomic xylanase gene (Mxyl) which was expressed in *Bacillus subtilis* extracellularly that simplifies downstream processing and checked the bleaching ability of the released xylanase. In the pulps which were treated by this recombinant enzyme, they observed a reduction in the kappa number while an increase in brightness and 24% reduction in chlorine consumption [87]. Also, J. Haarhoff et al [34] evaluated the bio-bleaching effect of hemicellulases produced by thermophilic fungi on a eucalyptus kraft pulp and after XECEDED bleaching sequence they observed a 10.5% reduction in Kappa number and an increase in brightness of 1.1% [34].

A.L. Torres et al [41] evaluated a novel xylanase obtained from the isolated *Bacillus sp.* strain BP-23 which was active enough at high pH (72% and 35% activity at pH 9.5 and 11.0, respectively) on the production of a bleached pulp with 89% ISO without loss in viscosity. They could save 30% of chlorine dioxide consumption and 1% more brightness in the fixed bleaching chemicals consumption. In the evaluation of fiber morphology after enzymatic treatment they observed cracks, flakes, filaments and peeling of the fiber cell wall on the surface of the fibers which justified them as the functionality of the enzyme which have found their way into the fiber to access their substrates [41].

Apart from facilitating lignin removal during bleaching, xylanase is helpful in the bleaching steps by removing the hexuronic acids (HexA) which adversely affect brightness stability [157]. Based on the practical research performed to investigate this hypothesis, it seems that xylanases reduce the HexA content in pulps [158, 159, 163, 175, 1]. In this regard Cristina Valls et al. observed that a commercial xylanase increased the HexA removal by 15% with respect

to the control treatment, whereas only a 2.5% increase in HexA removal was found for the new laboratory-developed xylanase [83]. However, in the study of Qijun Meng et al. [157] it was observed that hot acid pretreatment was able to remove four-times more hexuronic acid than xylanase pretreatment on unbleached eucalyptus kraft pulp which is the most popular pulp in pulp and paper industry containing an extended amount of hexuronic acid, although this high reduction was with the cost of notable reduction in viscosity of the pulp and its strength [157].

In addition, theoretically, Roncero proved that xylanases reduce the HexA content because they are able to hydrolyze hemicellulose-containing hexuronic groups [157, 158].

Table 6- Different xylanases declared in different resources with potential to be used as an aid in pulp bleaching.

Name	T	pH	Some information
Cartazyme HS-10	50 °C [160]	4.8 [160]	Resulted in a 31% reduction in chlorine consumption and a 30% reduction in total organic chlorine content in the extraction stage effluent, with an increase in the brightness, tensile strength and burst factor by 3%, 26% and 32% [160].
	35-55°C [180]	3.0-5.0 [180]	Pulp bleaching [180]
	45-55 [161]	4.5-5 [161]	Pulp bleaching [161]
	30-50 [263]	3-5 [263]	Bio-bleaching [263]
	35-55 [108]	3-5 [108]	- In comparison with Novozyme 473 and VAI Xylanase, maximum brightening effect was noted with Cartazyme HS-10 [108]. - Free of cellulase side activity [108].
	40-55 [16]	4-5 [16]	
			- Has β -Glucuronidase activity of 0.071 mU/ml [181]. - Declared activity: 10.000 U/g [180].
Cartazyme NS-10	~ 55 [29]	6–10 [29]	- Performance similar to Pulpzyme HC and Ecopulp [29]. - 0.5 mg of enzyme per gram of pulp [29]. - a significant decrease in the consumption of both ClO_2 and H_2O_2 [29].
			- Bio-bleaching [161] - Decreased kappa number from 10.5 to 9.4, increased brightness from 37.3 to 41.1% ISO, but decreased viscosity from 40.4 to 39.7 cP. [161]. - In comparison with Pulpzyme: NS-10 charge (10.7 mg protein/kg o.d. pulp) was lower than Pulpzyme HC (46 mg protein/kg o.d. pulp) [161]. - In comparison with Pulpzyme: for Pulpzyme the viscosity after treatment was constant, but Cartazyme NS-10 decreased the viscosity. [161]
Cartazyme HT	60-80 [264]	7-9 [264]	Pulp Bleaching [264]
	60-70 [263]	5-8 [263]	Pulp Bleaching [264]
Cartazyme PS-10			pulp bleaching [62]

			- Performed best on <i>P. pinaster</i> kraft pulp and could improve the bleaching capacity and some papermaking properties [62].
	40-65 [160]	6.5-9.5 [160]	- High xylanase activity [160] - Minimum cellulase activity [160]
Cartazyme MCX-A			a mixture of cellulase, activity 1.76 IU/g and xylanase, activity 0.001 IU/g
Cartazyme SR-10			Pulp bleaching [182]
Cartazyme 9407			Pulp bleaching [77]
Cartazyme MP			Pulp bleaching [77]
Irgazyme 40-4X		6-7 [162]	- Between Irgazyme 40-4X and pulpzyme HB and pulpzyme HC, Irgazyme 40-4X had the most concentrated xylanase activity (0.358 nkat/nl), followed by Pulpzyme HB (0.259 nkat/nl) and pulpzyme HC (0.095 knat/nl). Also it led to higher brightness. And it was the most successful bleach booster [162]. - It has negligible cellulase and mannanase activity [162]. - It is not a pure xylanase enzyme. It probably contains other enzymes which boost bleaching [162].
			Pre-bleaching/fiber modification [184]
	35-70 [263]	6-8 [263]	
	50-60 [263]	7-8 [273]	
Irgazyme 10A			Pulp bleaching [77]
VAI xylanase	65-75 [263]	6-7.5 [263]	
	65 [266]	6.5 [266]	- Can be stored for weeks without any loss of activity [266]. - Bleaching aid of kraft pulp [266].
	50-60 [16]	5-7 [16]	
	high T [268]	neutral pH [268]	The first commercial preparation from thermophilic mold <i>T. lanuginosus</i> (<i>Termomyces lanuginosus</i>) (a thermophilic fungus which does not secrete any cellulases [108]) which induces a 2.5 brightness point increase or a 31% decrease in chlorine consumption [268].
			Free of cellulase side activity [108]
Pulpzyme HA	below 55	up to pH 7	- First fungal xylanase [267]. - It's difficult to adjust the mill conditions [267]. - Minor amounts of cellulolytic activity [267].
	50-55 [263]	6-8 [263]	
		4-5	- A certain amount of cellulase activity [269]. - The activity at pH 7 is only 40% of the optimum [269]. - Can have very undesirable effects on pulp qualities such as pulp strength [269]. - Rapidly inactivated above pH 7-8 [269].
			- A mixture of xylanase (500 XYU/g) and cellulase (about 300 EGU/g) [270]. - Chlorine sensitive [270].
			Pulpzyme HA is acidic and Pulpzyme HC has a broad pH range [165].
			- Presence of endoglucanases and glucosidase [166]. - 21 kDa xylanase of <i>T. reesei</i> [166].

			-The first commercially available xylanase for use in bio-bleaching of wood pulps [167].
Pulpzyme HB	up to 55 [266]	neutral pH [266]	- Cellulase-free [266] - Bacterial origin [266]
	up to 55 [265]	neutral pH [265]	- Free of cellulase activity [265].
Pulpzyme HC		9- 9.5 [168]	Significant decrease in consumption of ClO ₂ and H ₂ O ₂ [168].
	40-65 [267]	6.5-9.5 [267]	Alkaline, thermostable xylanase [267]
			Produced by genetically modified species [169]
			Having negligible contaminating cellulase [170]
			Gives oligomers with DP in the range 4-5 or 6-7 as major reaction products [271].
Bleachzyme F	45-50 C [273]	6-6.5 [273]	
ECOPULP			Pulp bleaching
			- Thermostable xylanase [272] - Suitable for solubilizing and partial depolymerizing of the arabinoxylans [272].
			Specially designed to improve the bleachability of woody kraft pulps at high temperature [175].
			Ecopulp TX200A released the highest amount of reducing sugars (24.8 g/l glucose equivalent) after 1 hour hydrolysis [176].
Multifect XL	55-60	5-5.5	Pulp bleaching
			produced through recombinant expression of some species [272].
	50	4.8-5.2	- Derived from a genetically modified strain [189]. - Molecular weight: 21 kDa approximately [189] - Low cellulose side activity [189]
Optipulp L-8000	55 [263]	6.5 [263]	Pulp bleaching [263]
Ecozyme			Pulp bleaching [77]
Sumizyme X	55	5.5	Pulp Bleaching

As a summary, bio-bleaching is the main application of hemicellulases in practice so far, applying them as bleach boosting agents.

6-3-4-2- Hemicellulases on sulfite pulp

The mechanism of bio-bleaching of sulfite pulps with xylanase differs from that of kraft pulps [45, 217]. L.P. Christov et al [113] examined the ability of a crude enzyme preparation of *Aureobasidium pullulans*, containing xylanase and xylosidase (ratio of xylanase to xylosidase activity 20: 1), in removing pentosans from sulfite dissolving-grade pulps. They observed at the maximum 31% removal of pentosans, and the main released product was xylose due to the presence of xylosidase in the enzyme preparation [113]. They also applied a purified xylanase as well as acetyl xylan esterases on sulfite dissolving pulp as a pre-treatment to a single-stage hydrogen peroxide delignification. They obtained similar results as before and

observed that the enzymes hydrolyzed only a limited portion (less than 30%) of the acetylglucuronoxylan in the pulp. They observed different kinds of oligomers released from different xylanases from different families. They also concluded that acetyl xylan esterases do not play an important role in bio-bleaching of eucalypt sulfite dissolving pulps because they did not observe an additional brightness obtained when endo-xylanases were used in conjunction with acetyl xylan esterases [45].

In another research on sulfite pulp, they examined pretreatment with two xylanases; one from *C. subvermispota* and other *A. pullulans*. They observed improved alkali solubility and brightness, although the former negatively affected the cellulose content of dissolving pulp and a combination of both resulted in saving of 63% in active chlorine consumption and increased the brightness up to 93% ISO [71].

In addition, G.M Gübitz et al [53] evaluated the performance of different xylanases, mannanases and endoglucanases from seven different microorganisms on a softwood sulfite dissolving pulp. They could find an optimum combination of enzymes which worked synergistically the best, considering this observation that using endoglucanase increased solubilization of hemicelluloses by 21% for xylan and 9% for mannan [53]. The endoglucanase treatment probably caused a partial hydrolysis and loosening of the cellulose structure thereby increasing the hemicellulose accessibility to the synergistic action of xylanase and mannanase on pulp [45]. They explained this might show that in a sulfite pulp, certain xyans and mannans are in a close association with each other. Interestingly, the effect of enzymes' combination in solubilization of mannans was better than xylan comparing with the use of single enzymes, which can suggest that mannan in the bleached pulp might be shielded or closely associated with the xylan. They could solubilize between 20 and 26% of the xylan present in the original bleached pulp with xylanase or mannanase and between 8 and 20% of mannan [53].

Up to 50% of the xylan present in bleached pulp could be removed by xylanases from fungi such as *Aureobasidium pullulans* [98], *Schizophyllum commune* [154], *Saccharomonospora viridis* [155] and *Trichoderma harzianum* [53, 156]. Gamerith and Strutzenberger suggested that the remaining xyans in sulphite pulps are structurally like the native xylan in the wood but with less substituents. So, it is probable that sulfite pulps need a more complete range of hemicellulases to tackle with the solubilization of hemicelluloses as much as possible.

L. P. Christov et al [98] studied the bleaching effect of repeated treatments with xylanase and alkali or oxygen on unbleached sulphite pulps in order to produce dissolving pulp. They observed in the sequence of XOXOXO (triple-repeated xylanase and oxygen treatments), a substantial improvement: K number by 60%, total lignin content by 29%, and brightness by 18 points [98].

Table 7 summarizes some results obtained from applying hemicellulases on sulfite pulp.

To conclude, it seems that there is less interest in applying enzymes on a sulfite pulp than on a kraft pulp. The remained hemicelluloses in the kraft pulps have been modified significantly by cooking, which seems to facilitate the action of enzymes.

Table 7- Applying hemicellulases on sulfite pulp.

Enzyme	Results
Xylanase Xylosidase [113]	31% removal of xylans. No effect while using acetyl xylan esterase. Release of xylose.
Different xylanases [71]	Improved alkali solubility. Improved brightness up to 93% ISO. Saving 63% of active chlorine consumption.
Different xylanases Mannanase Endoglucanase [53]	Increasing solubilization of xylans by 21% and mannans by 9%. solubilizing of 20 and 26% of the xylan with xylanase or mannanase and between 8 and 20% of mannan.
repeated treatments with xylanase [98]	Improvement of brightness by 18 points

6-3-4-3- Hemicellulases in order to produce dissolving pulp

In the process of dissolving pulp production, the major points are the purity and the reactivity of the pulp. So, applying endoglucanase in order to increase the reactivity of the pulp and improve its morphology and xylanase in order to decrease the remained content of hemicelluloses in the pulp has been investigated [2]. Endoglucanase (EG) has been used by some researchers in order to increase the reactivity of the pulp and adjust its viscosity [11, 218, 219, 220, 221, 222]. After kraft cooking there is still some alkali-resistant hemicelluloses in the pulp [118, 46], which decrease the pulp reactivity in the viscose process. The parameters influencing the reactivity of the cellulose are the purity, the fiber accessibility and morphological parameters such as length, crystallinity, porosity, degree of polymerization and adsorption properties of cellulose [2]. A combination of enzymatic pretreatment and alkali extraction post-treatment is of interest [11].

Dissolving pulps are almost pure cellulose of high brightness and uniform molecular weight distribution but they can still contain some traces of hemicelluloses, lignin and extractives [11, 36, 223]. Excessive amounts of hemicellulose (> 10% [36]) in dissolving pulp can seriously affect the swelling of the pulp in mercerization, the viscose filterability and drainage of sodium hydroxide during steeping, the completion of the reactions during xanthation and the strength properties of the viscose end product [223]. Therefore, a complete removal of residual hemicelluloses from dissolving pulp would facilitate its processing and improve the quality of the final product [45].

Dissolving pulps are used for production of viscose, cellulose ethers and esters and the products like textiles, plastics, cellophane packaging, sponges, sausage castings, photographic films and filter cigarettes [53, 224]. Figure 31 represents different applications of dissolving pulps. They are produced usually by the acid sulfite cooking or pre-hydrolysis kraft (PHK), among which the sulfite process is today dominant.

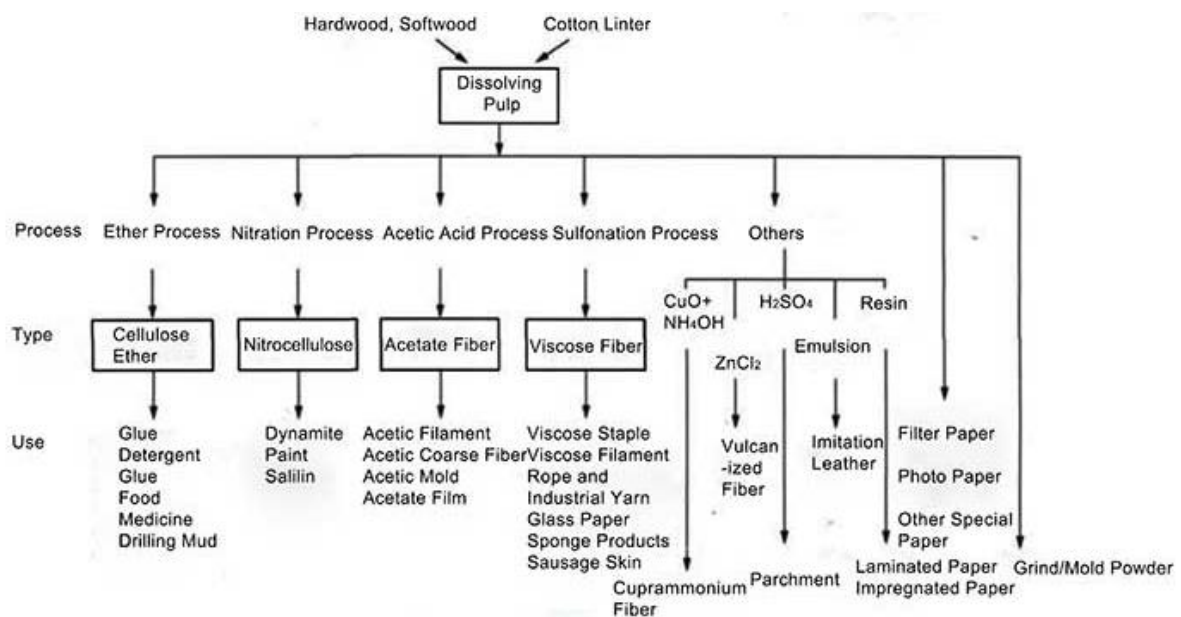


Figure 31- After-processing methods and use of dissolving pulp [14].

In this regards, Verena Gehmayr et al. investigated the effects of various enzymatic treatments on a commercial oxygen-delignified *Eucalyptus globulus* paper-grade kraft pulp in a totally chlorine free bleaching sequence in combination with refining techniques with the double objective of reduction of chemical consumption in alkaline and ozone bleaching steps, by means of xylanase pretreatment (X), and of the adjustment of final pulp viscosity utilizing endoglucanase post-treatment (EG), using the following sequence, (X)-CCE-A-ZP-EG, for the

production of a novel high-purity dissolving pulp [11]. They applied Pulpzyme HC as xylanase. They could remove 46% of the initial xylan and the brightness of pulp increased by 8.6% ISO with an observation of higher reactivity of the pulp towards xanthation [11].

In addition, Viviana Köpcke et al [36] applied a commercial xylanase (Pulpzyme HC) on ECF-bleached hardwood kraft pulps and obtained the solubilization of 37-47% of the initial xylan in the pulps, dependent on the type of the wood. In order to improve the reactivity and viscosity of the cellulose in the next steps, they applied endoglucanase. Furthermore, the endoglucanase inhibited the hornification effect produced by the removal of hemicelluloses. The most optimum sequence in order to produce dissolving pulp for eucalyptus was xylanase-CCE-endoglucanase.

Verena Gehmayr et al. concluded that although the reduced alkalinity of CCE step (70 g NaOH L⁻¹ compared to 100 g NaOH L⁻¹) was adequate to reach the target residual xylan of the pulp, the treated pulps showed a broader molecular weight distribution and the xylan recovered from CCE-lye was highly degraded compared to the pulp extracted at an elevated alkalinity of 100 g NaOH L⁻¹. Thus, they recommended xylanase pre-treatment just to be applied when the xylan fraction in the caustic lye is not further needed as polymeric reusable material. With xylanase, they obtained a saving in the consumption of chemicals in the next steps and with endoglucanase they obtained a pulp with adjusted viscosity proper for reactivity of fibers in the viscose production. At the end they recommended the (X)-CCE-A-ZP-EG sequence to produce dissolving pulp from kraft pulp [11]. Figure 32 represents the concept that they used in their studies, named as Hem-Extra-process.

They could solubilize 46% of the initial xylan with xylanase treatment (the xylan content went from 22.5% to 12.1%), and observed that the molecular weight of the remained xylyans in the pulp decreased substantially [40]. The combination of xylanase pretreatment and subsequent CCE at reduced alkalinity could decrease the xylan content to 4.9% [40].

The same sequence has been used by Heléne Almlöf Ambjörnsson et al [9] on kraft pulp in order to produce dissolving pulp, but they used NaOH-ZnO. Their results indicated that although the crystallinity and the specific surface area of the dissolving pulp did not change during the enzymatic pre-treatment, the solubility of cellulose in NaOH/ZnO solution increased from 29% for the original pulp fibers to 81% for the enzymatic treated fibers with minimal effect on the degree of polymerization. Furthermore, although the xylan and

glucomannan content in the dissolving pulp was very low, xylanase could reduce the xylan content by 23%, and the glucomannan content by 7%. Strangely while the xylanase dosage was increased the final extent of dissolution decreased and cellulose was also degraded [9].

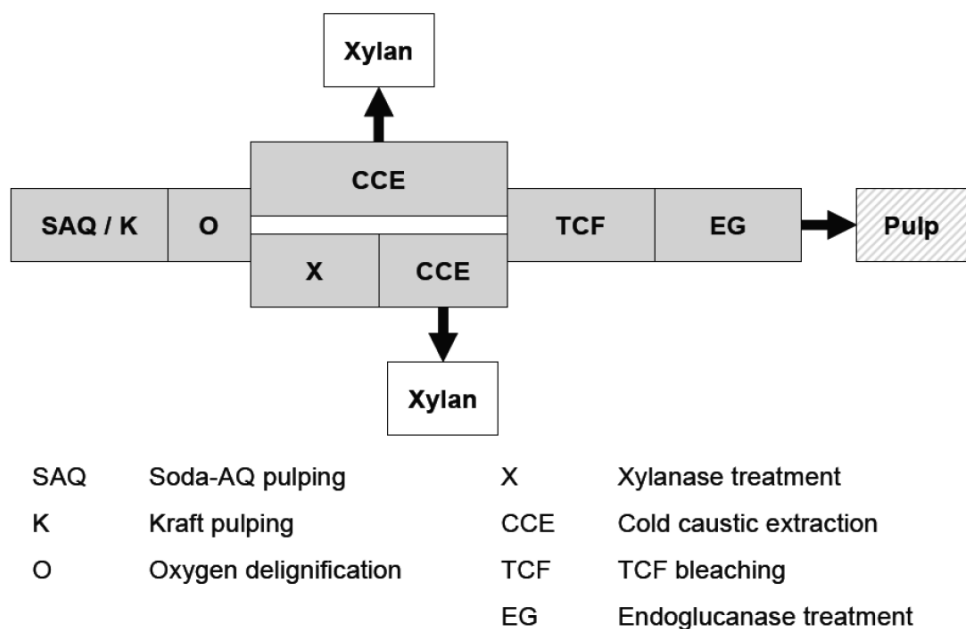


Figure 32- Concept of the Hem-Extra-process [40].

In summary, production of dissolving pulp from kraft pulp with the help of hemicellulases pre-treatment has been investigated by some research groups. Regarding the main issues of such a conversion, namely the reactivity of cellulose and the hemicelluloses content, a post-treatment with endoglucanase (after CCE) with the aim of increasing the reactivity and adjusting the viscosity, could produce a pulp almost with the same quality as dissolving pulp. Although this pre-treatment gave different results depending on some parameters like the origin of the pulps, it seems that with adding the benefit of valorizing the hemicelluloses in between, such a procedure is worth being investigated more extensively regarding pulp origins, types of enzymes and operational parameters along with downstream processing.

6-3-5- Hypotheses for the resistance of hemicelluloses present in pulp to be removed by enzymes

In the process of bleaching of kraft pulp, the enzymatic pre-treatment solubilizes only 1-2% of dry weight, or around 10% of pulp xylan. In softwood treatments, the solubilization is even lower [64, 225, 228]. In another study it has been observed that the enzymes hydrolyzed only a limited portion (less than 30%) of the acetyl-glucuronoxylan present in the pulp [45]. It has been reported that the degree of pentosan removal was limited to 31% [113] and further

higher enzyme dose and prolonged incubation did not enhance hemicellulose hydrolysis significantly [87, 53].

There are some hypotheses explaining the reason why not all hemicelluloses can be removed by the enzymatic hydrolysis, even though the enzymes possess the characteristics with which theoretically it should be possible. Christov and Prior (1993, 1996) suppose that it could be due to partial inaccessibility of the substrate due to various reasons such as enzyme size [229], fiber porosity [230], median pore size [231], and accessible surface area of pulp [45, 234]. They justified the limited removal of hemicelluloses by the fact that their enzymes' size was approximately 50 kDa and penetration into the inner layers of secondary cell wall could be the main problem [116]. It was estimated that pores with a size of about 40–50 Å or greater would be enzymatically accessible [45, 234, 235]. They explained that as xylan in hardwoods is dispersed in larger pores than in softwoods [235] and that during pulping macro-pores are created [236, 229], the enzymes can more easily penetrate into the layers of cell wall. The porosity of sulfite hardwood pulp would be greater than that of kraft, however the extraction of xylans with enzymes is limited. However, the term of porosity is a property that can differ largely from tree to tree even in one type of treated pulp [238]. Therefore, the uneven pore size distribution throughout pulp fibers might be a factor restricting the complete removal of hemicelluloses from dissolving pulp [45, 53]. Regarding kraft pulp it has been reported that pulping process increases the average pore size with the removal of lignin and hemicelluloses, but still the median pore width in kraft pulp fibers is in the range of 40-50 Å [64, 97].

Inaccessibility has been mentioned as the main reason by many researchers [64]. G.M Gübitz et al [53] while working on sulfite pulp studied the combination of xylanases, mannanases and endoglucanases, and observed that even in the best combination, there is a limitation in removal of hemicelluloses. The fact that the enzymes could extract more hemicelluloses from unbleached pulp than from bleached pulp strengthened their hypothesis suggesting that hemicelluloses in the bleached pulps are less accessible compared to the unbleached pulps, due to rearrangement of the molecular structure of hemicelluloses [53]. The same results were obtained [53, 154, 239] by other research groups suggesting that the residual xylan remaining after bleaching might be either chemically modified to restrict enzymes action or, more probably, the more accessible hemicellulose portion had already been removed during bleaching or extraction. However, a reverse result was obtained from a kraft pulp in which it

was found that five times more xylan (54%) was solubilized from a bleached kraft pulp than from an unbleached pulp by a xylanase preparation treatment [53, 156].

In kraft pulping, also, due to rearrangement of the molecular structures and changes in composition and localization of hemicelluloses, the existence of intermolecular hydrogen bonding in precipitated hemicelluloses on the surface of kraft fibers could make this reconfigured structure more resistant against enzymatic hydrolysis. Also, some hemicelluloses are located inside the fibers and hardly accessible [64].

Anne Kantelinen et al. [64] investigated the enzymatic solubilization of fiber-bound and isolated birch xylans from wood and from kraft pulp. They used four enzymes; endo-xylanase, β -xylosidase, α -glucuronidase and acetyl xylan esterase. They found inaccessibility and the structure of hemicelluloses as the main reasons for only partly-solubilization of hemicelluloses. They found out that the fiber-bound xylans were considerably less accessible to enzymes than the isolated xylans and low solubilization of fiber-bound xylans is mainly due to poor accessibility of enzymes to the fibrous material. Despite high enzyme loadings, the degree of hydrolysis of fiber-bound substrates did not exceed 20% of the theoretical value. In contrast to the fiber-bound substrates, the isolated xylans were readily hydrolysable. However, a maximum of 62% of the initial xylan of isolated xylans was solubilized in the enzymatic hydrolysis. The reprecipitated xylan isolated from the pulping liquor was hydrolyzed less efficiently than the other isolated substrates, corresponding to 72% of the degree of solubilization of pulp xylan. This can also be related to the amount of side-groups and reprecipitated structures, resulting in partial aggregation and thus limited accessibility [64]. In addition, the problem of limited solubility of isolated pulp xylan and reprecipitated xylan in the buffer comparing to isolated wood xylan existed due to their lower side-group contents [64].

Accessory enzymes had no additive effect on the solubilization of kraft pulp or reprecipitated xylan. The solubilization of xylan isolated from pulp was, however, slightly increased by the combined use of xylanase and accessory enzymes, which can be explained by the presence of methyl-glucuronic acid groups. The inefficiency of the accessory enzymes to enhance the solubilization of reprecipitated xylan indicated that this xylan was almost pure poly-xylose. Hence, xylan isolated from kraft pulp originated at least partly from the inner layers of the pulp fibers [64]. The low hydrolysis of xylan in the inner layers of kraft pulp fibers must

therefore be due to fiber architecture, low porosity and the presence of cellulose and residual lignin [64].

The same reasons have been mentioned for mannan. M. Rättö et al [52] hydrolyzed fiber-bound and isolated galactoglucomannans with mannanases and accessory enzymes. Mannanases exhibited different specificities on fiber-bound. Even at high dosage of enzymes, the degree of hydrolysis of fiber-bound substrates did not exceed 10% of the theories. They suggested poor accessibility of the substrates as the reason for this limitation [52].

Another reason that has been suggested as a limiting factor in removal of hemicelluloses is the presence of lignin-hemicellulose complexes [45, 53]. G. M. Gübitz et. al [56] found out that half of the remaining hemicellulose present in the pulp appeared to be entrapped within the cellulose matrix while the other half was associated with lignin-carbohydrate complexes. With different analyses, the presence of two types of lignin-carbohydrate complexes, a galactoglucomannan-lignin complex (DP 50-60) and a xylan-lignin complex (DP >200) was revealed [56].

The adsorption of the enzyme on the substrate is another important factor reflecting efficiency of the enzyme and can be among the limitation factors in removal of hemicelluloses by hemicellulases. P.J. Gerber et al [13] studied the adsorption of a xylanase and a mannanase onto bleached hardwood and softwood kraft fibers. They found out that the presence of cellulose binding domain influenced the adsorption of enzymes whereas the adsorption of the mannanase onto wood fibers with a substrate binding domain was as high as 90%. The maximum adsorption of the mannanase and xylanase without binding domains was less than 20% [13]. However, most of the xylanases do not possess a specific cellulose binding domain and that can provoke a limitation in removal of hemicelluloses. Thus, xylanases and mannanases could have different adsorption behaviors on fibrous substrates. Due to the lack of a cellulose binding domain in certain hemicellulases, ionic strength and pH appear to play a large role in the adsorption [13].

While in some articles it is mentioned that the hemicelluloses in bleached pulp are more accessible, compared to unbleached ones, due to partial removal of lignin and hemicelluloses [156], in some other an inverse observation is reported [154] suggesting the remaining pentosans in bleached pulps are well shielded by other pulp components and therefore not easily susceptible to enzymatic attack. But despite all these contradictions, the common fact

between them is the presence of a ceiling in the removal or solubilization of hemicelluloses by enzymes [113].

The wood species, method of pulping, the accessibility of pentosans and their quantity in pulp, the penetration capabilities [225] and substrate specificity of enzymes, the inhibitory action of bleaching chemicals, the linkage of xylan to lignin [240] and cellulose [241] by covalent and hydrogen bonds, respectively, may be the factors contributing to the difficulties in removing xylan by enzymes from dissolving pulp [113].

As a summary, all the studies have shown that hemicellulases could not solubilize all the hemicelluloses of pulps. The solubilisation is limited and the ceiling depends on various parameters such as pulp origins, pulping method or enzyme properties and origins. This limitation is standstill even with elevated dosage of enzymes or longer incubation times. The main reasons justified by many research groups relates to the accessibility of substrates (hemicelluloses) to enzymes (hemicellulases). The highly modified structure of hemicelluloses and their connection to other components of the fibers have been mentioned as the main reasons of inaccessibility along with the structural properties of the enzymes like their size (the preliminary of diffusion) and binding domains as a determining parameter in their performance.

6-3-6- Effect of the operational parameters

The conditions in which the enzymes are active, particularly the optimum conditions, are an extremely important parameter which affects the way into industrialization of an enzyme. One of the most important part of efforts in enzyme technology is promoting the enzymes that can tolerate severe conditions like extreme pH values and temperatures and preferably without the use of any solvents or buffers because the use of another chemical will require an extra separation step which is costly. In addition, it is preferable to have short incubation time that will decrease the energy consumption and maintenance requirements.

The usual pulp consistency that is used by researchers is 10% because this is the usual pulp consistency used in the bleaching line of pulps. Pulp consistency has an impact on the contact between enzyme and substrate. It has been reported that higher pulp consistencies (in the range of 9 or 10%) provide a closer contact between enzyme and cellulosic fibrils. However, it

has been reported that the residual xylanase activity after enzymatic treatment of the pulp is higher at 2.5% [242] pulp consistency than at 9% [98, 239].

As the hemicellulases should target just hemicelluloses, it is important not to have cellulase activity in the enzyme preparation. A slight increase in viscosity of pulp can indicate the absence of cellulase activity: indeed, the elimination of small chains will artificially increase the overall degree of polymerization [41]. In some studies, it is mentioned when the enzyme preparation is free of cellulase activity [71, 157, 83].

Furthermore, although the use of buffer in the enzymatic treatments guarantees the stability of the pH, the presence of salts can interfere with the subsequent chromatographic analysis [45] and from industrial point of view it is costly.

The state of the pulp is an important factor as well. Once-dried fibers exhibit lower hemicellulase adsorption than never-dried fibers. Drying the fiber drastically diminishes the adsorption capacity of kraft pulps. In a study, never-dried fiber adsorbed 2–3 times more enzymes than once-dried fiber [13].

Table 8 summarizes different operational parameters investigated in different studies.

Table 8- Different operational parameters investigated in different studies.

Operational parameter	Value
Temperature	22-56°C [160]
	27°C [19, 71]
	30-55°C [98]
	37°C [34]
	40°C [83, 113, 52]
	50°C [41, 53, 157]
	50-55°C [30]
	55°C [19, 71]
	60°C [47, 40, 36, 11, 83]
	70°C [83]
	80°C [75]
pH	4.5 [113, 98]
	4.7 [71]
	4.8 [53]
	5.0 [30, 52]
	5.5 [41]
	6.0 [30, 52, 19]
	6.5 [157]
	7.0 [34, 47, 75, 40, 36, 11, 30]
	7.5 [30]
	8 [19, 160]
Incubation time	1 h [71, 157]

	90 min [75]
	2 h [83, 19, 40, 36, 11]
	3 h [98, 30, 47]
	4 h [34]
	10 h [53]
	24 h [52, 113]
	2 weeks [71]
Pulp consistency	1% [13, 160]
	1.94% [34]
	2% [52]
	2.5% [113, 98]
	3% [40, 36, 11]
	5% [53]
	9% [71, 98]
	10% [45, 47, 157, 30, 83, 75, 19]

E. Valcheva et al [160] studied the kinetics of enzyme action of Cartazyme NS-10 on a hardwood kraft pulp prior to bleaching. They proposed an equation that could describe the performance of the enzyme (Figure 33 and 34) [160]. They investigated various kinetic equations, such as Arami-Erofeev topochemical equation, the Prout-Tompkins topochemical equation, the three-dimensional diffusion equation, and the Brown et al.'s power equation to describe the kinetic curves for the determination of the equation describing the process best [243]. It was found that a modified Prouty-Tompkins topochemical equation describes the obtained kinetic curves with the highest degree of precision, which can be presented by the following equation [160]:

$$\frac{\alpha}{(1 - \alpha)} = (kt)^k$$

where coefficient k is characterized by the initial rate v_0 below a reaction time of 1 min, the linear character of the kinetic curves might be predicted. The power factor k depends on the nature of the heterogeneous reaction system. This kinetics equation makes it possible to control the process of enzyme treatment of kraft pulp [160].

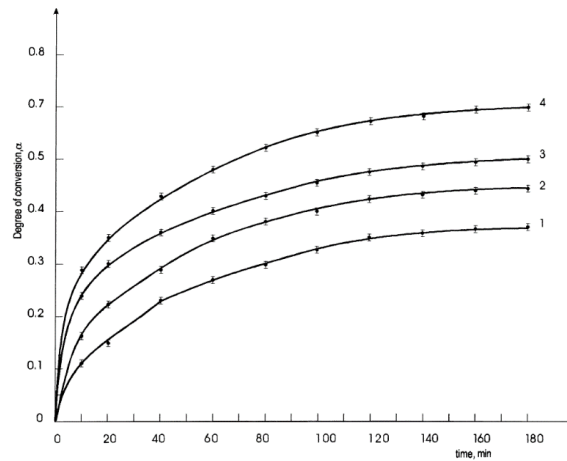


Figure 33- Kinetic curves of enzyme reaction at the temperatures of: (1) 22°C; (2) 34°C; (3) 45°C; (3) 56°C with a Cartazyme concentration of 125 XU/g pulp [160].

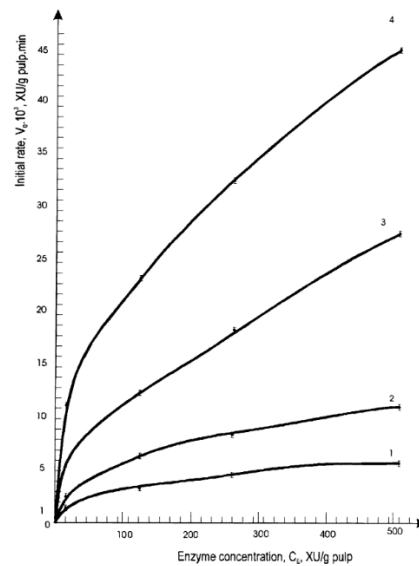


Figure 34- Dependence of initial conversion rate on enzyme concentration at temperatures: (1) 22°C; (2) 34°C; (3) 45°C; (3) 56°C [160].

7- Products from hemicelluloses

Hemicelluloses have potential to be used in different applications as follows:

- From sugar monomers, examples of possible products are:
 - Fermentation of hemicellulosic hydrolysates to fuel ethanol [112]. A high yield of fermentation is demanded to justify the feasibility of the process [112].
 - Production of xylitol, the major product of xylose [112], which is a five-carbon sugar alcohol [244] and due to its high sweetening properties, non- and anti-cariogenicity property and microbial growth inhibition capacity, it has a big application in pharmaceuticals and food industries [112, 245, 246].

- Production of other value added products like butanediol that is applied as a solvent, liquid fuel, and as a precursor of many synthetic polymers and resins [68], lactic acid which is widely used in food, pharmaceutical and textile industries and biodegradable plastics, furfural which is used for the production of a wide spectrum of important non-petroleum derived chemicals and resins, butanol as an advanced biofuel and an industrial solvent in products such as lacquers and enamels, bio-hydrogen as an ideal energy alternative for the future [112].
- From oligomers:
 - Xylo-oligosaccharides (XOs) which may have variable proportions of substitute groups like acetyl, uronic, and phenolic acids, have applications in food and pharmaceutical such as low-calorie sweeteners [112, 517] (The sweetness of xylobiose is equivalent to 30% that of sucrose, and the sweetness of other xylooligo-saccharides is moderate and possess no off-taste [517]), in low-pH juices and carbonated drinks [247], soluble dietary fiber, prebiotics, functional foods [517], synbiotic foods (which are both prebiotic and probiotic) [517] and so on (figure 33) [112]. Their water activity, antifreezing activity are competitive with the equivalent products in the market [248]. Other properties like their odour, non-cariogenic [249, 250, 251, 252] and low-calorie are of interest [517, 253, 254]. They are being used commercially as functional foods in Japan in which the regulations are rather different from US and Europe [517, 255].

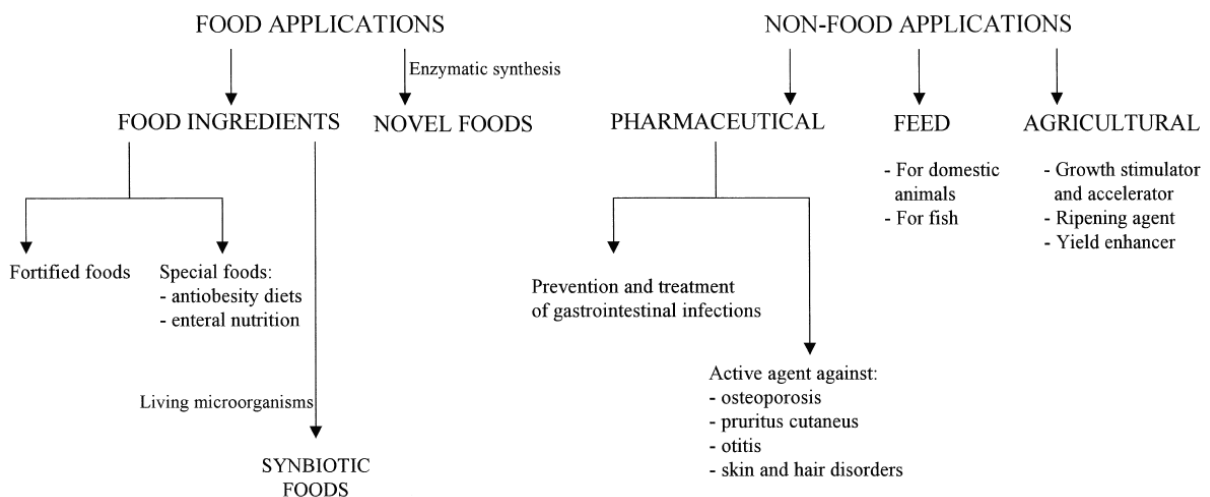


Figure 35- Applications of xylooligosaccharides (XOs) [517].

8- Conclusion

Nowadays, applying clean technologies is in the center of attention in different industries in order to decrease the release of pollutants in the environment. Maximizing the revenues of a production line by valorizing the value-added precious side products is also looked for. In the case of the cellulose industry, trying to valorize the wood components instead of burning half of the wood content with the black liquor would improve the economy model of the mills and ensure their perennation in some cases.

Kraft pulping is the dominant pulping method producing paper-grade pulps. Among various current and potential value-added products in pulp and paper industry, hemicelluloses are of the important ones, as they represent up to 30% of wood mass, and up to now they have not been valorized except for the production of energy. There are two ways under study by several research groups to have access to hemicelluloses when considering the kraft process: either they can be partially extracted by an autohydrolysis process prior to the kraft cooking, or they could be extracted from paper pulps which still contain from 15 to 30% of hemicelluloses.

In this study we will focus on the extraction of hemicelluloses from bleached paper pulp by using enzymes alone, or in combination with cold caustic extraction. The choice of bleached pulps as raw material should ensure to have access to purer hemicelluloses than from an autohydrolysis process applied on wood: indeed, bleached pulp does not contain lignin any more, and hemicelluloses in bleached pulps should be free of acetyl groups, and only few uronic acid groups should remain.

The choice of using enzyme, is because we aim at extracting rather pure hemicelluloses in their oligomeric form for a possible future usage as prebiotics for example. Acidic processes are thus excluded as acid hydrolysis is prone to depolymerize oligomers to monomers and to degrade sugar monomers into furans. Acidic process could also degrade cellulose. The advantage of using enzymes is that pure hemicelluloses should be obtained, as enzymes are selective.

Xylanase and mannanase, are the two enzymes that will be investigated. These enzymes have already been tested in the pulp and paper industry, as bleaching boosting agents. But still there are many challenges in applying hemicellulases to valorize hemicelluloses from kraft

pulps, among them the limitation in the extraction of remained hemicelluloses which is why we will also study a combination between enzymes and a CCE treatment.

The study is divided into two parts: the first part will investigate the effect of the two enzymes on bleached softwood and hardwood pulps, alone or in combination with CCE, and the second part will study the structure of the oligosaccharides extracted.

Chapter 2 Materials and methods

- Materials

1-1- Pulp

Two types of pulps were used; namely fully bleached kraft hardwood and softwood pulps. Both were industrial pulps received from Fibre Excellence company. Based on the manufacturer information, they were, each, a mixture of different hardwoods and softwoods, respectively and were bleached by a ODEDD bleaching sequence.

These feed pulps were never-dried pulps in order to prevent the negative consequence of hornification that comes from drying of the pulp. They were received in the 40 kg big containers of wet pulps.

While received, the pulps were soaked for 15 min in the big containers with a filter situated at the bottom and then were washed thoroughly with tap water. Soaking and washing were repeated several times until reaching transparent water in the outlet of the containers. Then the pulps were centrifuged, in several batches, until reaching a pulp consistency between 30 and 40% and then preserved in the freezer at -20 °C in the packages of 200 g.

1-2- Enzymes

Two enzymes were used: xylanase and mannanase. Both were provided by Novozymes, Denmark. The xylanase solution was concentrated monocomponent endoxylanase with an orange color, and an activity of 5200 FXU/g. The mannanase solution was much diluted and it was a monocomponent endomannanase with an amber color and with an activity of 0.06 MIUM/g. Their optimum operational conditions were, for both enzymes, a temperature between 70-80°C and a pH close to neutral namely approximately 6. The range of pH in which both enzymes were active was mentioned as 4-9. The two enzymes were very robust in terms of temperature and pH profile; they were thermophilic and alkali tolerant. The dosage recommended by company for xylanase was > 100 ml/t pulp and for mannanase a dosage of 15 L/t pulp in order to remove the hemicelluloses.

2- Methods

2-1- Enzymatic treatment

Enzymatic treatments were performed in different conditions with different objectives. However, the general condition and method were the same. In order to solubilize

hemicelluloses, the two enzymes were used in different arrangements on both never-dried hardwood and softwood bleached kraft pulps: each one alone, the two enzymes together or in two consecutive steps. In each one alone, there were four sets of experiments: xylanase on hardwood and softwood, mannanase on hardwood and softwood. While using two enzymes together, there were two experiments: the mixture of enzymes on hardwood and softwood pulp, and in two consecutive steps, there were four sets of experiments: xylanase then mannanase on hardwood pulp, mannanase then xylanase on hardwood pulp, xylanase then mannanase on softwood pulp, mannanase then xylanase on softwood pulp.

2-1-1- pH and temperature

Since the optimum temperature and pH of the enzymes had been indicated by the producers, the same has been used for all the experiments: temperature of 75°C (the temperature in the middle of the range was chosen, since the range of 70-80°C had been defined by producers as the optimum range of temperature) and pH value of 6. The very high temperature and the pH out of range of 4-9 could make the enzyme inactive with denaturing their three-dimensional structure and eliminating their performance.

In order to keep the temperature of each experiments fixed at 75°C, a water bath was used. In order to fix the pH at 6, a buffer was used. A 0.05 M citrate buffer was prepared with a mixture of 0.05M citric acid and 0.05M of sodium while adjusting the pH at 6. Using the buffer guarantees the stability of pH since the enzymes react sensitively to pH values. However, some experiments were done in distilled water instead of buffer. The reason of doing so was that the pH of buffer (representing the optimum pH required for enzymes) was close enough to neutral and in fact it was in the range of distilled water's pH and on the other hand the presence of ionic buffer containing charged mineral materials that interfered with some analyses particularly MALDI-ToF and chromatography tools. So it was decided to use the water instead of buffer in those experiments. The variation in pH before and after manipulations with distilled water was measured with pH meter and since the effect was.

2-1-2- Incubation time

The enzymatic treatments were done in different incubation times: from 2 hours to 72 hours (2 h, 4 h, 8 h, 16 h, 24 h, 32 h, 48 h and 72 h) were investigated on different enzymatic treatments and for some analyses, the incubation times of 30 minutes and 1 hour were tested as well.

2-1-3- Pulp consistency

Regarding the pulp consistency during enzymatic treatment two main pulp consistencies of 5% and 10% were used. However, the main pulp consistency which have been chosen for almost all experiments was 10%. This value of pulp consistency is the usual pulp consistency which is used in the pulp and paper industry and therefore the results would be more reliable. Besides, pulp consistency of 10% was the value that had been proposed by the manufacture of provided enzymes. This value of pulp consistency needs agitation to ensure the accessibility of substrates to the enzymes.

2-1-4- General procedure

All the experiments were done in plastic bag. With the initial feed pulp of 20g (this weight corresponds to most of the experiments, although based on necessity, the treatments with the starting pulp of 10-250 g were done as well) and a desired buffer volume to reach to 10% consistency, the treatment were put into practice.

The starting materials (pulp and buffer) were pre-heated to facilitate the action of enzymes. In addition, in order to improve the dispersion of enzymes, the enzymes were added firstly to the buffer, well mixed and then this solution was added to the pulps. Since the manipulations were done in the plastic bag, next to addition of solution of buffer and enzyme to the pulp and sealing the plastic bag, all the culture content were well mixed by hand and the plastic bag was kneaded well enough to ensure a good homogeneity and then it was placed in the water bath ready at 75°C, during a desired incubation time. At the end of the incubation time, the content of pulp mixed with buffer and enzyme was placed in a small well-prepared water bath with boiling water during 10-15 minutes in order to inactivate the enzymes. After this step the content of manipulation was filtered; the hydrolysates were collected for further analyses and the treated pulp was washed thoroughly with distilled water in order to remove the buffer.

In the experiments in which the pulps were treated with two consecutive steps of enzymatic treatments, just after the first treatment the content of pulp as placed in a water bath containing boiling water in order to inactivate the enzyme which was already inside the culture and then after the next enzyme was added and the procedure was repeated.

2-1-5- Ultra-filtration

In some experiments, in case of necessity, the hydrolysates were ultra-filtered in an ultrafiltration cell (from Millipore Corporation) of 350 ml bottomed by a membrane disc of 1

kDa and a magnetic agitation of 100 rpm, with the tank volume of 5 L filled with distilled water at the pressure of 2 bar in order to remove the salts from the hydrolysates. The ultrafiltration terminated when the value of conductivity of the outlet liquid reached the conductivity of water and in most of the cases it took several days. The membrane was well rinsed before its first usage by floating with glossy skin side down (which then after was placed into ultrafiltration device with the glossy skin toward solution) in water for one hour, during which the water was changed three times. At the end of each procedure, the membrane disc was stored in a 10% ethanol/water solution in refrigerator.

2-2- Cold Caustic Extraction

Cold Caustic Extraction (CCE) was done generally on two sets of materials: Feed pulps, namely never-dried fully bleached kraft hardwood and softwood pulps, and on enzymatically treated pulps after enzymatic manipulations.

CCE was done with a NaOH solution of 11% while 110 g of NaOH was solubilized in 1 L of distilled water. Proportionally, 1 L of the soda solution was well mixed with 100 g of air-dried grinded pulps and the mixture put aside during one and a half hours at ambient temperature. The 10% pulp consistency was aimed in this step. After one and a half hour, the mixture was filtered and the CCE treated pulp washed thoroughly with a large amount of distilled water in order to remove all the salt remaining in the pulp and while the pH of the washed liquid in the outlet reached to the pH value of neutral, the washing step stopped. This treated pulp was left behind, either in the freezer or was air-dried depending on the objective of the experiments and analyses.

The collected liquids containing hemicelluloses then were neutralized while gradually adding a solution of HCl 32%. While the pH reached about 7, no more solution of HCl was added and at that moment the color changed from pale yellow to white (even though the change in color started earlier but the color change at that moment is considerable) and the appearance and precipitation of some substances, namely part of hemicelluloses mostly xylans, was observed. After that, this suspension was centrifuged in the centrifugation for 20 min at 4000 rpm in several batches. This centrifugation separated two parts in each vial; one which was precipitated seated at the bottom of the vial with the color of entirely white (rather jelly) and the other the supernatant situated on the top which was almost colorless in the form of a

transparent liquid. These two parts were collected separately and each part was kept in the freezer for further analyses.

Again, while needed, the ultrafiltration step with the same condition as mentioned before, was done on both parts.

2-3- Combination of enzymatic treatment and Cold Caustic Extraction

After termination of enzymatic treatments, the pulps were treated by cold caustic extraction. CCE treatments were applied on enzymatically-treated softwood and hardwood pulps, both, in two different incubation times of 4 and 72 hours, using hemicellulases (xylanase and mannanase) in different arrangements.

After each sequence of enzymatic treatment, the enzyme(s) was/were deactivated while boiling the content and then filtration was performed. The extracted hydrolysates were stored and then the treated pulps were treated in a CCE step which was similar to CCE treatment of starting pulps. The treated pulps were measured rapidly for their water content and then a solution of NaOH was prepared, the concentration of which was calculated in order to reach 11%, taking into account the remaining water in the already-enzymatically-treated pulp. The CCE was done at ambient temperature and during 1.5 hour.

3- Analyses of pulps and of hydrolysates

3-1- Saccharidic composition of the samples using High-Performance Anion Exchange Chromatography

3-1-1- Sample preparation

Carbohydrate content was measured, after acid hydrolyses, by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Dependent on the type of the materials needed to be analyzed, one mild simple acid hydrolysis or a two-step acid hydrolysis were performed. For the mild one, which was done on the soluble oligomers of hemicelluloses, 5 ml of hydrolysates was mixed with 1 ml of sulfuric acid 24% and then this preparation was autoclaved during 1 hour in an autoclave at high temperature and pressure; the temperature of 120°C and pressure of 1 bar. For the pulps, two steps of acid hydrolyses were done before analysis. Firstly, 350 mg of the grinded pulps were mixed with a 3 ml-solution of 72% sulfuric acid and were put in a water bath at 30°C for 1 hour and then with adding 84 ml of distilled water the concentration of sulfuric acid declined to 24% and then the

same procedure was done in an autoclave, the same as explained for the mild acid hydrolysis step.

3-1-2- Analysis

High-performance anion exchange (HPAE) was used in order to separate monosaccharides. Coupled with pulsed amperometric detection (PAD), it permits direct quantification of non-derivatized carbohydrates at low-picomole levels with minimal sample preparation and cleanup. This chromatography takes advantage of the weakly acidic nature of carbohydrates to give highly selective separations at high pH using a strong anion-exchange stationary phase. Pulsed amperometry detects only those compounds that contain functional groups that are oxidizable at the detection voltage employed.

Although anion-exchange chromatography has been used extensively to analyze acidic carbohydrates and glycopeptides, it has not been commonly used for analysis of neutral sugars. However, examination of the pK_a values of the neutral monosaccharides listed in table 1 shows that carbohydrates are in fact weak acids. At high pH, they are at least partially ionized, and thus can be separated by anion-exchange mechanisms.

Table 9- Dissociation constants of some common carbohydrates (in water at 25°C) [12].

Sugar	pK_a
Fructose	12.03
Mannose	12.08
Xylose	12.15
Glucose	12.28
Galactose	12.39
Dulcitol	13.43
Sorbitol	13.60
α -Methyl glucoside	13.71

The columns designed for doing this analysis permit the separation and analysis of mono-, oligo-, and polysaccharides, dependent on the requirements.

To do the analysis, sugar fucose was used as the external standard whose concentration was exactly the same for all sample vials, as well as the standard vials (20 mg/L).

Consequently, all the monomers in the samples, including five main sugars of hemicelluloses namely as glucose, xylose, mannose, galactose and arabinose, were quantified by high-performance anion exchange chromatography with pulsed amperometric detection [HPAEC-PAD, Dionex ICS 5000 system with a CarboPac™ PA10 column at 25°C (Thermo Fisher, Waltham, MA, USA)] and with the eluent of NaOH 2 mM with the flow rate of 1 ml min⁻¹.

3-2- Measurement of Viscosity of pulps for the determination of their degree of polymerization

Measurement of viscosity was done after solubilization of the grinded pulp in the solution of cupriethylene diamine [8]: after complete dissolution, measurement of the time needed for this solution to move between two scales in a particular glassware designed for measurement of viscosity. This travel time gives us the data to calculate the viscosity and the degree of polymerization of the correspondent pulp.

The obtained intrinsic viscosities were converted into the respective values of DP in the following equation (Brown and Wikstrom, 1965) [256]:

$$\overline{DP}^{0.905} = 0.75[\eta]_{\text{CuEn}} (\text{cm}^3 \text{g}^{-1})$$

All the DPs have been quantified after several times repetition.

Although the never-dried pulps were under different kinds of treatments throughout the project, the DP of the pulps was measured on the pulps that were dried in the open air and after almost complete dryness, whereas their water content was less than 10%, they were grinded by a device namely as forplex and then the standard procedure was followed.

3-3- Gel Permeation Chromatography for the determination of the molecular weight distribution of the pulp's polysaccharides and of the poly and oligo-saccharides present in the hydrolysates

This analysis was done on two sets of materials: 1) the pulp (initial hardwood pulp and treated hardwood pulps), 2) hemicelluloses hydrolysates. The first set is based on cellulose and the second on hemicelluloses for which the device and method are different.

There are different average molecular weights: M_n , M_w , M_z and M_p . M_n is the number average molecular weight which is the statistical average molecular weight of all the polymer chains in the sample. M_w is the weight average molecular weight which takes into account the molecular weight of a chain in determining contributions to the molecular weight average. The more massive the chain, the more the chain contributes to M_w . M_z is the higher average molecular weight and is increasingly more sensitive to high molecular weight polymers and accordingly are increasingly more difficult to measure with precision.

The polydispersity index is used as a measure of the broadness of a molecular weight distribution of a polymer, and is defined by division of M_w to M_n . The larger the polydispersity

index, the broader the molecular weight. A monodisperse polymer where all the chain lengths are equal has an $M_w/M_n = 1$. The best controlled synthetic polymers (narrow polymers used for calibrations) have M_w/M_n of 1.02 to 1.10.

3-3-1- GPC of the pulps

3-3-1-1- Preparation of samples

The method for measurement of molecular weight distribution was based on direct dissolution of cellulose in a mixture of DMAc/LiCl (8%) through solvent exchange. DMAc and LiCl are both hygroscopic and the traces of water adsorbed from the air humidity affect negatively on the manipulation.

The air-dried grinded samples were swelled in milli-Q water and then dried with methanol with several repetitions in order to be sure of the whole drying and the entire absence of water. The samples were then dissolved in DMAc and then after in the mixture of DMAc/LiCl (8%) until the whole dissolution of fibers.

3-3-1-2- Analysis

The analysis was done in the system of ion-exchange chromatography of ICS-300 (Reagent Free™ – RFIC™). This chromatography works based on separation of ions and polar molecules and their affinity to the ion exchanger and retains sample molecules on the column based on their ionic interactions. The prepared samples of dissolved pulp were filtered and then injected to the device.

3-3-2- GPC of hemicelluloses contained in the hydrolysates

The hydrolysates of enzymatic treatments were freeze-dried and then were analyzed for their distribution of molecular weight by size exclusion chromatography (SEC) on a Biogel P2 (Biorad) column at 50°C, with deionized H₂O at 0.5 ml min⁻¹ as eluent, coupled to a RI detector.

3-4- Measurement of degradation products, formic and acetic acids by HPLC

Degradation products, namely furfural (F), hydroxymethylfurfural (HMF), formic acid (FA) and acetic acid (AA), were quantified by HPLC on a Varian Hi-Plex H ligand exchange column (Varian, Palo Alto, CA, USA) at 65°C, with 5 mM H₂SO₄ at 0.6 ml min⁻¹ as eluent and with refractive index (RI) detection.

3-5- MALDI-TOF MS

MALDI-TOF MS (matrix-assisted laser desorption/ionization) is an ionization technique which produces ions from large molecules using a laser energy absorbing matrix. It is applied for analysis of large organic molecules and biomolecules. It is a three-step process. Firstly, the sample is mixed with a matrix material and applied to a metal plate. Then after, a pulsed laser irradiates the sample, triggering ablation and desorption of the sample and matrix material, and finally the sample molecules are ionized by being protonated in the hot plume of ablated gases and then they can be accelerated into whichever mass spectrometer is used to analyse them. MALDI-TOF (time of flight mass spectrometer) is the most widely used mass spectrometer, mainly due to its large mass range. The TOF measurement procedure is also ideally suited to the MALDI ionization process since the pulsed laser takes individual 'shots' rather than working in continuous operation.

The samples were analysed by Autoflex Speed (Brüker Daltonics) in mode reflectron and linear positive. Mode reflectron detects better and more precisely the lower chains of released oligomers and the linear positive mode goes further and makes it possible to detect the bigger molecules and proportionally is less precise. The matrix which has been used was dihydroxy benzoic acid (DHB). The matrix was diluted to 50 mg/ml in water. This concentration has been changed in some analysis in order to extract better results and additionally, in some analyses the solvent has been replaced by DMSO or TA30 (Acetonitrile/H₂O/TFA with the distribution of 30%, 70% and 0,1% respectively). Samples were diluted in water in order to being analysed with DHB/TA30 or in DMSO in order to being analysed with DHB/DMSO. Then the samples were placed on the target and were dried in ambient temperature and at the end were analysed.

4- Morphology of fibers

The samples of initial pulps and treated pulps were analysed for their physical structure of fibers through two types of microscopy: optical and scanning electron microscopy.

The optical microscopy which uses of visible light to magnify the objects was applied with different resolutions from 10× to 50× which was not enough to detect the changes in the structure of fibers.

Scanning electron microscopy (SEM) is among the powerful tools widely used to investigate the lignocelluloses surfaces through surface characterization, morphology, and analysis of microstructure. While using SEM, the surface characteristics like erosions, deconstruction and re-localization of the cell wall components can be investigated. Furthermore, it is likely to estimate the accessibility of the substrates to the enzymes in enzymatic treatments [257]. Apart from the advantages obtaining from this analysis there are some weaknesses which should be considered in the description of the resulted images, having potential to limit the application of this apparatus. For examples, the samples for SEM should be conductive. Additionally, the electron beam may damage the samples. In order to make the samples conductive, they can be coated with a vaporized metal (e.g., gold) or carbon [526, 258].

The FEI quanta 200 device was applied for SEM at an accelerating voltage of 10 kV. The samples were dried and were mounted using a sticky 100% carbon disc and then metalized in the carbon layer of 10 nm.

In order to measure the length and width of fibers, Morfi analyzing was applied the principle of which is based on taking photo by a camera. The taken photos are then analyzed by a software. The initial pulps and the treated pulps were well dispersed in water and analyzed by Morfi analyzer. The suspension of sample is poured inside a tank. There is a pump that circulates this suspension and its passage from a capillary through which the photos are taken.

Chapter 3 – Study of the extraction of hemicelluloses from bleached kraft pulps - Comparison of the effects of enzymes, CCE and their combination

Introduction

The first objective of this chapter is to study the ability of enzymatic treatments, cold caustic extraction and combination of both treatments to extract hemicelluloses from bleached softwood and hardwood kraft pulps. These treatments were chosen for different reasons: they should enable to recover hemicelluloses as oligomers, they should not affect cellulose, and enzymatic treatments are considered as eco-friendly treatments.

The second objective is to evaluate the effects of these treatments on the degree of polymerization and molecular weight distribution of the polysaccharides and on the morphology of the fibers.

3-1- Characterization of the starting pulps

Never-dried fully bleached hardwood and softwood kraft pulps were used, kindly provided by Fibre Excellence St Gaudens pulp mill.

The Degree of Polymerization (DP) of the pulp polysaccharides was measured based on viscosity measurement. The average DP of the polysaccharides was 1 130 for the hardwood pulp and 1 380 for the softwood pulp.

The length and the width of the fibers were quantified. Table 10 shows the average length and width of the fibers for both starting pulps.

Table 10- The average length and width of the fibers in the starting pulps.

	Length (μm)	Width (μm)
Starting hardwood pulp	670.0 \pm 4.4	24.3 \pm 0.2
Starting softwood pulp	1104.0 \pm 4.4	30.5 \pm 0.3

The figures 36 and 37 show the distributions of length and width in both pulps. As expected, softwood pulp has a much higher number of fibers in the bigger classes both for width and length (above 30 microns and 1.30 mm, respectively).

All the experiments have been repeated three times and all the reported results are the average of them.

Regarding the saccharidic composition of the starting pulps the results of high-performance anion exchange (HPAE) with pulsed amperometric detection (PAD) (HPAE-PAD) quantified the content of cellulose and hemicelluloses in each pulp (table 11). HPAE-PAD was performed

Chapter 3 Study of the extraction of hemicelluloses from bleached kraft pulps

after acid hydrolysis of the grinded pulp to be sure that all the polymer chains have been hydrolyzed to their monomer units.

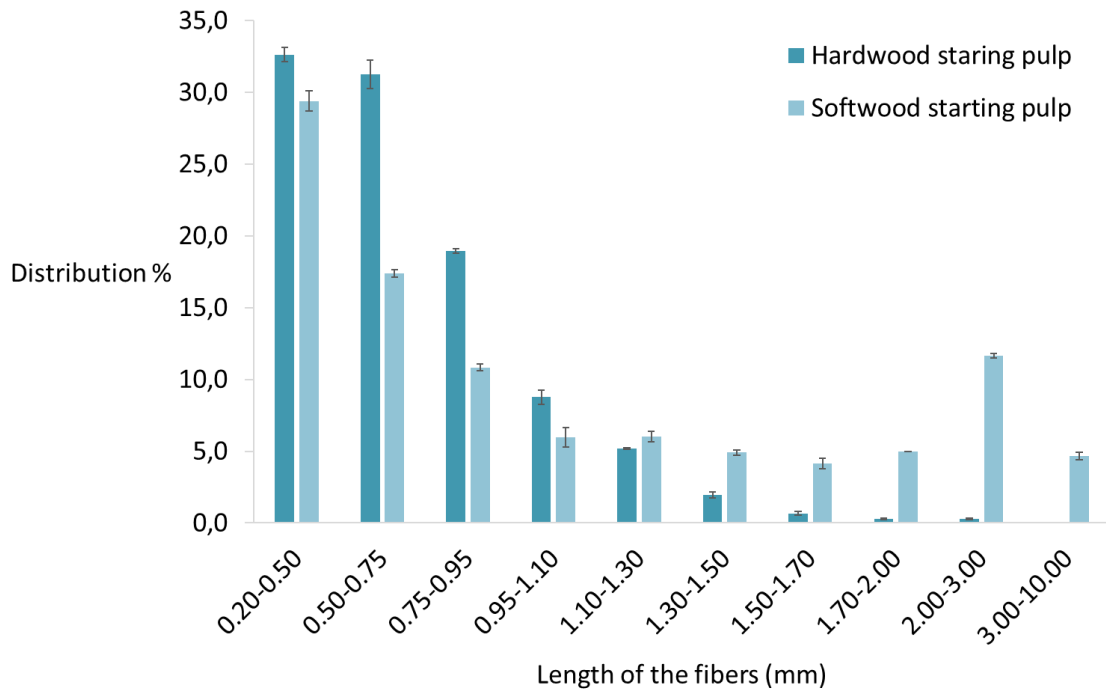


Figure 36- Distribution of fiber length in both starting pulps.

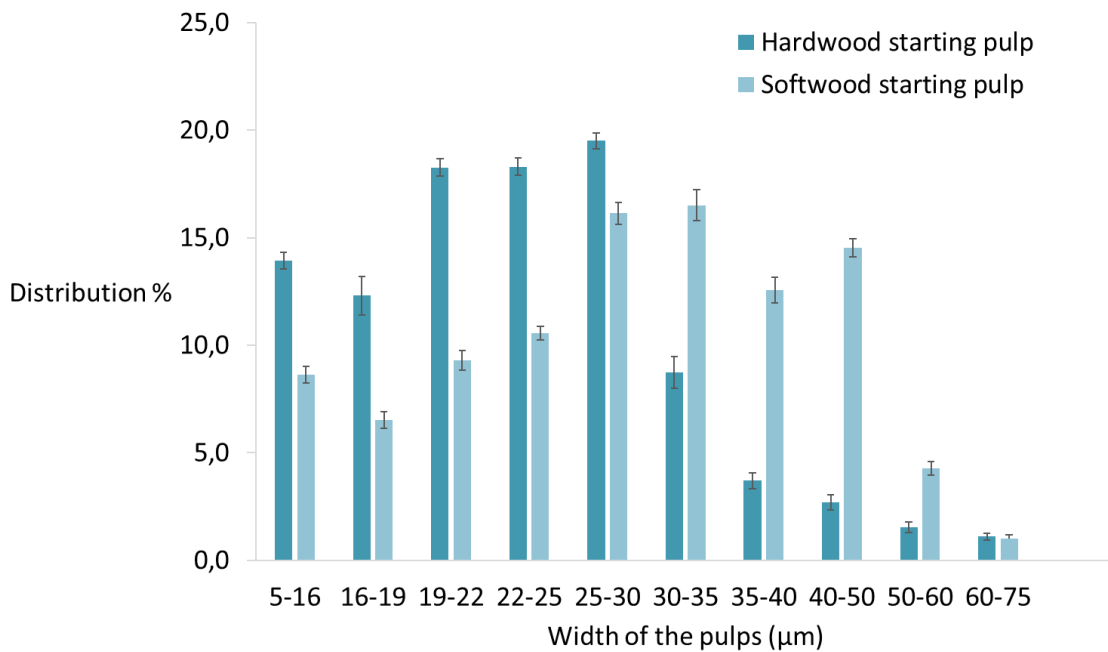


Figure 37- Distribution of fiber width in both starting pulps.

In all the calculation, the amount of degraded sugars has been considered. Measurement of degradation products, due to acid hydrolyses, has been done by doing the analysis of HPLC on a Varian Hi-Plex H ligand exchange column. The degradation products in these experiments

include furfural (F) and hydroxymethylfurfural (HMF) in which the former comes from C5 sugars and the latter comes from C6 sugars. The analysis showed that the procedure used to completely hydrolyze the pulp into monomers only yielded a very small amount of furfural (0.7% for hardwood and 0.4% for softwood) which has been taken into account in all the calculations.

Table 11- The cellulose and hemicellulose content of starting pulps.

	Cellulose content	Hemicellulose content	
Starting fully bleached hardwood kraft pulp	75.7%	24.3%	
		Xylan %	91.9%
		Glucomannan %	8.1%
Starting fully bleached softwood kraft pulp	86.3%	13.7%	
		Xylan %	60.4%
		Glucomannan %	39.6%

3-2- Effect of CCE on pulp characteristics

Cold Caustic Extraction (CCE) is a well-known process used to extract hemicelluloses. The objective is to compare the CCE extraction yield of hemicelluloses with the enzymatic treatment one's and the selectivity in dissolution of hemicelluloses. The CCE treatment was done by using 11% NaOH on pulps, which has been shown to be the optimum dose for the extraction of hemicelluloses [228]. In addition, the manipulation was done at ambient temperature which avoids the degradation of cellulose and hemicelluloses [232, 40, 239, 237, 228].

3-2-1- Effect of CCE on Degree of Polymerization (DP)

The CCE treated pulps were checked for their DP values. The thoroughly washed treated pulps were air-dried and after that, their DP values were quantified. It was observed that DP values were increased for both pulps (figure 38). The measured DP of the CCE treated hardwood pulp was 1290 and 1470 for the treated softwood pulp (to be compared to the initial DP values of hardwood and softwood polysaccharides of 1130 and 1380, respectively). A slight increase in degree of polymerization during CCE of bleached pulps has been reported by different studies like on fully bleached softwood kraft pulp [228] and on hardwood dissolving pulp [49]. The CCE treatment dissolves some hemicelluloses that have a much shorter DP than cellulose, which explains this increase in DP. This is one of the advantages of CCE treatment that just dissolves hemicelluloses and short chain cellulose and there is no cellulose degradation.

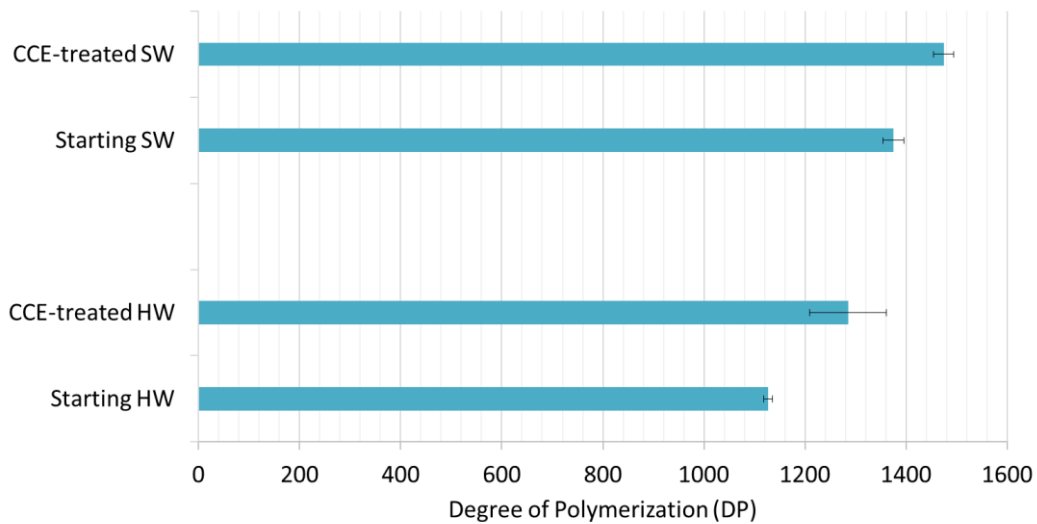


Figure 38- DP values of starting and CCE-treated hardwood and softwood polysaccharides.

3-2-2- Effect of CCE on Molecular Weight Distribution

Molecular Weight Distribution (MWD) has been quantified by Gel Permeation Chromatography (GPC). Figure 39 shows the distribution of molecular weight of the hardwood kraft pulp before and after CCE treatment.

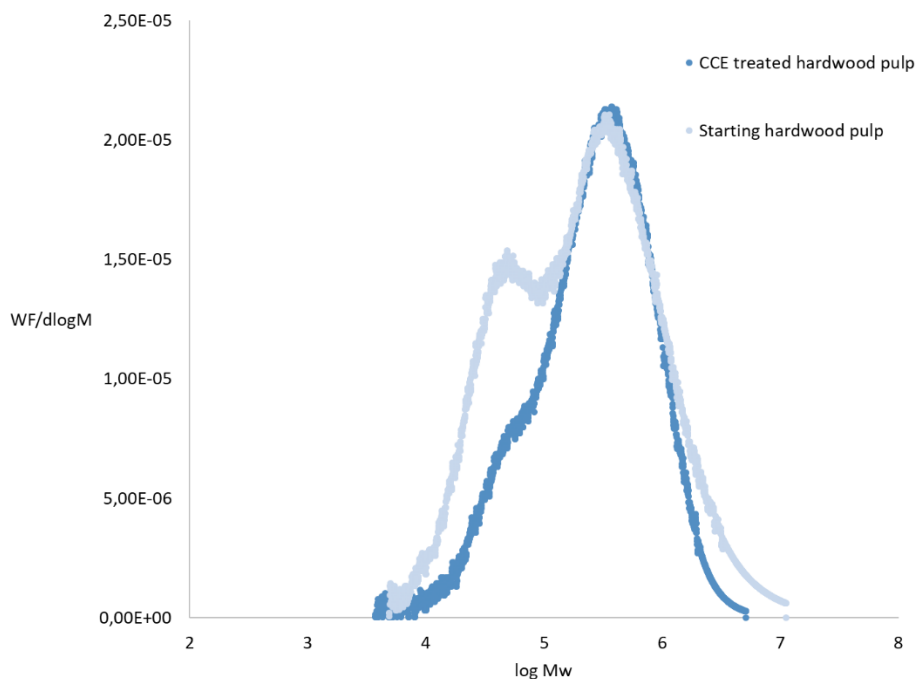


Figure 39- Molecular weight distribution of hardwood pulp before and after CCE treatment.

It can be observed that the lower peak, representing the molecules of hemicelluloses, was almost removed during the CCE-treated pulp. The decline of low molecular weight hemicellulose peak during CCE treatment of kraft pulps has been visualized by chromatography analyses in different studies, indicating the hemicellulose extraction efficiency of this treatment [234, 235].

Polydispersity was calculated for the two pulps. It is calculated by the division of M_w (weight average molecular weight) by M_n (number average molecular weight) (M_w/M_n). The polydispersity index is a parameter determining whether the molecular weight distribution is narrow or broad. The higher the polydispersity index, the broader the curve of molecular weight distribution. The number average molecular weight is the statistical average molecular weight of all the polymer chains in the sample:

$$M_n = \frac{\sum N_i M_i}{\sum N_i}$$

,where M_i is the molecular weight of a chain and N_i is the number of chains of that molecular weight. On the other hand, the molecular weight distribution is calculated by the following equation:

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i}$$

Compared to M_n , M_w takes into account the molecular weight of a chain in determining contributions to the molecular weight average. The more massive the chain, the more the chain contributes to M_w .

Considering these facts and based on the results of GPC for both pulps, the polydispersity index shows a decrease from 7.2 to 4.0 for hardwood starting pulp to CCE-treated hardwood pulp, respectively, which can be explained by the removal of small molecular weight hemicelluloses and thus a narrower distribution. It has been studied by others that applying CCE increases the uniformity of the distributions clued by lower residual hemicellulose content in the pulps [234].

3-2-3- Composition of CCE-treated pulps

CCE treatment objective is to remove some hemicelluloses from pulps. The results in figure 40 show that CCE treatment increased the percentage of cellulose from 75.7% to 82.2% for starting and CCE treated hardwood pulps, respectively, and from 86.3% to 91.3% for starting and CCE treated softwood pulps, respectively, with a standard deviation of 1.3% and 1.1% for hardwood and softwood CCE-treated pulps, respectively. Thus, the percentage of hemicelluloses went from 24.3% to 17.8% for starting and CCE treated hardwood pulps, respectively, and from 13.7% to 8.7% for starting and CCE treated softwood pulps, respectively. This corresponds to 26.7% decrease in hemicellulose distribution for hardwood

pulp and 36.5% decrease in hemicellulose distribution for softwood pulp. These results show that the CCE treatment removed more hemicelluloses from the softwood than from the hardwood fully bleached kraft pulps under the conditions used in the CCE treatment.

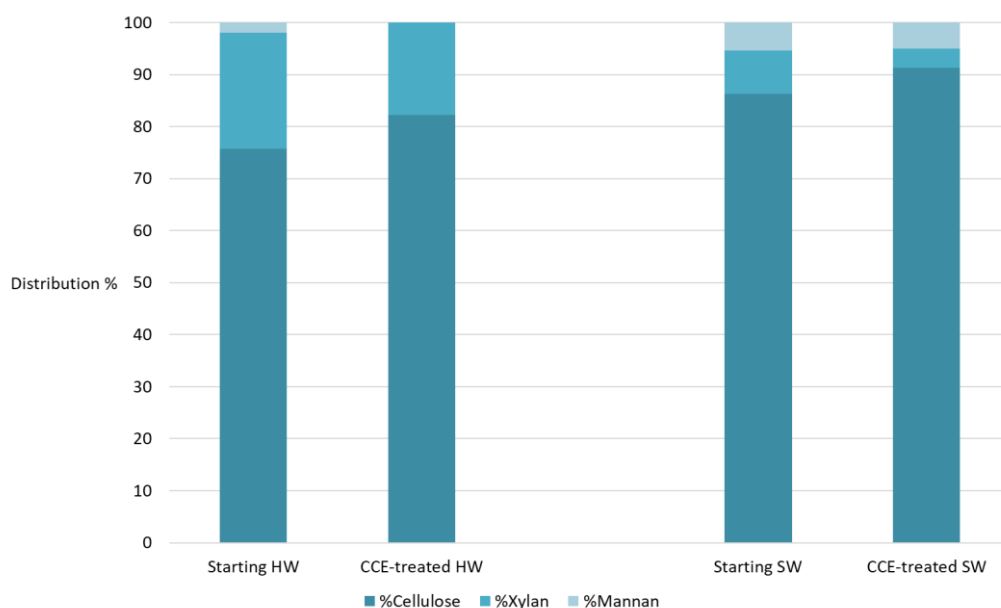


Figure 40- The difference in cellulose and hemicellulose distributions before and after CCE treatment.

In the literature, different studies obtained different results based on the starting materials and the treatment conditions. A CCE treatment on fully bleached softwood kraft pulp at 35°C could obtain a pulp with 93.7% purity of cellulose [228]. In another study with the effective alkalinity of 9% on a ECF-bleached hardwood kraft pulp, the content of hemicelluloses decreased from 18% to 4% [235].

Figure 41 shows the changes in xylan and mannan distributions in both pulps before and after CCE treatment. CCE treatment modified the composition of xylan and mannan in both pulps. In hardwood pulp, the mannans were completely removed by CCE, and xylan content was decreased by 20.2% whereas in softwood pulp, CCE had almost no effect on glucomannans, but removed xylylans significantly (55% decrease in mass). Table 12 summarizes the cellulose and hemicellulose content and the types of hemicelluloses in the starting and CCE-treated pulps.

When comparing these results with the literature, in a ECF fully bleached softwood kraft pulp, the content of glucomannans and xylylans have decreased from 7.4% to 4.7% and from 6.7% to 1.6%, respectively, after a CCE treatment at 35°C and with 11% NaOH [228]. In another study, CCE treatment of an oxygen-delignified never-dried birch kraft pulp at 20°C with the effective alkalinity of 120 g l⁻¹ could decrease the content of xylan from 23.8% to 5.2%, regardless of the

formation of cellulose II [234]. Regarding the softwood pulp, our results confirm well the result of literature, although there is a difference in the temperature of the treatment, but regarding the hardwood pulp, in our case, different results have been obtained, although there is a difference in concentration of NaOH and the type of starting hardwood pulp, both the species and the bleaching sequence.

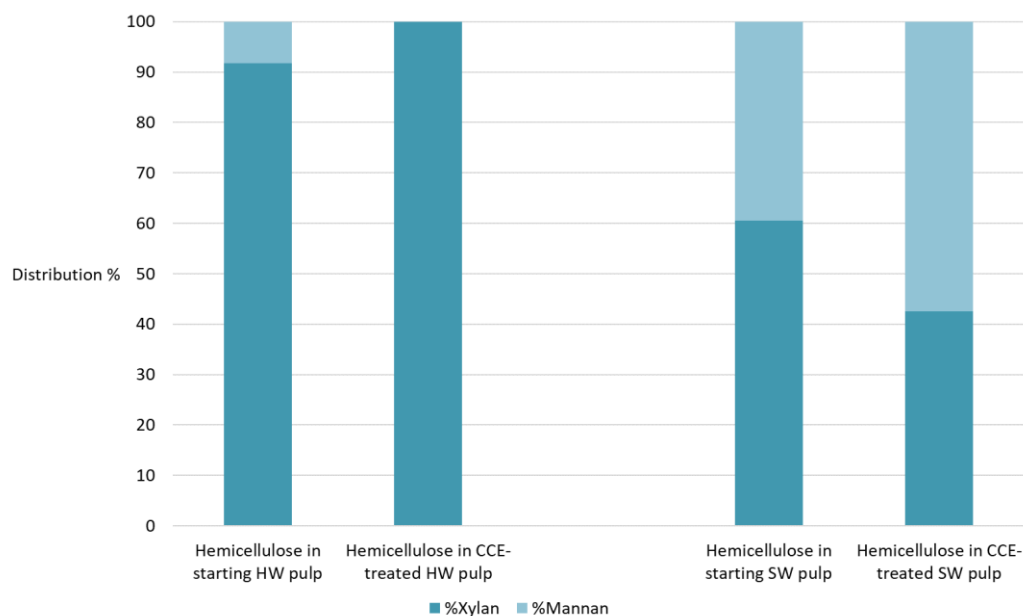


Figure 41- Changes in xylan and mannan content due to the CCE treatment.

Table 12- Composition of hardwood and softwood pulps before and after CCE treatment.

Type of pulp	%xylan in pulp	%glucomannans in pulp	Total % of hemicellulose in pulp
Hardwood pulp	22.3	2.0	24.3 +/- 1.6
Hardwood pulp + CCE	17.8	0.0	17.8 +/- 1.3
Softwood pulp	8.3	5.4	13.7 +/- 1.2
Softwood pulp + CCE	3.7	5.0	8.7 +/- 1.1

3-2-4- Comparison between DMSO and CCE treatments in extraction of hemicelluloses

As CCE treatment alone had limited effect of hemicellulose extraction, it was decided to compare it with DMSO extraction, as it has been described as a selective treatment to extract xylans from pulp [191]. In DMSO method a sample of 6 g of hardwood pulp was treated with 130 mL of DMSO, at 24°C for 24 h, under nitrogen atmosphere and with constant stirring. After this treatment, the hydrolysate was filtered through a polystyrene membrane (porosity 60 μm) and washed with ~20 mL of distilled water. The supernatant liquid was added to 600 mL of ethanol at pH 3.5 (adjusted with formic acid) and left for 12 h at 4°C. The precipitated hemicelluloses were isolated by centrifugation (10 min at 4500 rpm) and washed 5 times with methanol. The xylans were dried in a vacuum oven for 72 h at 30°C.

Chapter 3 Study of the extraction of hemicelluloses from bleached kraft pulps

According to the results (table 13) which is based on the analysis of treated pulps, the DMSO was more efficient in removing hemicelluloses than CCE. While in our case the DMSO treatment could extract 36.9% of the initial xylan, based on correspondent content, this value for CCE treatment was 20.2%. Based on the other studies, an average of 65% xylan extraction yield has been reported from MFC (microfibrilated cellulose) [191] and 46% from a eucalyptus wood [55].

Table 13- A comparison between CCE and DMSO treatments based on correspondent treated pulp.

	%Cellulose	%Hemicellulose	
Starting hardwood pulp	75.7	24.3	
		Xylan %	91.9%
		Glucomannan %	8.1%
CCE-treated pulp	82.2	17.8	
		Xylan %	100%
		Glucomannan %	0.0%
DMSO-treated pulp	85.9	14.1	
		Xylan %	100%
		Glucomannan %	0.0%

The DP of the treated hardwood pulps with CCE and DMSO was measured and compared with the starting hardwood pulp (figure 42). The results show similar DP values for both treated pulps which shows that the two methods are rather selective towards small chains polysaccharides.

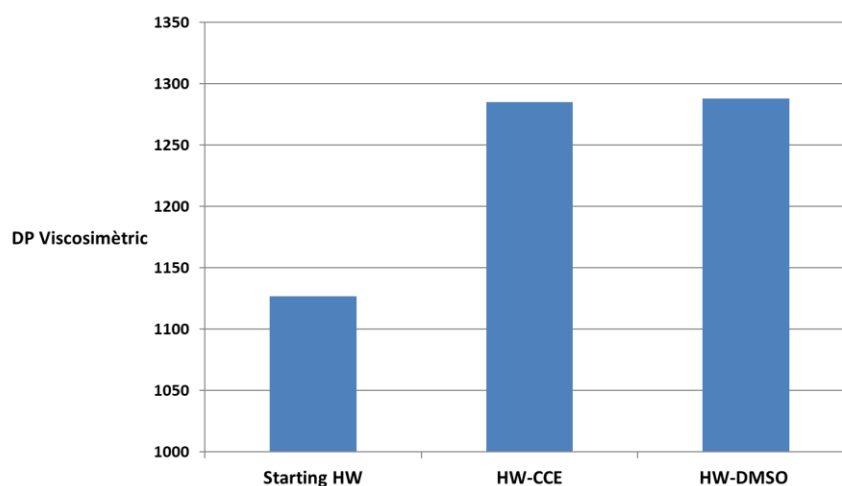


Figure 42- The DP values for CCE and DMSO treated hardwood pulps.

Even if the DMSO treatment worked better on the hardwood pulp to remove xylans, significant amounts of xylans was left. Furthermore, this method could be questionable from an industrial point of view: it is indeed better to use caustic soda rather than a toxic solvent. For these two reasons, only CCE treatment was considered in the following parts.

3-3- Enzymatic Treatments

The enzymatic treatments were done in order to extract the hemicelluloses as much as possible without degrading the cellulosic fibers to be able to valorize hemicelluloses in the kraft pulp mill to add value to the production line on the one hand, and on the other hand in order to help in the production of dissolving pulp with the removal of hemicelluloses.

All the enzymatic trials have been done on never-dried pulps to avoid the hornification side effects.

The two enzymes used in the enzymatic treatments were xylanase and mannanase and they were used separately or in consecutive steps or together in one-step. The temperature used for all experiments was 75°C and the pH was 6 and these values were selected for these enzymes based on the recommendations of the manufacturer of the enzymes (Novozymes). The pH was regulated by using a citrate buffer at the value of 6. It should be noted that in some experiments the buffer was not used because it disturbed some of the subsequent analysis.

In order to find the maximum functionality of each enzyme, they have first been used separately on each type of pulp. Four sets of experiments were prepared: xylanase on hardwood, xylanase on softwood, mannanase on hardwood and mannanase on softwood. The dosage of the enzymes and the duration were varied in order to find the maximum capacity of the enzymes. The incubation time varied from 2 to 72 hours. For xylanase, two dosages of 600 ml/ton of pulp and 1000 ml/ton of pulp and for mannanase 100 L/ton of pulp and 200 L/ton of pulp were applied. Thus, 32 main experiments were done for each pulp. For each experiment the extraction yield of hemicelluloses was calculated by quantifying the hemicelluloses in the hydrolysate solution obtained directly after the treatment, by filtering the pulp, without washing the pulp, each one in comparison with the initial amount of hemicellulose in the starting pulp. The values that are given in the following parts thus underestimate the quantity of hemicelluloses extracted by the enzymes, but it was decided to do so given the high number of experiments. The quantity of hemicelluloses solubilized after the enzymatic treatments and left in the pulp according to our procedure represents about 20% of the quantity present in the hydrolysate (this will be described in the next part).

The experiments that were duplicated or triplicated, are shown with error bars. It was not possible to repeat all the experiments due to the high number of them.

3-3-1- Optimization of the xylanase treatment: effect of incubation time and charge of enzymes

Figure 43 shows the extraction yields of xylan from hardwood bleached kraft pulp that was treated with Xylanase 600 ml/ton and Xylanase 1000 ml/ton of pulp. No mannan extraction was observed. It can be observed that there was an increase in extraction yield with the incubation time and after 48 hours the extraction reached a plateau.

It is observed that there was no meaningful difference between the performance of xylanase in two dosages of 600 ml/ton and 1000 ml/ton. A maximum of around 15% of xylan extraction was reached, in average.

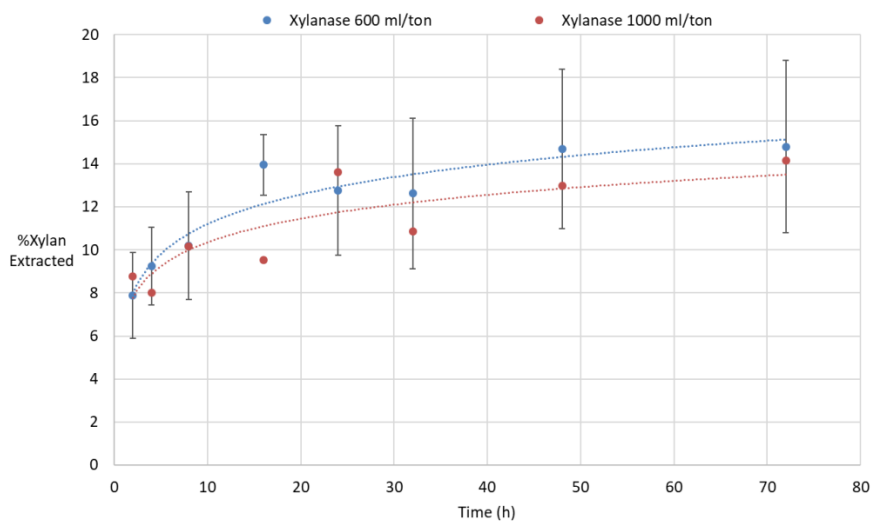


Figure 43- Comparison of two xylanase dosages (600 and 1000 ml/ton) on hardwood pulp.

As explained before, the results are based on the pure hydrolysates. In order to check the effect of washing following the extraction of pure hydrolysates, 10 g (expressed as dried pulp) of the xylanase-treated-hardwood pulps obtained after pressing of the pulps at the end of the enzymatic treatment, were washed in two consecutive steps: first with 50 ml and then with 100 ml of distilled water. The results showed a substantial increase in extraction of xylans (figure 44). This extra washing enabled to extract the amount of oligomer that were dissolved during the enzymatic treatment, but that still remained in the water surrounding the fibers. According to that, the extraction of xylan could increase by about 20%. There was no effect of the incubation time on the additional amount of oligomers collected.

The xylanase treatment was also tested on softwood pulp. Again, two dosages were applied: 600 and 1000 ml/ton. The xylan extraction yield increased over 20% (figure 45). Again, no mannan extraction was observed. This confirms the specificity of the enzyme. The same trend

as with hardwood is observed here. There is no special difference between the efficiency of the two dosages.

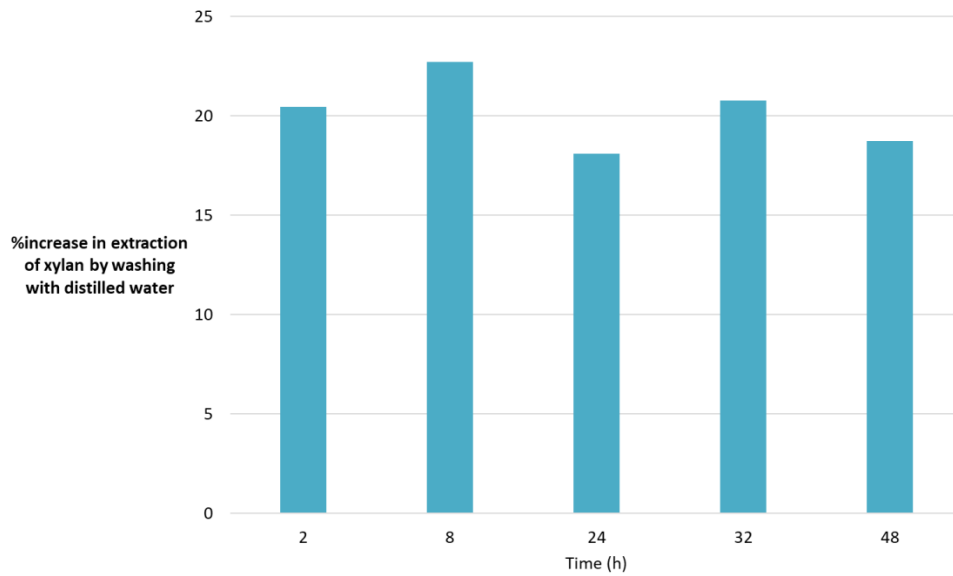


Figure 44- The increase in extraction of oligomers of hemicelluloses with an extra washing step when applying xylanase on hardwood pulp in different reaction times.

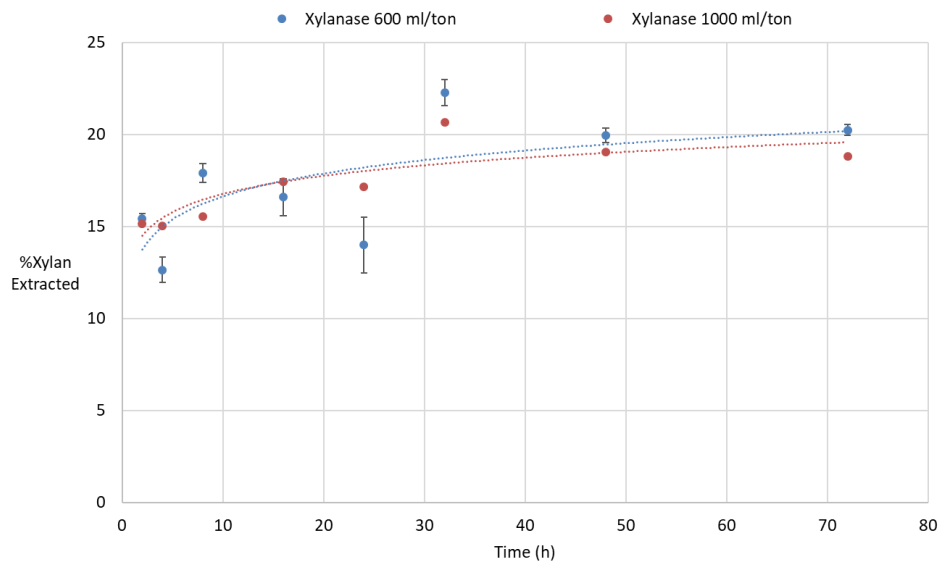


Figure 45- Comparison of two xylanase dosages (600 and 1000 ml/ton) on softwood pulp.

In the literature, it has been reported that xylanase pre-treatment of an oxygen delignified eucalyptus kraft pulp solubilized 46% of the initial xylan to a content of 12.1%. The enzymatic treatment was done at 3% consistency and for 120 min and the treated pulp was washed with hot deionized water [11, 40]. In another study 47% of the initial xylan was solubilized in the birch kraft pulp, whereas 37% was solubilized in the eucalyptus kraft pulp with the treatment in 3% pulp consistency during 3 hours [36]. Furthermore, solubilization of 20% of a birch kraft pulp xylan during 24 hours has been reported [64]. Xylanase treatment on a softwood

dissolving pulp solubilized about 25% of xylose and less than 1% of mannose, with 5% pulp consistency during 10 hours and a following-step washing with a 10% NaOH solution after incubation [56]. The operational conditions, as well as, the type of enzyme and the starting pulp and in addition the following probable sequences affect substantially the final results.

3-3-2- Optimization of the mannanase treatment: effect of incubation time and charge of enzymes

Mannanase acted differently from xylanase: the use of mannanase led to the removal of both xylan and mannan for hardwood and softwood pulps (figure 46 - 49). It is clearly observed that the extraction yield for both xylan and mannan for hardwood pulp was less than 1% (figure 46 - 49). It can be said that the yields are negligible and there is no meaningful trend while incubation time increased. Figures 47 and 48 represent the extraction yield of xylan and mannan when the hardwood pulp was treated with mannanase with the dosage of 200 L/ton. The results were not better than with the lower dosage.

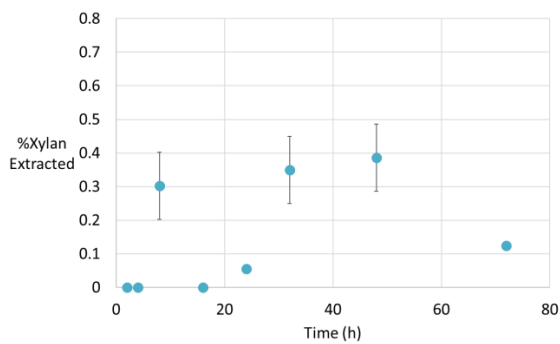


Figure 47- The extraction yield of xylan when applying mannanase 100 L/ton on hardwood pulp.

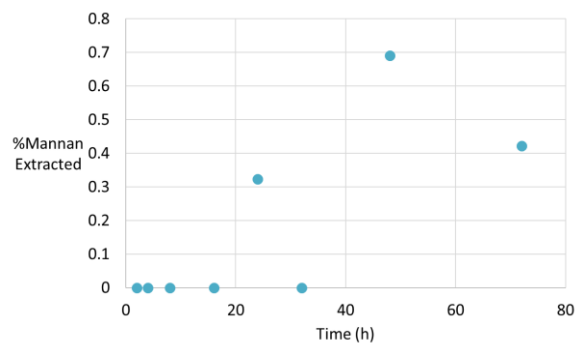


Figure 46- The extraction yield of mannan when applying mannanase 100 L/ton on hardwood pulp.

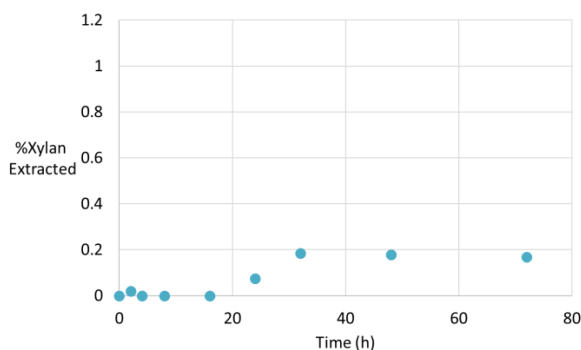


Figure 49- The extraction yield of xylan when applying mannanase 200 L/ton on hardwood pulp.

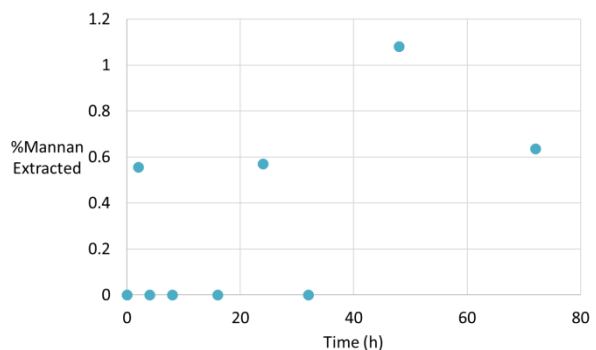


Figure 48- The extraction yield of mannan when applying mannanase 200 L/ton on hardwood pulp.

The release of xylan and mannan while applying mannanase can bring up different hypotheses. The first hypothesis is that the preparation of enzyme can contain xylanase activity. The second explanation could be linked to the internal arrangement and structure of

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xylans and mannans. While applying xylanase no release of mannan is observed and while applying mannanase both release of xylan and mannan is observed. This could suggest that the location of mannan is in the inner layers behind the xylans and the very low yield can justify this inaccessibility as well. In addition, while the enzyme at the end can have access to its substrate, here oligomers of mannans, their release needs the release of xylan. At the end the whole process brings up the hypothesis of very difficult accessibility of substrate for mannanase. It should be considered that hardwood itself contain low amount of mannans as well and this can make harder the accessibility.

The softwood pulp showed more % release of hemicelluloses than hardwood pulp with mannanase. Figures 50 and 51 represent the extraction yield of xylan and mannan when the softwood pulp was treated with mannanase with the dosage of 100 L/ton, respectively.

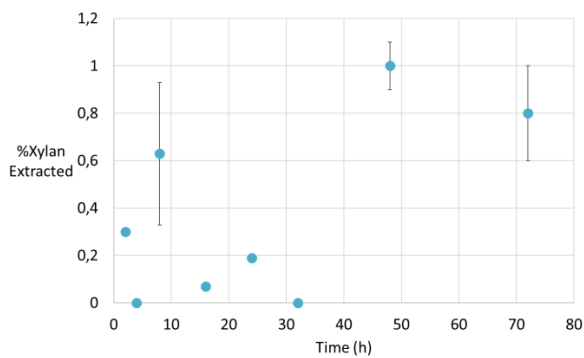


Figure 51- The extraction yield of xylan when applying mannanase 100 L/ton on softwood pulp.

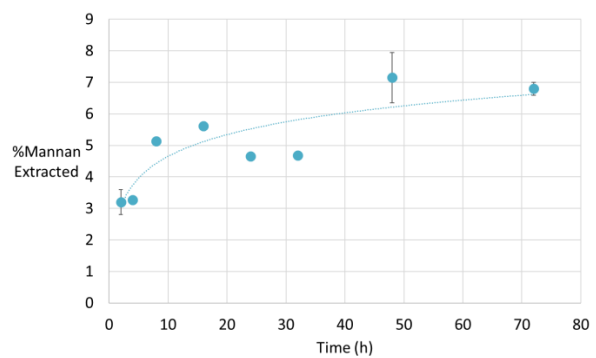


Figure 50- The extraction yield of mannan when applying mannanase 100 L/ton on softwood pulp.

It is clearly observed that the extraction yield of mannans is increased compared to hardwood and this could be due to the different structure and arrangement of hemicelluloses in hardwood and softwood pulps. The xylan extracted yield was below 1%.

A higher dosage of mannanase was tested (figures 52 and 53).

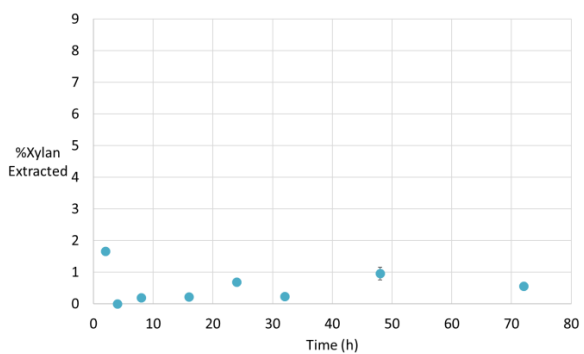


Figure 53- The extraction yield of xylan when applying mannanase 200 L/ton on softwood pulp.

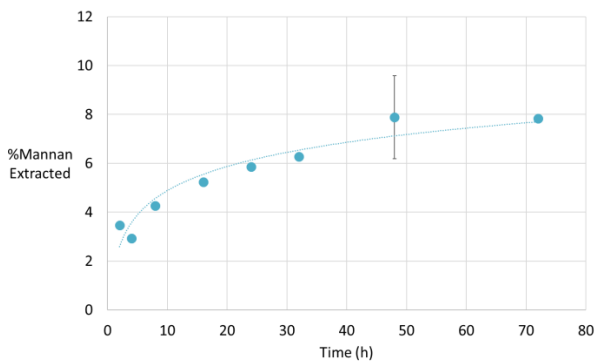


Figure 52- The extraction yield of mannan when applying mannanase 200 L/ton on softwood pulp.

Doubling the dose of mannanases did not improve the results significantly.

Higher dosages of mannanase were tested for 48 h of incubation time which has been shown as the best incubation time for both hardwood and softwood pulps. The experiments, that were done on softwood pulp only (figure 54), show that increasing the enzyme charge had no effect on the yield of hemicelluloses extracted suggesting that the dosage of 100 L/ton is sufficient.

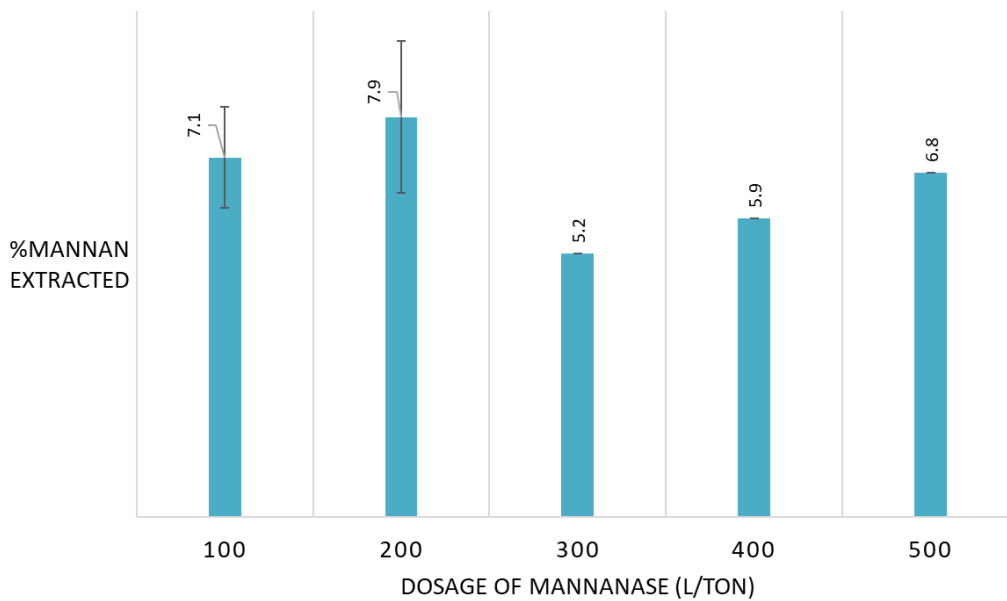


Figure 54- Comparison of different dosages of mannanase on softwood pulp at 48 h.

Regarding results obtained in the literature, applying mannanase on a softwood dissolving pulp at 5% pulp consistency during 10 hours enabled solubilization of around 40% of mannose, 10% of glucose and 1% of xylose. The results have been obtained after washing of treated pulp with a solution of 10% NaOH [56]. Furthermore, on a pine kraft pulp, a mannanase could solubilize 7.3% of the initial mannans with a treatment during 24 hours [52].

3-3-3- Summary of the enzymatic treatments

Table 14 summarizes the best results obtained so far, when applying xylanase and mannanase on our hardwood and softwood bleached pulps: xylanase extracted 14.8% of the xylans contained in the hardwood pulp and 22.3% in the softwood pulp and mannanase extracted only 1.1 % of mannans from hardwood pulp and 7.9% from softwood pulp.

Table 14- The best extraction efficiency of each enzyme.

	%Xylan extracted	%Mannan extracted
Xylanase on hardwood pulp	14.8%	0.0%
Xylanase on softwood pulp	22.3%	0.0%
Mannanase on hardwood pulp	0.4%	1.1%
Mannanase on softwood pulp	1.0%	7.9%

Table 15 summarizes the corresponding process conditions.

Table 15- The best efficiencies considering dosage and incubation time.

	Best yield	Dosage	Incubation time
<i>Xylanase on hardwood pulp</i>			
Xylan extracted	14.8%	600 ml/ton	72 h
Mannan extracted	0.0%	-	-
<i>Xylanase on softwood pulp</i>			
Xylan extracted	22.3%	600 ml/ton	32 h
Mannan extracted	0.0%	-	-
<i>Mannanase on hardwood pulp</i>			
Xylan extracted	0.4%	100 L/ton	48 h
Mannan extracted	1.1%	200 L/ton	48 h
<i>Mannanase on softwood pulp</i>			
Xylan extracted	1.0%	100 L/ton	48 h
Mannan extracted	7.9%	200 L/ton	48 h

Comparison of the two pulp species shows that extraction of hemicelluloses from our softwood pulp was easier than from our hardwood pulp (figures 55 and 56), which was also the case when the cold caustic extraction was applied.

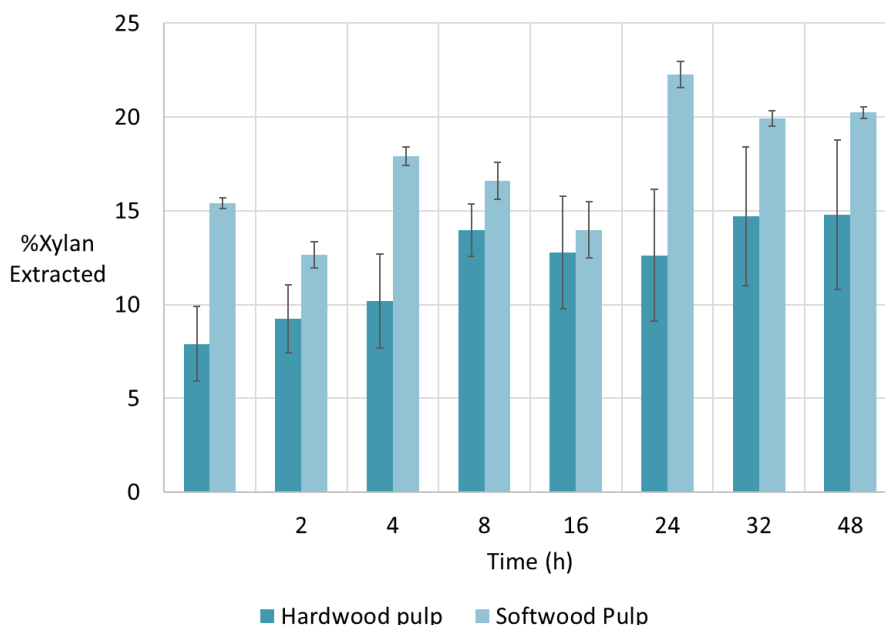


Figure 55- Comparison of the extraction of xylans from hardwood and softwood pulp with xylanase (600 ml/ton) with varying time of incubation.

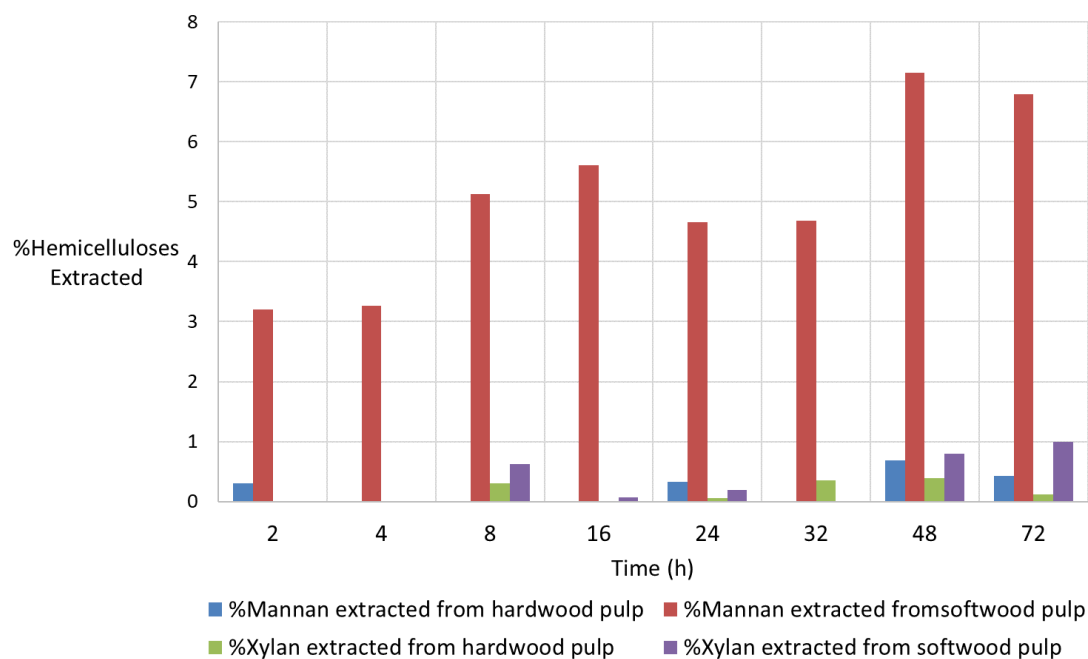


Figure 56- Comparison of the extraction of hemicelluloses from softwood and hardwood pulps with mannanase (100 L/ton).

3-3-4- Effect of pulp consistency on the extraction of hemicellulose with enzymes

Pulp consistency is an important parameter affecting the efficiency of the extraction and the performance of enzymes [98, 34, 36]. All the experiments above were performed at 10% pulp consistency suitable for industrial application. A pulp consistency of 5% was tested. Lower pulp consistency reflects an environment containing more liquid in the media and thus better homogeneity of enzyme.

The results showed that the pulp consistency of 5% was much more efficient than 10% (figures 57 and 58), and the difference could not just be explained that at lower consistency, more material could be recovered from the hydrolysate after pressing of the pulp. Indeed, as shown previously (diagram 9), the additional material collected after pulp washing represented 20% of the material contained in the hydrolysate before washing, whereas the differences obtained by using 5% consistency were above 30% (figure 57). They suggest that even though the performance of xylanase at 10% pulp consistency is reaching the plateau, it is not the case when 5% pulp consistency is used during the incubation times tested and it is more likely that more extraction of xylans could be obtained in longer incubation times. This confirms that the homogeneity of the media is a critical condition which affects substantially the efficiency of the enzymatic treatments. One hypothesis could be that using a lower pulp consistency, due

to a media containing more liquid, the enzyme can move more freely to find its substrate and the accessibility increases substantially.

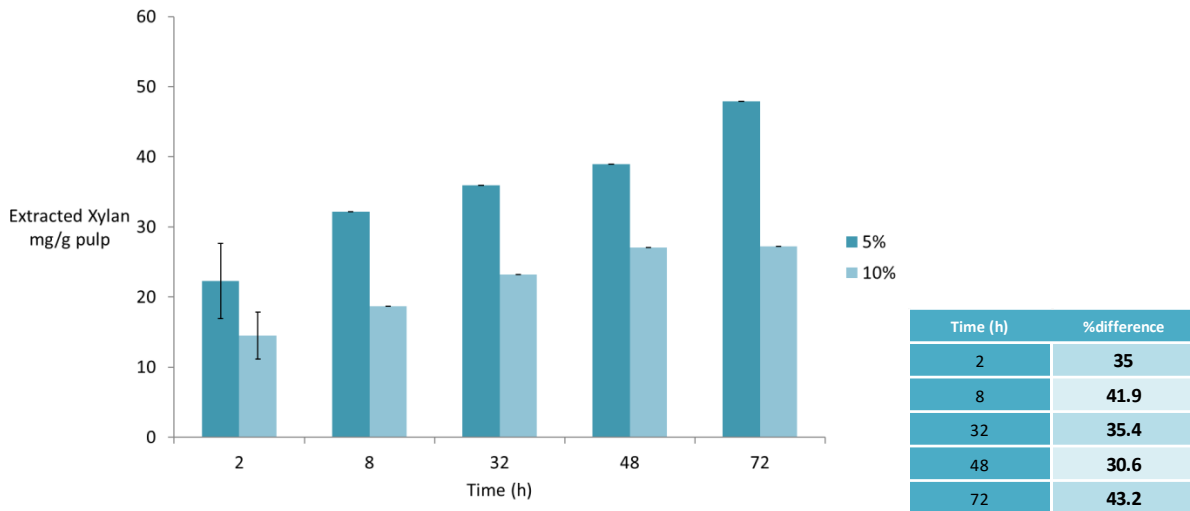


Figure 57- Comparison between pulp consistencies of 5% and 10% when xylanase is applied on hardwood pulp. (T=75°C, pH=6).

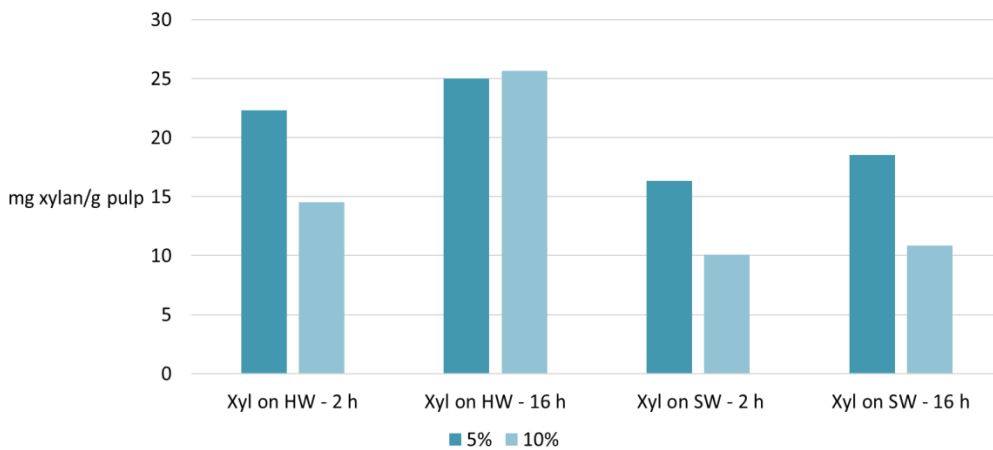


Figure 58- Comparison between pulp consistencies of 5% and 10% when xylanase is applied on hardwood and softwood pulps in 2 and 16 hours. (T=75°C, pH=6)

Different studies in the literature have reported conflicting results about the effect of consistency. In one study, pulp consistencies of 9% and 2.5% were compared, and in that case the higher pulp consistency gave better results: it has been concluded that the higher pulp consistency (9%) provides a closer contact between enzymes and pulp fibers, probably because of the reduced volume of the liquid phase, thus facilitating enzyme adsorption to pulp and the sequential hydrolysis of hemicellulose. In support of this suggestion, the residual xylanase activity in the enzyme filtrates after pulp treatment was found to be higher at 2.5% pulp consistency than at 9% [98]. In another study different pulp consistencies from 1 to 5% were tested for a eucalyptus kraft pulp, and they found that 5% pulp consistency was the most efficient. The probability of the closer contact between enzymes and pulp resulting in more

accessibility has been justified as the reason [34]. In another study, the effect of pulp consistency has been considered as an important economical parameter, meaning higher pulp consistency equals consumption of lower amount of chemicals and solvents. Increasing drastically the pulp consistency to 10%, for birch and eucalyptus kraft pulps, negatively affected all of the parameters studied [36].

3-3-5- Effect of the use of buffer on the removal efficiency of hemicelluloses with enzymes

Due to sensitivity of enzymes to the ionic power of the media, the value of pH of the trials is of main importance. In a certain range of pH, the enzymes are active and they keep their three dimensional (3-D) molecular structure, which guarantees their functionality, out of which they loose their 3-D structure and become deactivated. Applying a buffer keeps the pH of the media in a constant value and so provides the best possible conditions regarding the ions to the enzymes. However, presence of buffer can have some negative effects and one is that the ionic strength of the buffer itself can affect badly the activity of the enzymes. Nevertheless, at the end the presence of buffer brings more advantages than disadvantage.

As aforementioned, due to the interfering influence of buffer in some analyses, some trials were done without buffer, the optimum pH being close to that of the deionized water.

To study the effect of the buffer, three main sets of enzymatic trials were chosen: xylanase on hardwood pulp, xylanase on softwood pulp and mannanase on softwood pulp.

In the case of the use of xylanase on hardwood, the values of pH with and without buffer are given in table 16. For the experiments with buffer, as expected, the pH was constant before and after treatments, for different incubation times from 2 to 72 hours, and the maximum drop change in pH was for 72 h but in the range of the hundredth percentile scale. For the experiments without buffer, and with distilled water instead, the variations in pH was in the range of 1.0 or less. In these experiments, in the blank experiments (without buffer and without enzymes) the drop in pH was lower.

Table 16- The values of pH before and after xylanase enzymatic treatments for the trials with and without buffer.

Incubation time (h)	With Buffer		Without Buffer (with distilled water)		Blank Trials – Without enzyme and without Buffer (with distilled water)	
	pH _{Before}	pH _{After}	pH _{Before}	pH _{After}	pH _{Before}	pH _{After}
8	6.2	6.2	7.4	6.7	7.3	6.8
24	6.2	6.2	7.3	6.2	7.3	6.9
32	6.2	6.2	7.3	6.2	7.3	7.0

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The figures 59, 60 and 61 show the results and a comparison between the presence and absence of buffer at pH 6. Based on the results obtained, the enzymes showed good performance even in the absence of buffer.

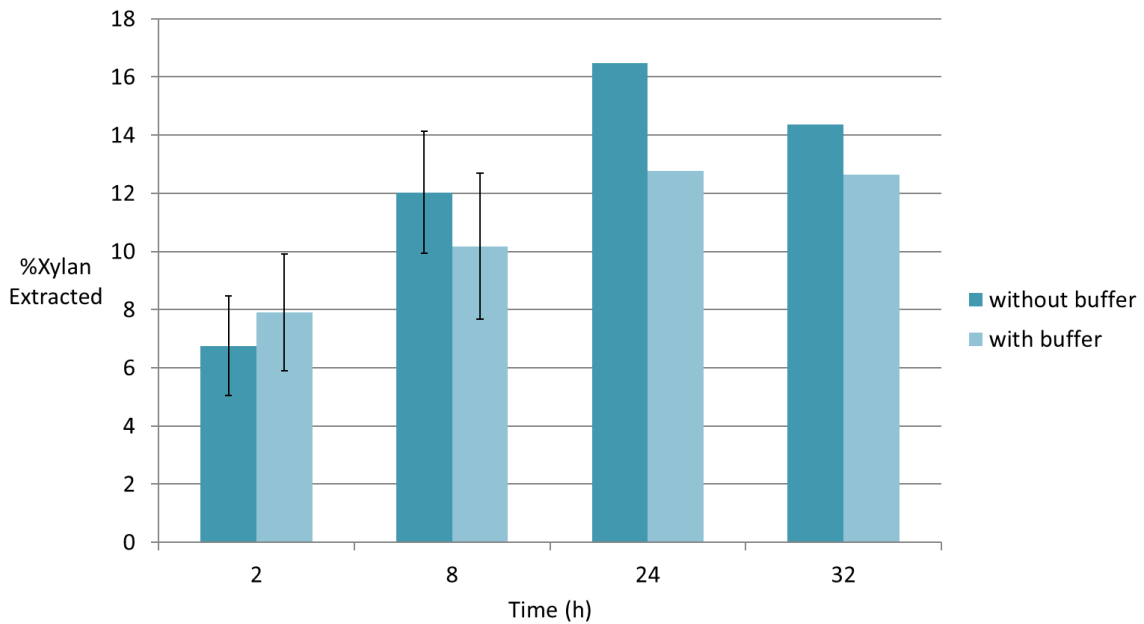


Figure 59- Effect of the use of buffer at pH 6 on extraction of oligomers of xylan, for the xylanase treatment applied on hardwood pulp.

When applying xylanase on hardwood pulp, the absence of buffer performed better in some incubation times. In general, there was no meaningful difference between presence and absence of buffer.

In the case of softwood pulps, results were better in the presence of buffer, in particular for longer incubation times, both for xylanase and mannanase. This is quite surprising, as the differences in final pH with or without buffer were about the same in the case of softwood and hardwood pulps.

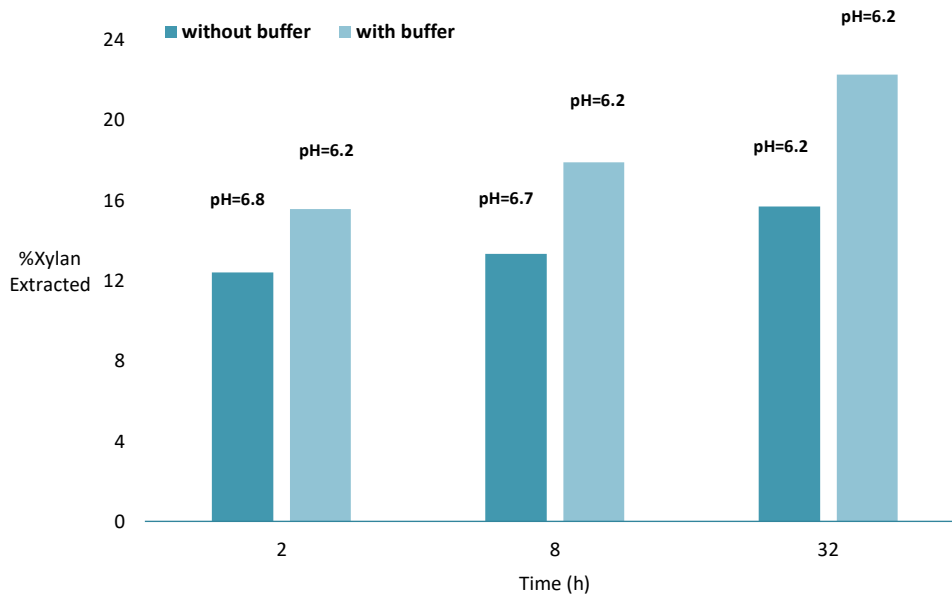


Figure 60- Effect of presence of buffer at pH 6 on extraction of oligomers of xylan, when xylanase is applied on softwood pulp. The values of pH after treatments are shown above the columns. The initial pH for the experiments with buffer: 6.2 and for without buffer: 7.3.

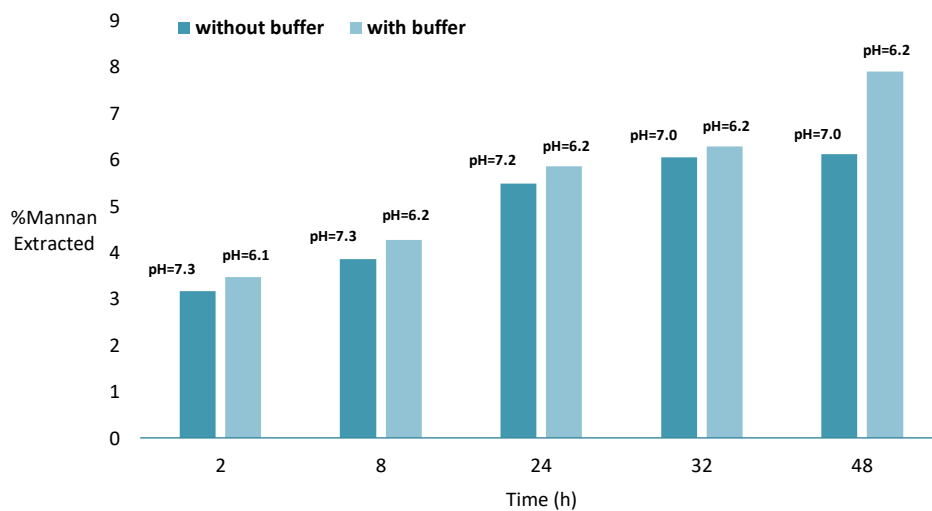


Figure 61- Effect of presence of buffer at pH 6 on extraction of oligomers of mannan, when mannanase is applied on softwood pulp. The values of pH after treatments are shown above the columns. The initial pH for the experiments with buffer: 6.2 and for without buffer: 7.4.

3-3-6- Effect of the enzymatic treatment on pulp degree of polymerisation (DP)

The DP of the three following pulps was measured: xylanase-treated hardwood pulp, mannanase-treated softwood pulp and xylanase-treated softwood pulp. The results of mannanase-treated hardwood pulp have been put aside because of no meaningful changes happening on the pulp regarding the DP of the treated pulp compared with the starting hardwood pulp.

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When applying xylanase on hardwood pulp, the results showed a slight increase in DP compared to the starting hardwood pulp (figure 62). The incubation time of 0 goes back to the starting hardwood pulp's DP. According to this diagram it is observed that there is a slight increase in the DP of the treated pulp after treating with xylanase. The logic of this increase is the removal of the small-chain hemicelluloses out of the structure of the fibers.

In the treatment of softwood with xylanase the same slight trend is observed. The figure 63 shows the changes in the DP of softwood pulps treated with xylanase in different incubation times.

The DP increases until 8 h and then there is a plateau until 72h. There is a slight trend of increasing and then decreasing and remaining stable of the DP. The results confirm that the preparation of enzyme used as xylanase seems to have pure activity of xylanase and does not degrade the cellulosic chains which is an advantage, for both hardwood and softwood pulp.

It seems that the increase of DP in softwood pulp was slightly higher than for hardwood. This could be explained by the size of hemicelluloses extracted from the fibers.

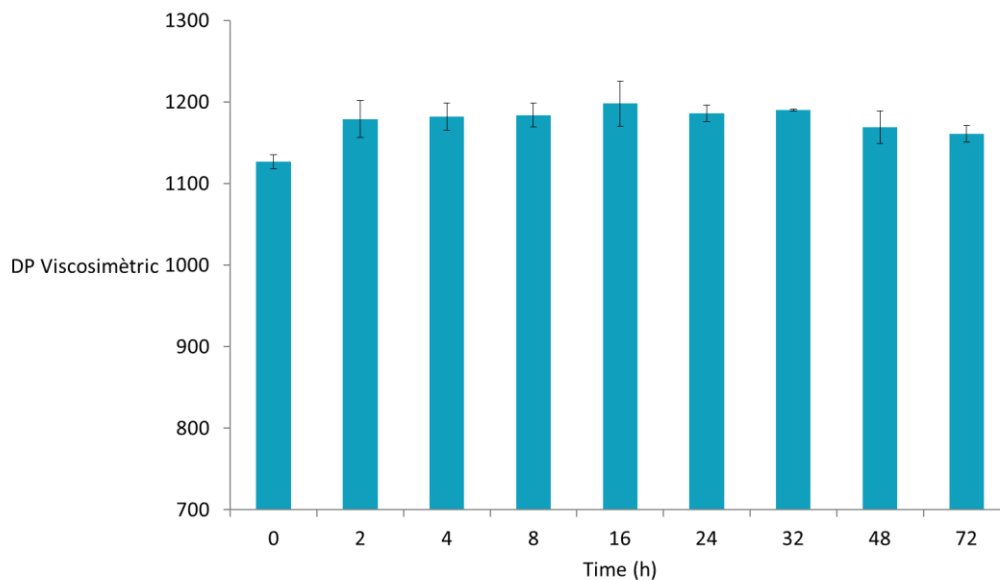


Figure 62- The changes in DP of the hardwood pulps treated by xylanase in different incubation times.

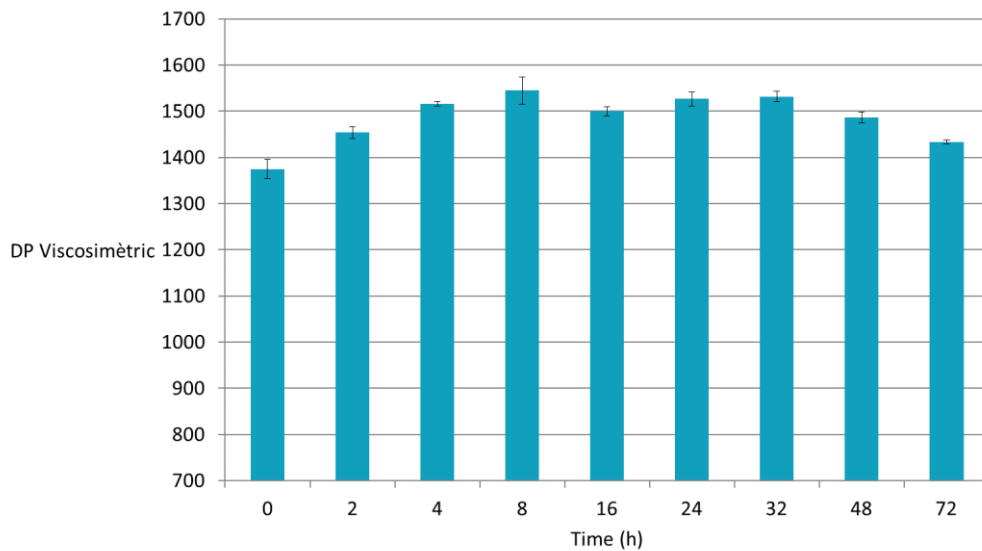


Figure 63- The changes in DP of the softwood pulps treated by xylanase in different incubation times.

The inverse trend is observed when using mannanase on softwood. Figure 64 shows the changes in the DP of softwood pulps treated with mannanase in different incubation times.

The results show a slight decrease in the DP of the softwood pulps treated with mannanase. This decrease can suggest the presence of cellulase activity in the preparation of enzymes used as mannanase. Firstly, a slight increase in the DP is observed which can reflect the very early removal of short oligomers of hemicelluloses by enzymes. After 2h the overall trend is decreasing. At the end the difference in the DP of the softwood treated pulp with mannanase is approximately 100 units. It should be noted that the ratio of glucose/mannose in the hydrolysate for softwood pulp was in the range of 0.19-0.26 and that could question the hypothesis of the presence of cellulase activity in the solution of enzyme (figure 65).

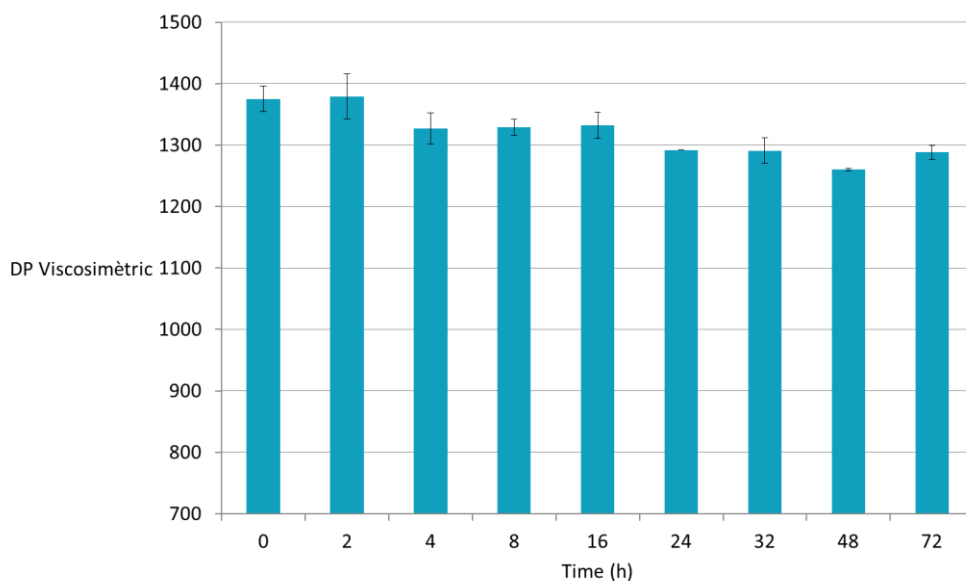


Figure 64- The changes in DP of the softwood pulps treated by mannanase in different incubation times.

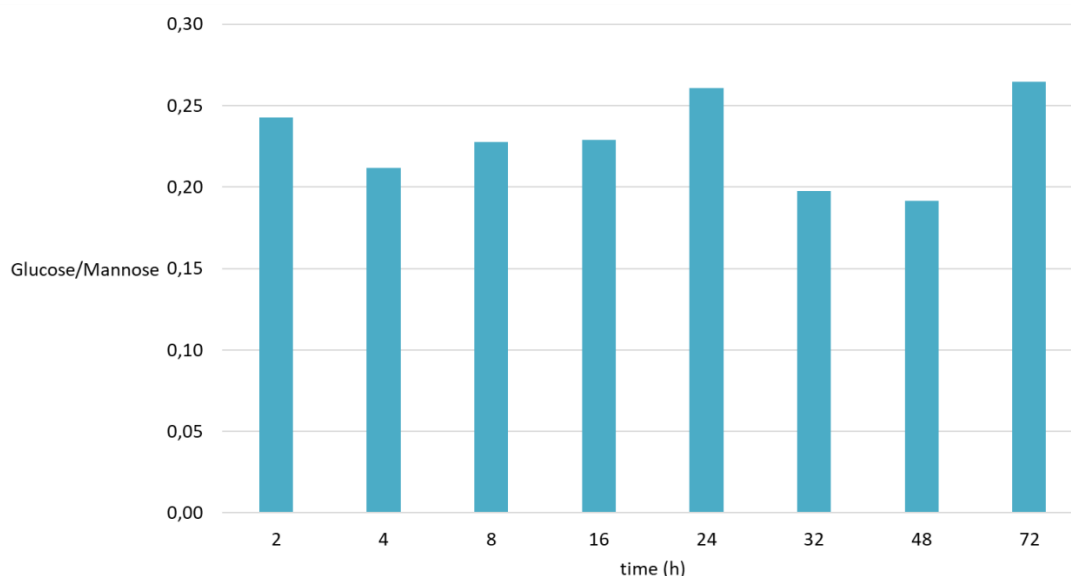


Figure 65- The ratio of glucose to mannose unit in the hydrolysate of treatment of softwood pulps by mannanase in different incubation times.

The continuous evolution of this ratio suggests no specific trend and it stands more or less in the same range. This does not necessarily exclude cellulase activity, as cellulase can decrease cellulose DP without necessarily producing soluble oligomers. However, mannanase treatment did not change the DP of hardwood pulp significantly. Thus, the reason for the DP decrease when applying mannanase of the softwood pulp is not clear.

3-3-7- Study of the combination of enzymes on the extraction efficiency of hemicelluloses from pulp

Different arrangements of enzymes were tested in order to study the effect of interaction between enzymes or their possible synergy. The arrangements that have been studied were: both enzymes together, xylanase and then mannanase, mannanase and then xylanase. These experiments were compared with each enzyme applied alone. All these arrangements were studied for both hardwood and softwood pulps and with two different incubation times of 2 and 4 hours. The general condition of all trials were as for the other enzymatic treatments, namely at temperature of 75°C, at pH of 6 using a buffer solution and a pulp consistency of 10%. Furthermore, the selected dosage of enzymes was 600 ml/ton for xylanase and 100 L/ton for mannanase.

Regarding the trials with two consecutive enzymes, the first enzymatic treatment was performed with one of the enzymes, and then the enzyme was deactivated by boiling the media culture at around 100°C for 10-15 minutes, and then the second enzyme was added. The results in figure 66 compare the effects of the enzyme applied alone and when both

Chapter 3 Study of the extraction of hemicelluloses from bleached kraft pulps

enzymes were applied together. A mixture of enzymes acted better upon the pulps either on softwood or on hardwood, and either for extraction of xylan or for extraction of mannan. It should be mentioned that the effect of mannanase on hardwood is not shown here because it had very low effect on hemicelluloses extraction. In addition, in the experiment of xylanase on softwood there was no release of mannan.

The effect of applying two consecutive enzymes was studied as well. So eight experiments were designed: applying both enzymes in the same time for 2 and 4 hours, 2 first hours xylanase and 2 second hours mannanase and lastly 2 first hours mannanase and 2 second hours xylanase and for both hardwood and softwood pulps.

Figure 67 shows the effect of this arrangement on extraction of xylan from hardwood pulp. In these experiments, no release of mannan was recorded.

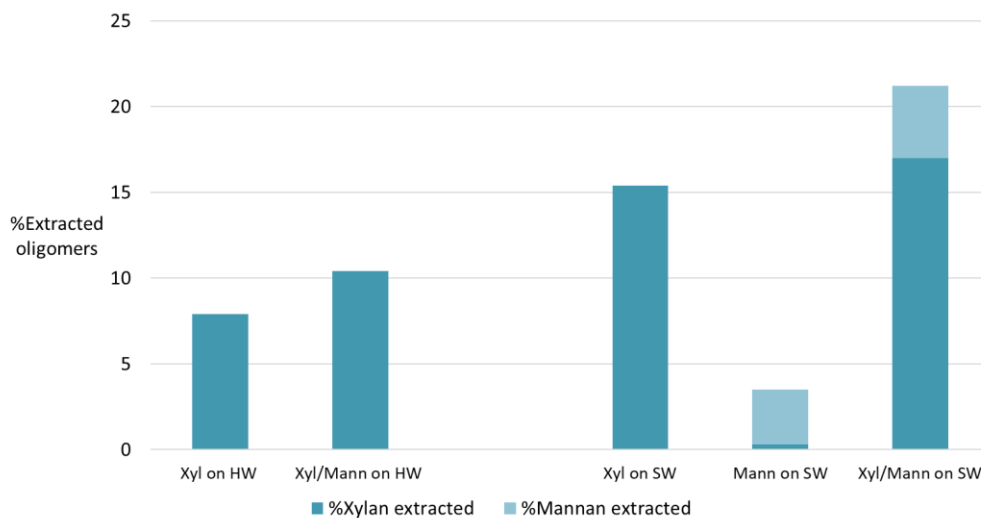


Figure 66- Effect of presence of both xylanase and mannanase on extraction of oligomers of hemicelluloses from hardwood and softwood pulps for 2 h.

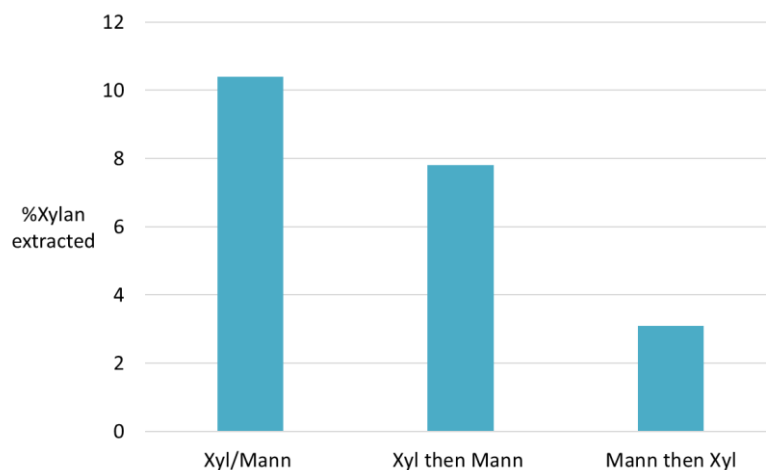


Figure 67- Effect of different arrangement of enzymes on extraction of oligomers of xylan in 2 h from hardwood pulp.

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The results show that it is better to apply both enzymes together. The results for softwood are presented on figure 68.

On softwood, the best results for extraction of xylan belongs to the sequence of xylanase and then mannanase and the best result for extraction of mannan belongs to mannanase and then xylanase which is almost equal to xylanase/mannanase, so in that case no synergy can be seen between the two enzymes. The fact that different results are obtained compared to hardwood pulp supports the fact that either the structure of hemicelluloses are different in both species, and/or that they do not have the same accessibility to enzymes.

The figures 69 and 70 put together all the results considering the incubation times of 2 and 4 hours in a same diagram, for hardwood and softwood pulp, respectively.

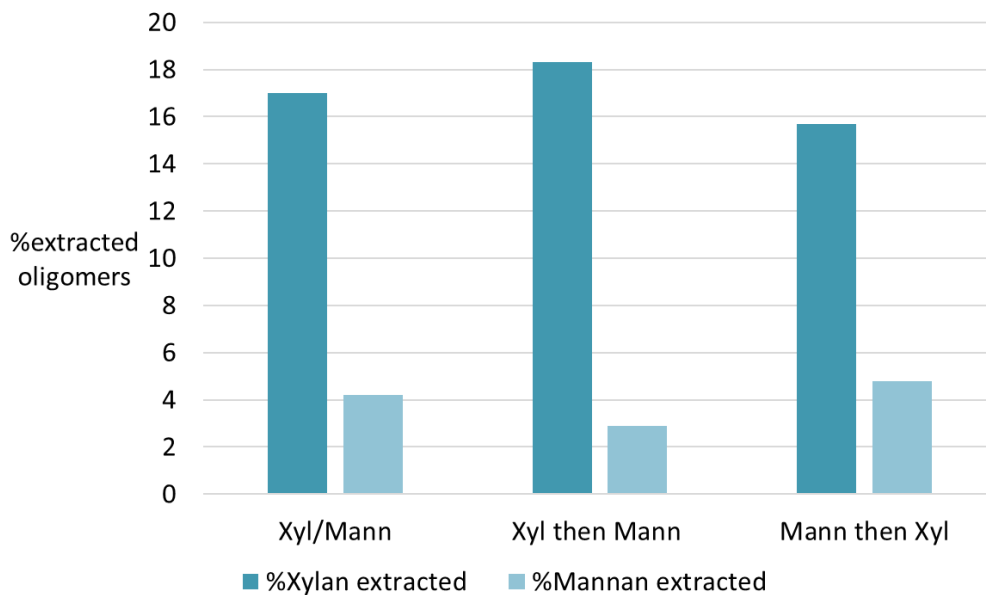


Figure 68- Effect of different arrangements of enzymes on extraction of oligomers of hemicellulose in 2 h from softwood pulp.

According to these results, it is confirmed that the best set regarding the extraction of hemicelluloses for hardwood is a treatment in which both xylanase and mannanase are present. In the case of softwood, there is no clear synergy between enzymes, so their combined or consecutive uses are both possible.

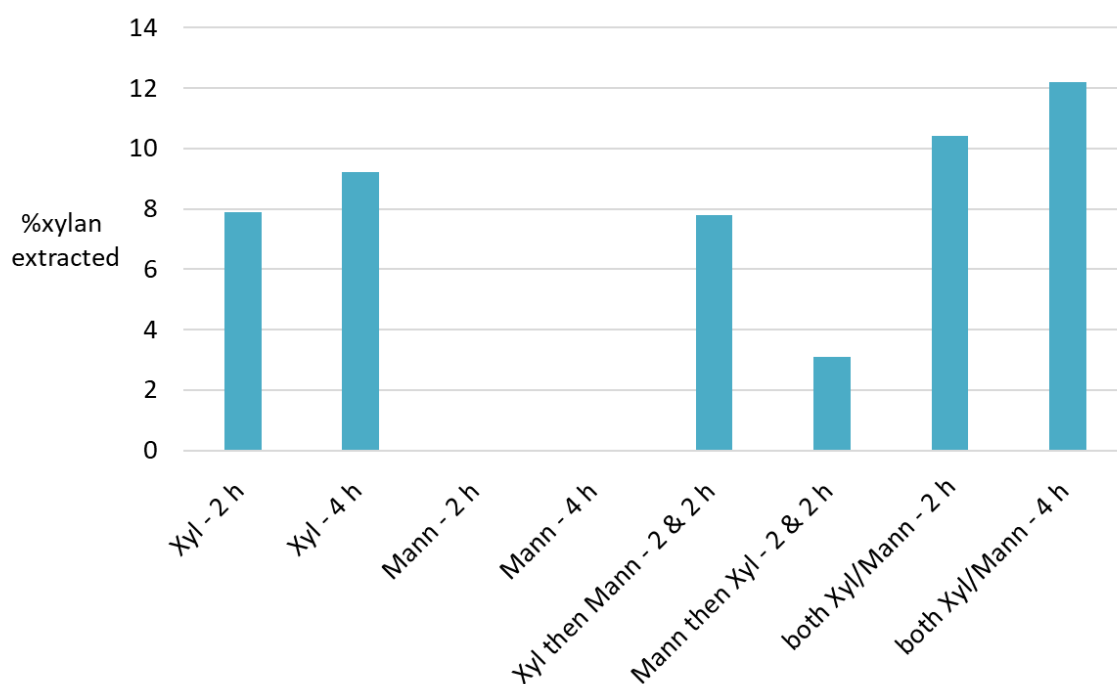


Figure 69- Effect of different arrangements of enzymes on extraction of oligomers of xylan in 2 and 4 h from hardwood pulp.

There are not many studies investigating the synergy between xylanase and mannanase. Synergic effect mostly has been studied between depolymerizing and side-group cleaving enzymes like for example acetylated xylan as a substrate [168, 115, 60]. However, in a study on a sulfite pulp it has been reported that a combination of mannanase and xylanase increased the amount of xylan solubilized by 11% and the amount of mannan solubilized by 50%, clearly indicating a synergistic effect, and suggesting that a certain portion of the mannan and xylan is located in close proximity with each other and that the mannan in the bleached pulp might be shielded or closely associated with the xylan [53]. In another study by the same group on a softwood dissolving pulp, again a combination of xylanase and mannanase extracted more xylan comparing to sequential treatment [56]. A softwood kraft pulp in a sequential incubation with xylanase and mannanase produced the best effect in largest final kappa number reduction and it was concluded that the mixtures of hemicellulase activities can be chosen to enhance pulp bleachability [565]. Furthermore, in a study on sugarcane bagasse the greatest amount of reducing sugar and largest degree of synergy was obtained using a combination of two enzymes (25% mannanase and 75% xylanase) with a NH_4OH pre-treated SCB (just in a comparison between these two enzymes) [566], whereas in another study on the pretreated sugarcane bagasse no effect of synergy of xylanase and mannanase has been observed [567].

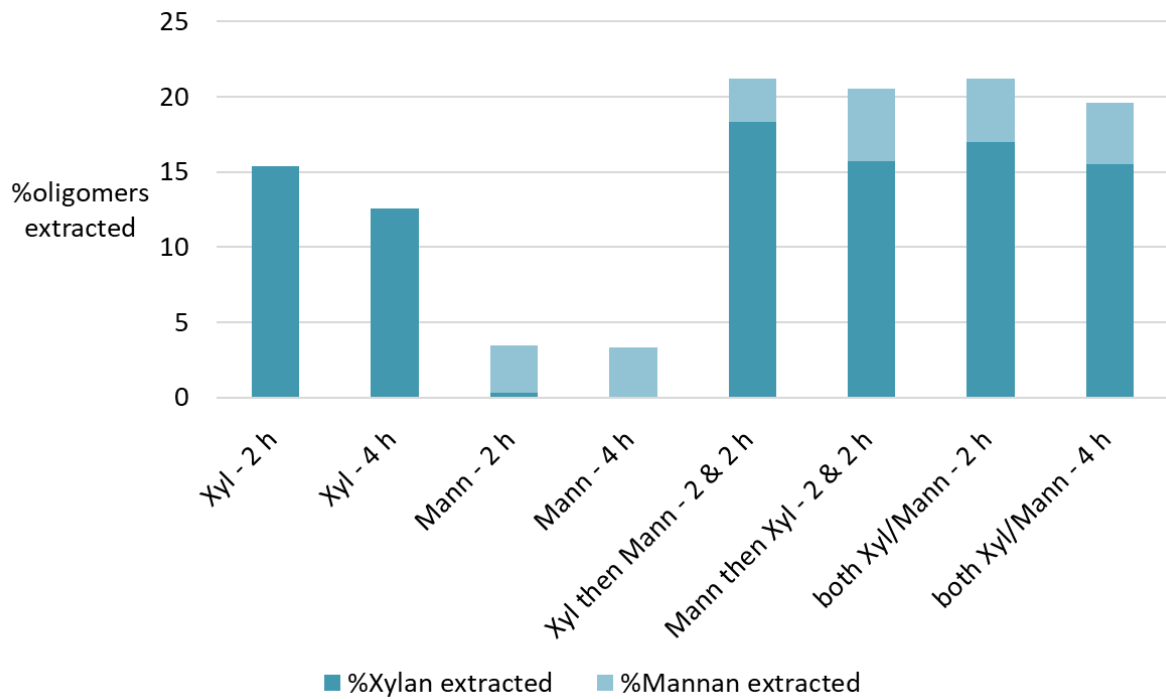


Figure 70- Effect of different arrangement of enzymes on extraction of oligomers of hemicelluloses in 2 and 4 h from softwood pulp.

It is argued that a main-chain cleaving enzyme will have enhanced activity if substituents are first removed through debranching enzymes [259], on the basis that the substituent poses a steric hindrance to the main-chain cleaving enzyme [563].

The DP of the treated pulps with different arrangements of enzymes has been measured (figure 71). We observe similar trend as obtained previously: increase in DP when using xylanase, which can be explained by the removal of small oligomers chains, and a slight decrease of DP when using mannanase.

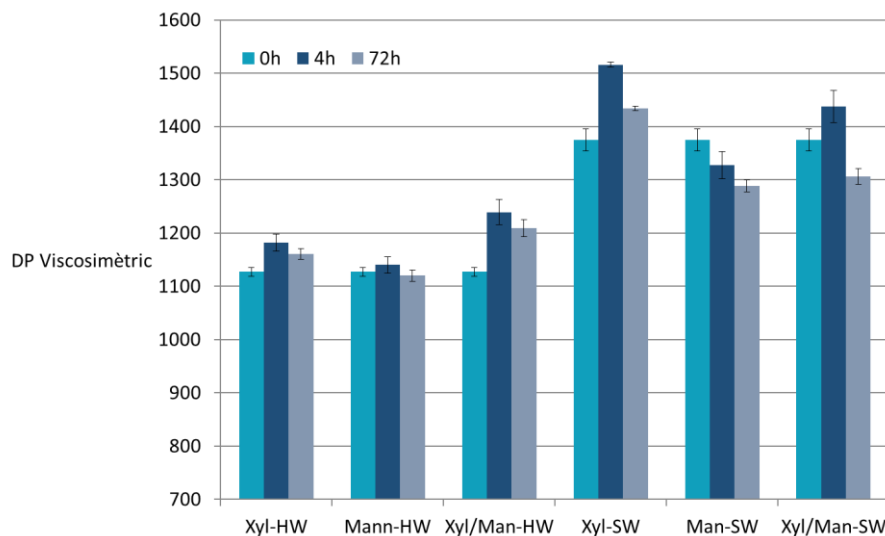


Figure 71- Comparison of changes in DP in hardwood and softwood pulps treated with xylanase and mannanase in different reaction times.

3-4- Combination of enzymatic treatments and CCE

The results obtained with enzymatic treatment alone showed that there are still significant amounts of residual hemicelluloses left, which is why it was decided to combine the enzymatic extraction with CCE. It is likely that CCE cannot remove all the hemicelluloses left in the structure of fibers after enzymatic treatment but the ones that are extractable maybe help us to know more about their structure.

CCE treatments have been done on enzymatically-treated softwood and hardwood pulps, both, in two different incubation times (4, 72 h), using xylanase and mannanase in different arrangements. The different arrangements of enzymes were as follows: xylanase alone, mannanase alone and both enzymes at the same time. Mannanase on hardwood was not done since the previous results did not show significant effect on hemicellulose removal.

After each sequence of enzymatic treatment, there was a step of deactivation in which the content was boiled and then filtered. The extracted hydrolysates were stored and then the treated pulps were treated with a CCE step which was similar to CCE treatment of starting pulps. The treated pulps were measured rapidly for their water content and then a solution of NaOH was prepared, the concentration of which was calculated in order to reach 11%, taking into account the remaining water in the already-enzymatically-treated pulp. The CCE was done at ambient temperature and during 1.5 hour. For this study the effect of the treatments was measured by the residual hemicelluloses in the pulps.

Figures 72 and 73 show the percentage of hemicellulose that remained in the hardwood pulps after two steps of treatments: enzymatic treatments (4 and 72 hours of incubation) followed by CCE treatment.

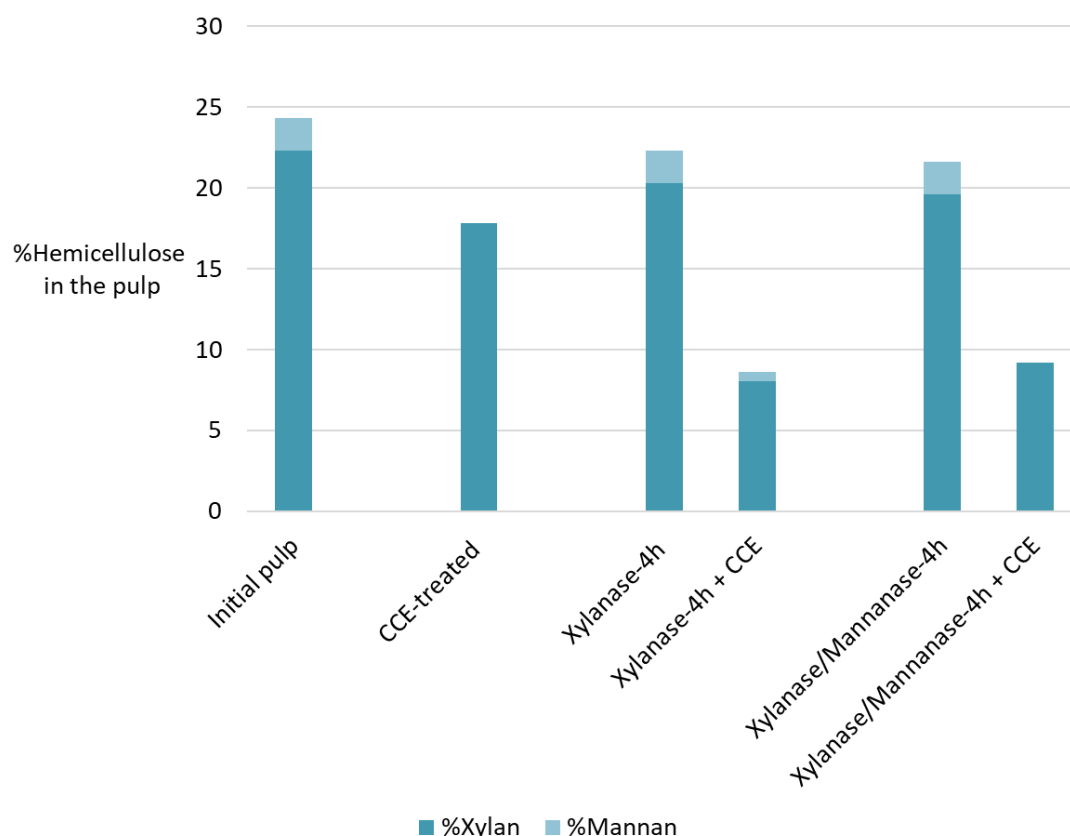


Figure 72- A comparison between the remained proportion of hemicelluloses in hardwood pulp after CCE treatment performed after enzymatic treatment in 4 h incubation time, with CCE treatment only and enzymatic treatment only.

According to the results, CCE treatment could extract more hemicelluloses from the hardwood original pulp than enzymatic treatments with different arrangements. It has been reported in a study that xylan pre-treatment of an oxygen delignified hardwood kraft pulp could decrease the amount of xylan from 22.5% to 12.1%, whereas a CCE treatment with 10% NaOH decrease this value to 4.2% [11, 40]. The same results have been obtained from another study with similar sets of experiments and objectives [36].

Furthermore, CCE could extract almost all mannan from the pulp, whereas the enzymatic treatment could not extract mannan from the original pulp.

The combination of enzymatic treatment and CCE was much more efficient to extract hemicelluloses from the hardwood pulp compared to CCE or enzymatic treatments only. This could be explained by the fact that enzymes might reduce the size of residual hemicelluloses, and/or make them more accessible.

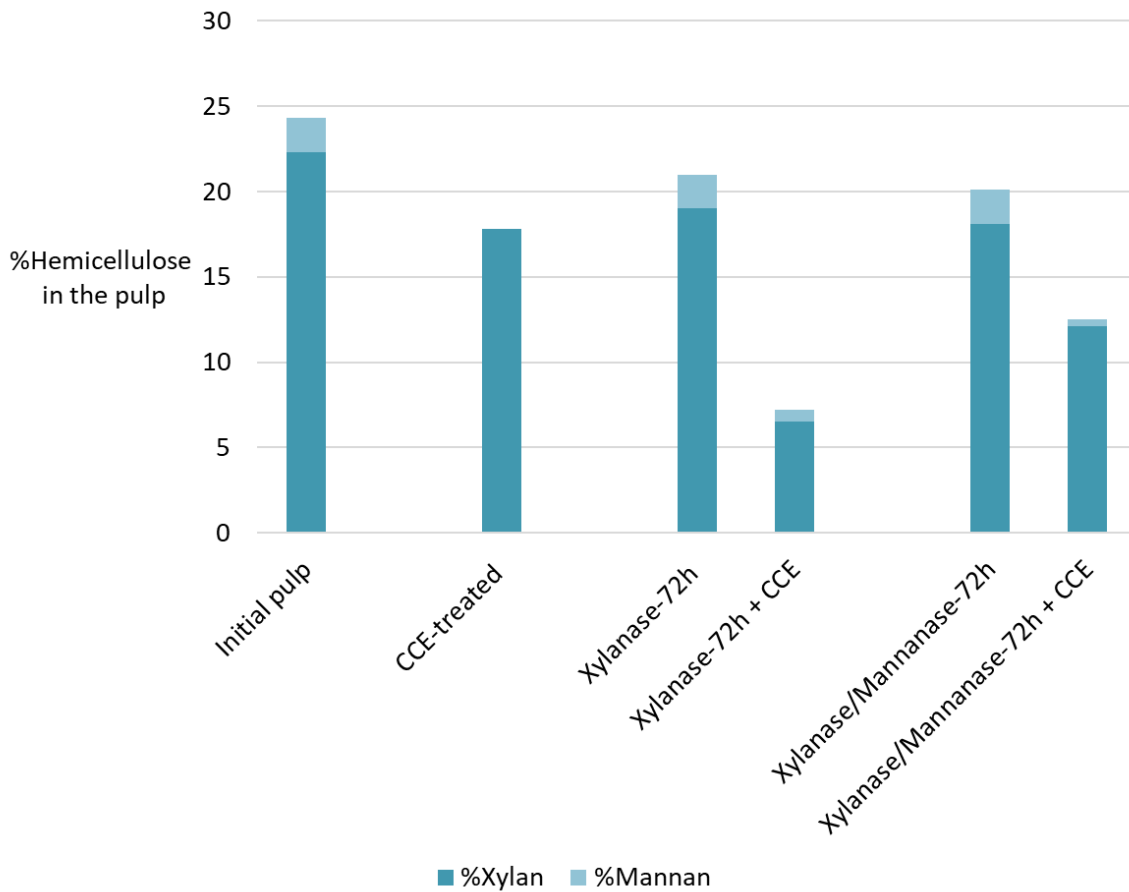


Figure 73- A comparison between the remained proportion of hemicelluloses in hardwood pulp after CCE treatment performed after enzymatic treatment in 72 h incubation time, with CCE treatment only and enzymatic treatment only.

The comparison between 4 and 72 hours' incubation times (Figures 72 and 73), showed that increasing the incubation time hardly improved the results (about 1% less hemicellulose for the xylanase-CCE treatments) or even gave a higher residual hemicellulose value for the xylanase/mannanase – CCE combination.

Figures 74 and 75 show the results obtained for the softwood pulp. In that case, the combination of enzymes and CCE did not work better than CCE alone, except when mannanase treatment was applied for 72 hours prior to CCE. It can be noticed that mostly only xylan was removed by these treatments for the softwood pulp. In our case, mannans seem to be recalcitrant to these treatments, even when most part of the xylans was removed. Thus it does not seem that mannan recalcitrance is linked to their inaccessibility due to the presence of precipitated xylans after cooking.

Figure 76 synthetize the effect of CCE on enzymatically treated hardwood and softwood pulp. Although there is no definite rule in different arrangements, it seems that the effect of CCE

treatment applied after enzymatic treatment was more efficient for hardwood pulp than for softwood pulp.

Another result can be observed: the CCE treatment was less efficient after the combined xylanase/mannanase treatment for 72 hours, compared to single enzyme treatment. We have no explanation for that result.

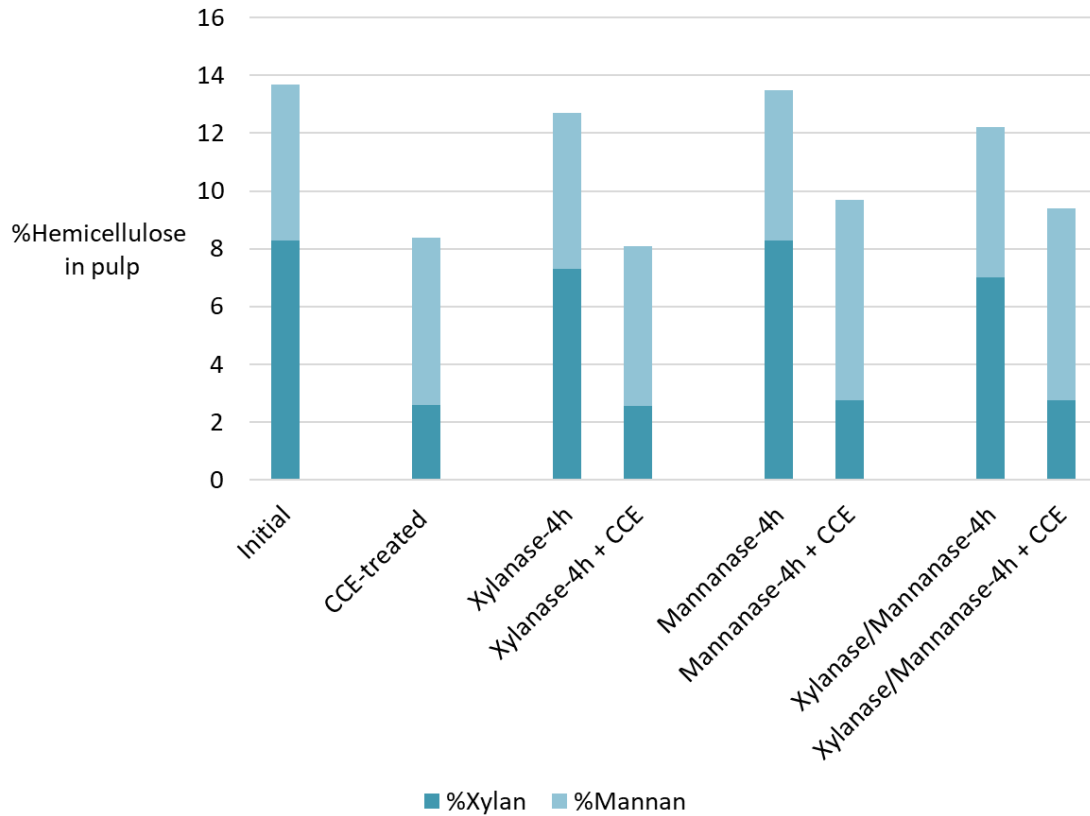


Figure 74- A comparison between the remained proportion of hemicelluloses in softwood pulp after CCE treatment performed after enzymatic treatment in 4 h incubation time, with CCE treatment only and

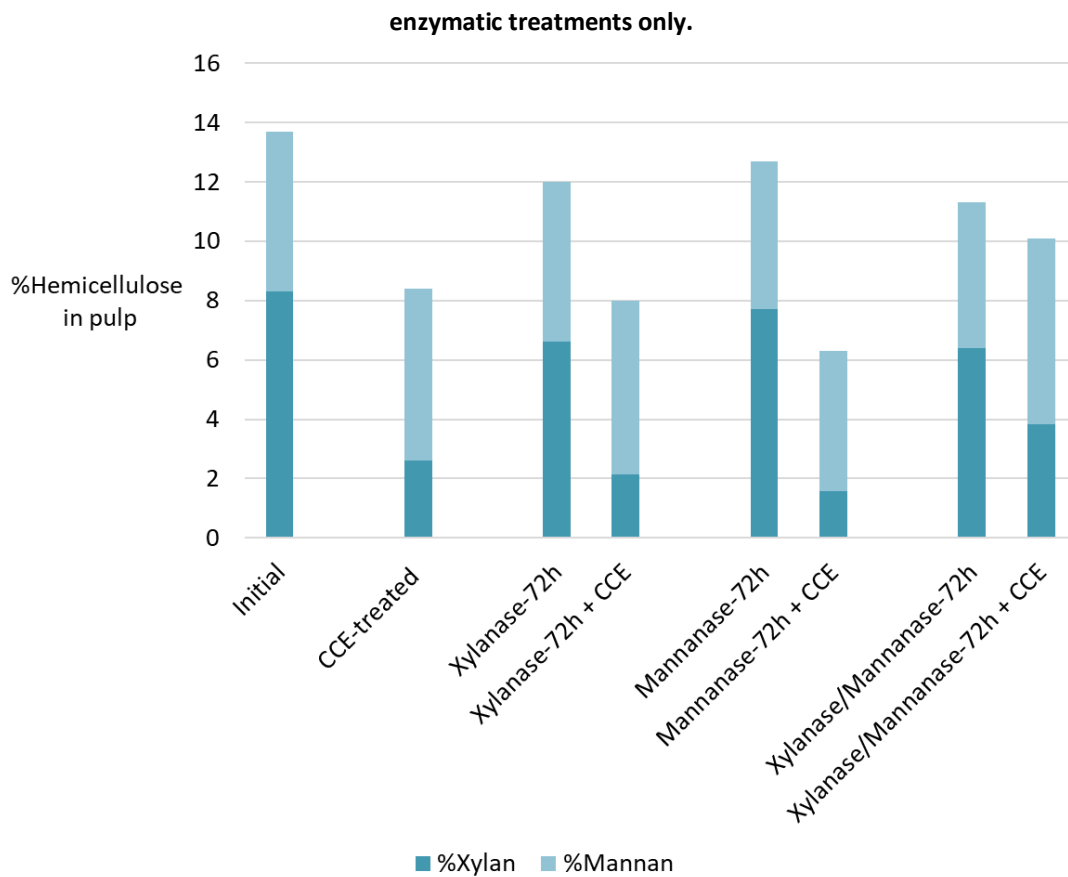


Figure 75- A comparison between the remained proportion of hemicelluloses in softwood pulp after CCE treatment performed after enzymatic treatment in 72 h incubation time, with CCE treatment only and enzymatic treatments only.

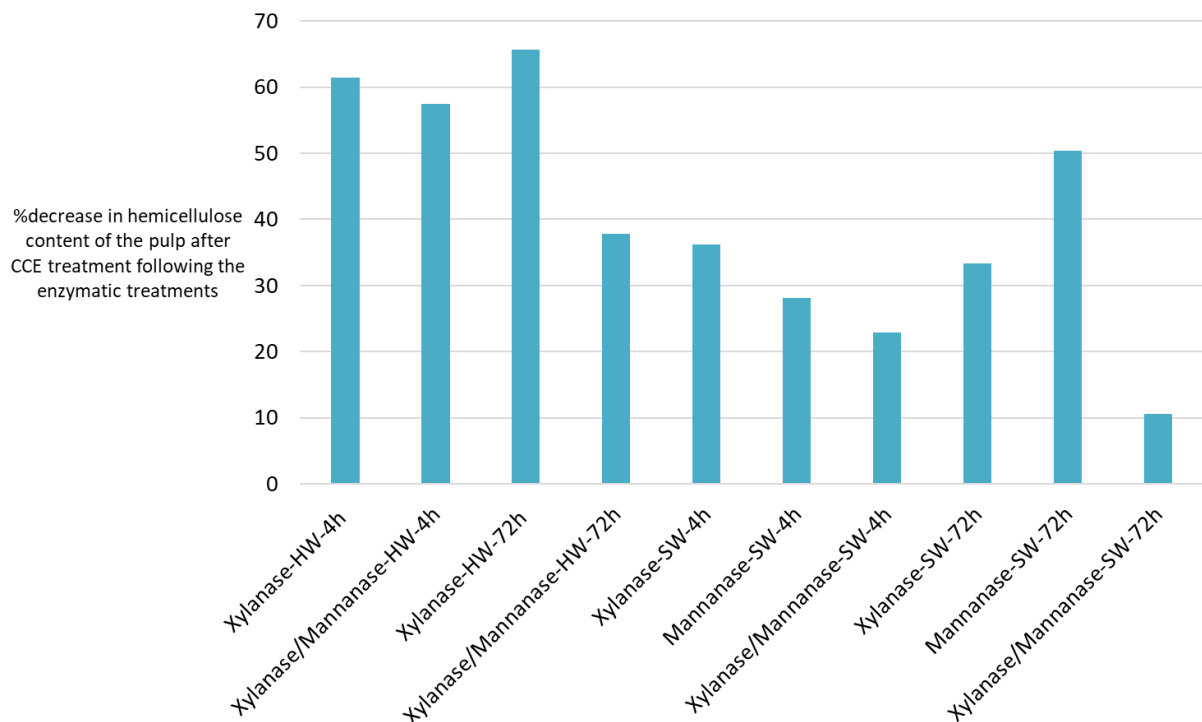


Figure 76- The effect of CCE treatment in decrease of hemicelluloses content in enzymatically treated pulps (in different arrangements of enzymes, in 4 and 72 hours' incubation times and for both hardwood and softwood pulps).

3-5- Effect of enzymatic treatments on molecular size distribution of pulps

3-5-1- Molecular size distribution of enzymatically-treated hardwood pulps

Figure 77 compares the molecular size distributions of the xylanase hardwood treated pulps (2 hours and 72 hours) and of the starting pulp: the partial removal of hemicelluloses by xylanase can be visualized by the shortening of the first peak. There is no substantial difference between the distributions of 2 hours and 72 hour xylanase treated pulps, which confirms results presented earlier in this chapter.

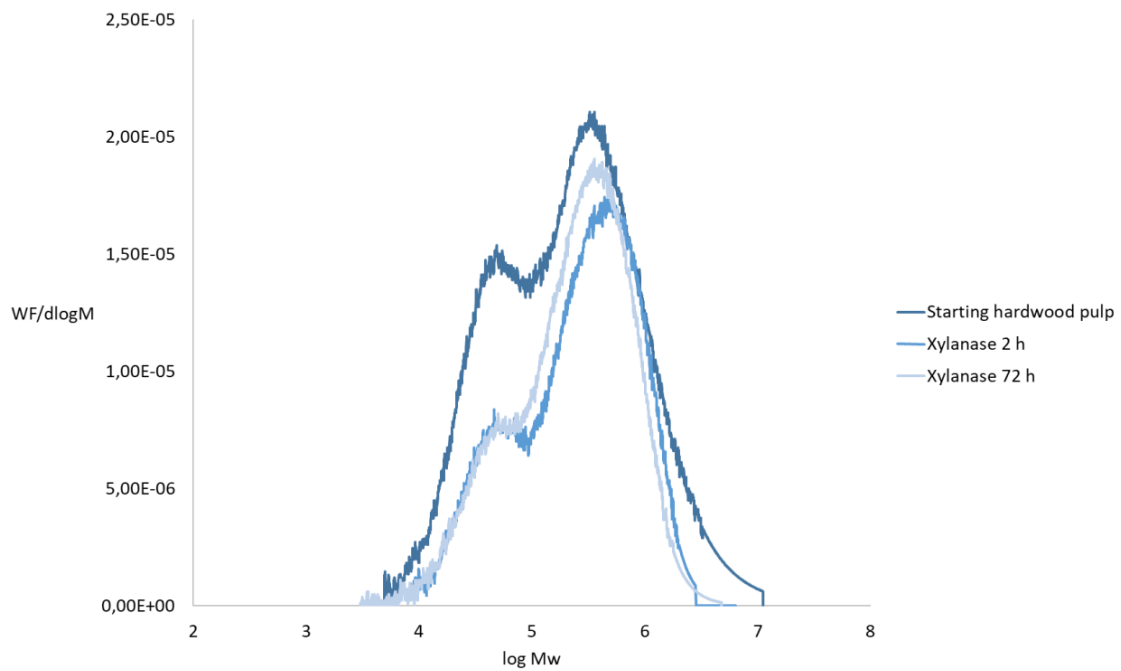


Figure 77- The difference between molecular weight distribution in starting hardwood and xylanase-treated hardwood pulps for incubations times of 2 and 72 h.

3-5-2- Molecular size distribution of CCE treated pulps compared to enzyme treated pulps

The comparison of molecular weight distribution of starting hardwood pulp, CCE-treated hardwood pulp and xylanase-treated hardwood pulp with incubation time of 72 hours is presented on figure 78. The peak corresponding to hemicelluloses is slightly more visible for the enzyme treated pulp than for the CCE treated pulp, which is in accordance with the fact that the CCE treatment removed hemicelluloses more efficiently than the enzyme treatment.

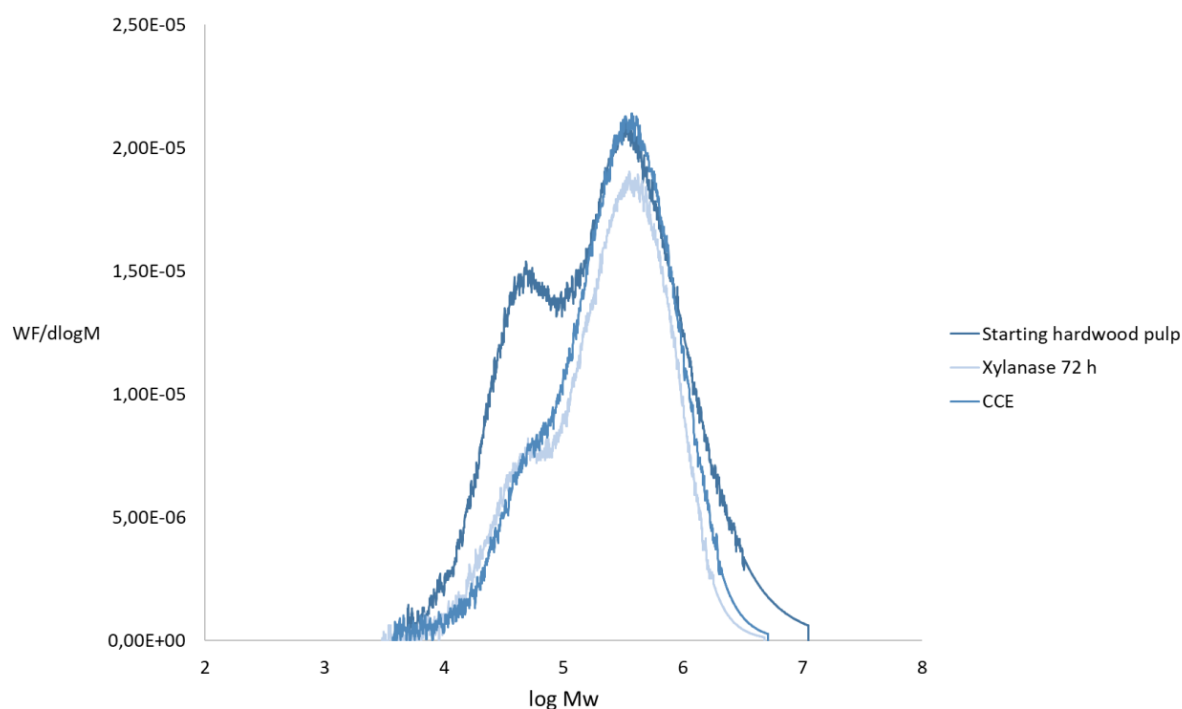


Figure 78- The difference between molecular weight distributions in starting hardwood pulp, CCE-treated hardwood pulp and xylanase-treated hardwood pulp with the incubation time of 72 hours.

3-5-3- Molecular size distribution of pulps treated with a combination of enzymatic treatment and CCE

Analysing the molecular weight distribution of the treated pulps with the combination of enzymatic treatment and CCE highlights the influence of these combinations in removing hemicelluloses and leaving an appropriate homogeneous distribution (figures 79 and 80). It is clearly seen that from initial hardwood pulp to xylanase 72h + CCE treatment, the distributions become more homogeneous and the hemicelluloses level decreases and at the end there is almost no hemicelluloses left in the final pulp. This is in accordance with the results obtained previously.

Some enzymatic experiments, as aforementioned, have been performed with different arrangements of enzymes. The molecular weight distribution analyses have been also done on the hardwood pulps treated by xylanase/mannanase (both in the same culture) in two different reaction times of 4 and 72 hours followed by CCE treatment (figure 81). The results show no meaningful difference between different arrangements, once mannanase is added to the preparation of enzyme. But still there is a more homogeneous distribution while the longer time of incubation is applied. Compared to the starting hardwood pulp, the best distribution leading to the highest removal of hemicelluloses belongs to xylanase 72 h followed by CCE treatment and then xylanase/mannanase 72 h followed by CCE treatment.

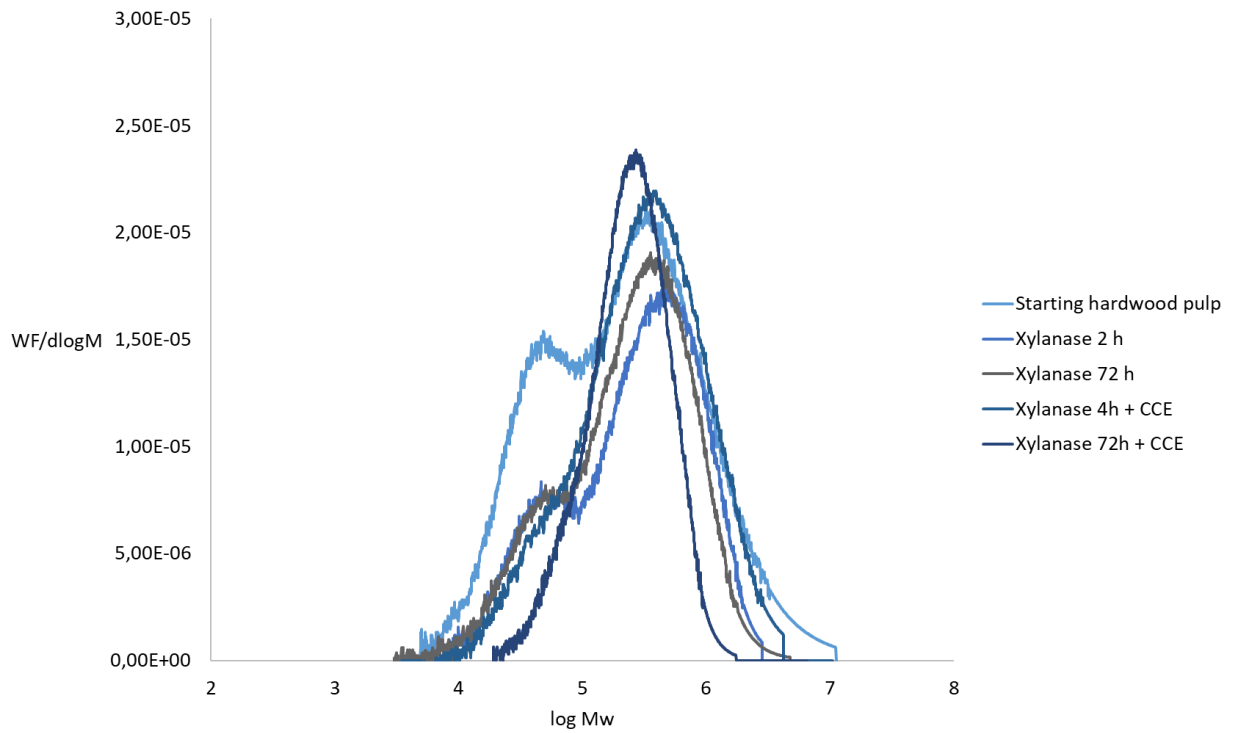


Figure 79- Comparison of molecular weight distributions between starting hardwood pulp, xylanase-treated pulp in two incubation times of 2 and 72 hours and the combination of xylanase treatments and CCE in two enzymatic incubation times of 4 and 72 hours.

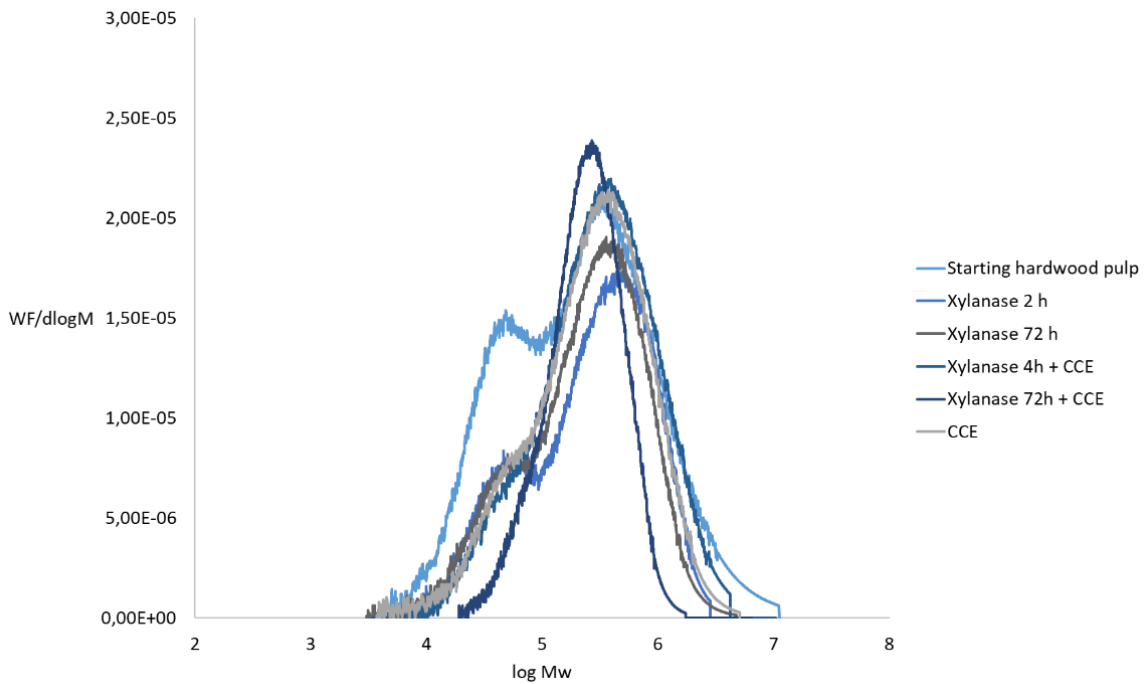


Figure 80- Difference between distributions of molecular weights in initial hardwood pulp, xylanase-treated pulps in two incubation times of 2 and 72 hours, the combination of xylanase treatments in two incubation times of 4 and 72 hours with CCE treatment and CCE treatment alone.

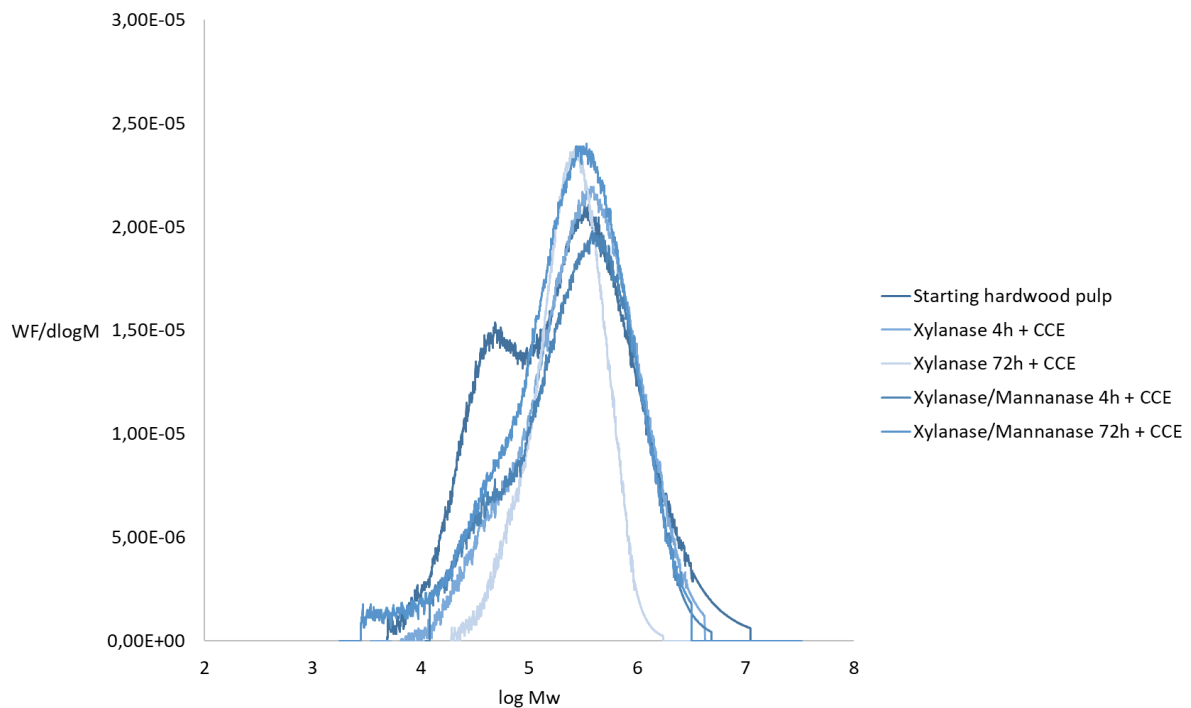


Figure 81- Comparison between molecular weight distribution of pulps treated by xylanase 4 h, xylanase 72 h, xylanase/Mannanase 4 h and xylanase/mannanase 72 h, all followed by CCE treatment.

Finally, the best set of experiments regarding the least remained hemicelluloses in the hardwood pulp with the most homogeneous distribution is the experiments of xylanase 72 h + CCE in which a very mild homogeneous distribution of molecular weight is represented by this analysis.

3-6- Effect of the different treatments on the morphology of the fibers

Enzymatic treatments are likely to affect the surface morphology of fibers due to removal of hemicelluloses or other components like lignin, which can cause fibrillation of fibers [83].

Optical microscopy and Scanning Electron Microscopy (SEM) analyses have been done on starting hardwood and softwood pulps and enzymatically-treated pulps, both with xylanase and mannanase and in two different incubation times of 2 and 72 hours.

In the first step, the optical microscopy analysis has been done to check out if there is a difference in the appearance of fibres that might be observable in the scale of optical microscopy. As expected in this scale of microscopy, no observable difference has been detected between starting and treated pulps.

Consequently, SEM was checked on four sets of enzymatically-treated pulps: xylanase on hardwood pulp, xylanase on softwood pulp, mannanase on hardwood pulp and mannanase on softwood pulp and each set in two different incubation times of 2 and 72 hours.

3-6-1- SEM of xylanase-treated fibers

3-6-1-1- Hardwood pulp

Studying the photographs obtained from SEM imaging does not show a big difference between the surface characteristics of starting pulps and enzymatically treated ones. However, a closer look to the fibers could bring out some changes on the surface of the treated fibers which is observable in some cases and in some parts.

A comparison between the SEM analysis of starting hardwood pulp and xylanase-treated hardwood pulps in 2 and 72 hours presents different phenomena. In starting hardwood pulp there are some surfaces which are rather smooth, although with the presence of some filaments, whereas xylanase-treated pulps show fibers that are less smooth with some visible fibrils that are separating from the fibers, as well as other small particles (figures 82-83).

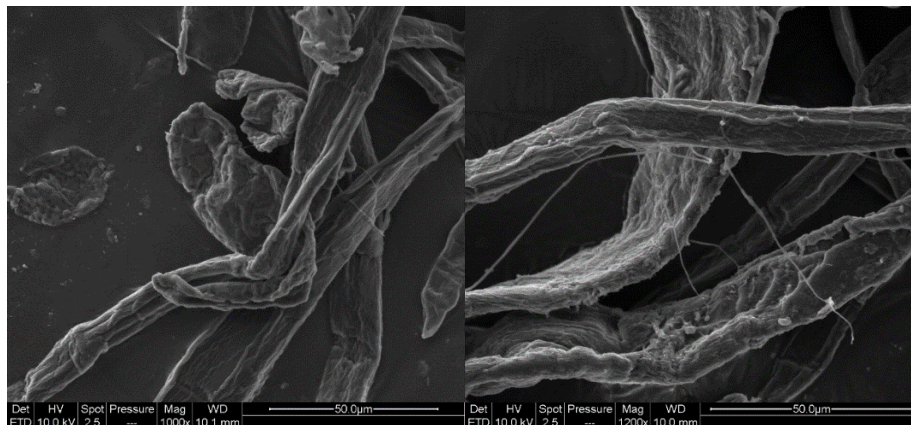


Figure 82- Comparison between the SEM analysis of starting hardwood pulp (left) and xylanase-treated hardwood pulp in 2 hours (right).

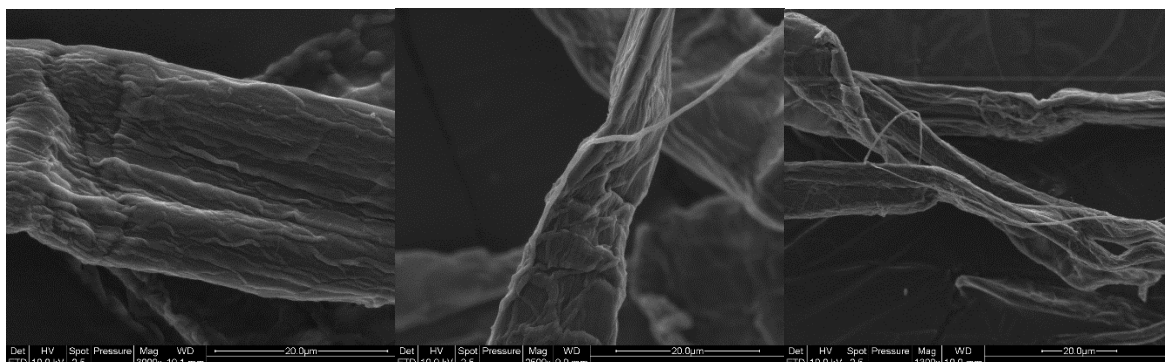


Figure 83- Comparison between the SEM analysis of starting hardwood pulp (left) and xylanase-treated hardwood pulp in 2 hours (center) and xylanase-treated hardwood pulp in 72 hours (right).

3-6-1-2- Softwood pulp

Similar to hardwood, softwood fibers show diversity in physical appearance (figure 84). There are some fibers that possess smooth surface and there are some others that have fibrils at the surface and there are some fibers on which seemingly some particles and some fibrils or other substances are being detached.

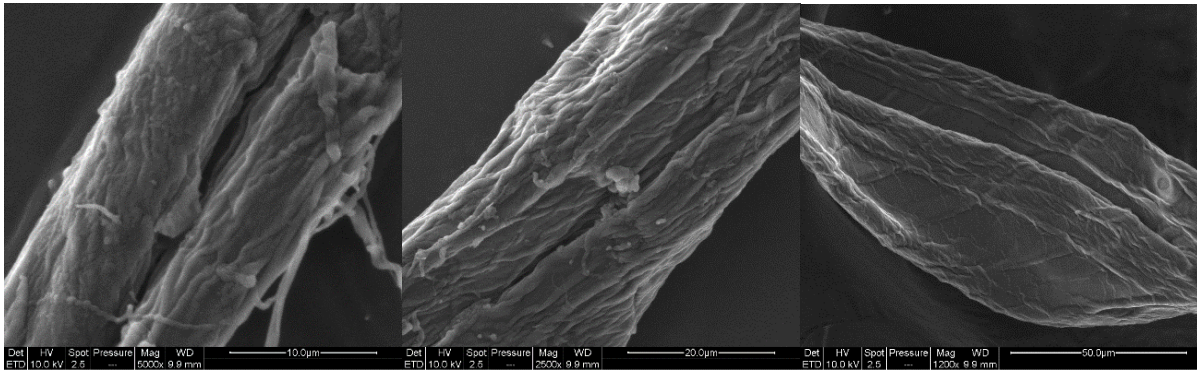


Figure 84- SEM analysis of starting softwood pulp.

In the 2 hours-xylanase-treated softwood pulp, a difference is observed with hardwood fibers: the softwood fibers have less fibrils that are beginning to detach from the fibers as well as less flakes and peeling effect (figure 85). In fact, seemingly the surface of softwood treated fibers with xylanase is more regular and smoother contrary to its starting pulp and even there exist some fibers that are completely smooth without any cracks in the surface but with some holes and effect of peeling on them. However, there are fibrils that appear on the surface without showing detaching effect. These fibrils exist in various orientations.

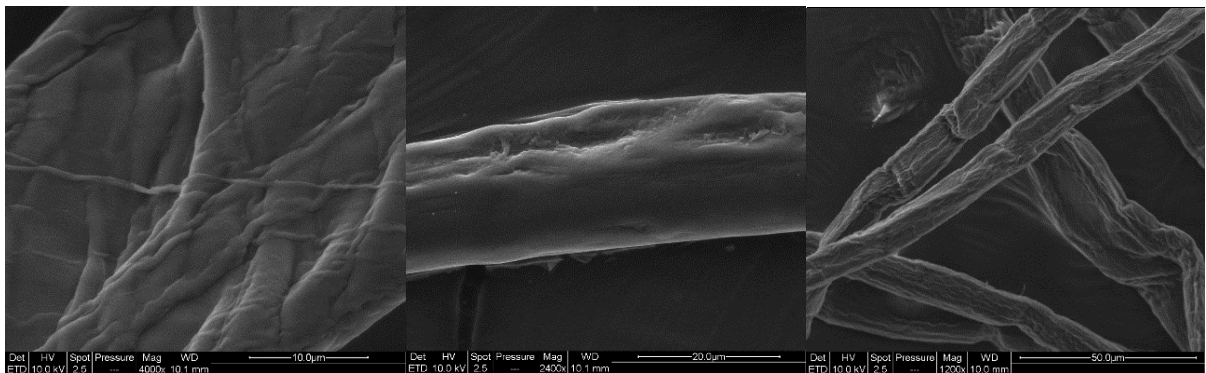


Figure 85- SEM analysis of xylanase-treated softwood pulp for 2 hours.

However, for treatment in 72 hours with xylanase, the surface of softwood fibers shows more irregularity and more roughness than treatment in 2 hours (figure 86).

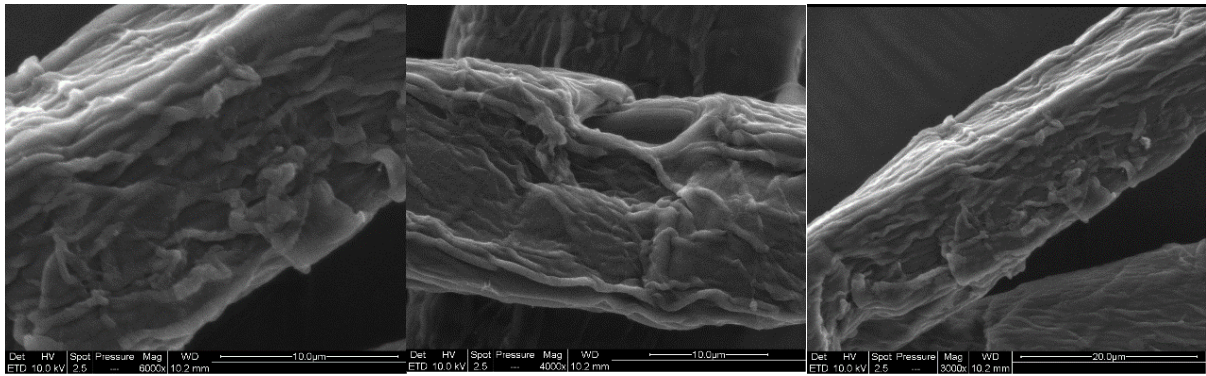


Figure 86- SEM analysis of xylanase-treated softwood pulp for 72 hours.

An overall comparison between the SEM images of starting softwood pulp and xylanase-treated softwood in 2 and 72 hours might suggest the fibers become smoother from starting state to the 72 hours of treatment with xylanase (figure 87).

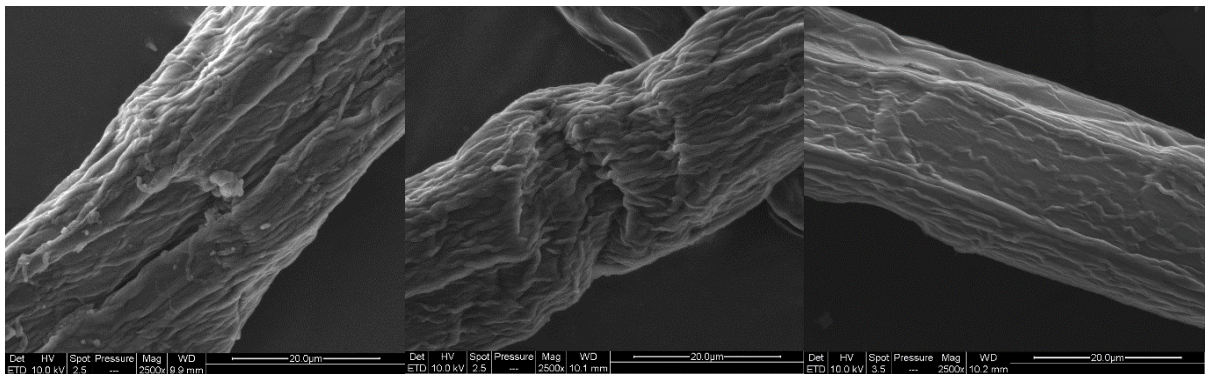


Figure 87- Comparison between the SEM analysis of starting softwood pulp (left) and xylanase-treated softwood pulp in 2 hours (center) and xylanase-treated softwood pulp in 72 hours (right).

3-6-2- SEM of mannanase-treated fibers

3-6-2-1- Hardwood pulp

The micrographs of hardwood treated pulp with mannanase during 2 hours show no significant change (figure 88) compared to the starting pulp.

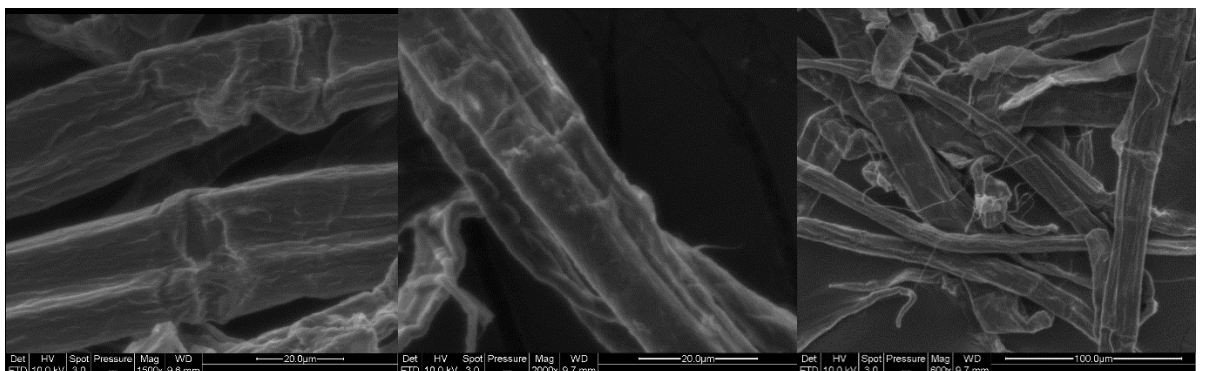


Figure 88- SEM analysis of mannanase-treated hardwood pulp for 2 hours.

However, for the same treatment lasting 72 hours, fibrillations and segregation of some particles can be observed (figure 89). These treated fibers show some changes in morphology even though no substantial hemicelluloses have been extracted.

These micrographs show very small particles which are separating from the main fibers, the fibrils which have been visible due to treatments, the peeling effect is observed and therefore all the signs of fibrillation, segregation, flakes, peeling and cracks are observed in the morphology of the treated fibers. These changes are much more pronounced than treatment after 2 hours of incubation. All this can show the activity of the enzyme, even if the hardwood includes a very little amount of mannans in its structure.

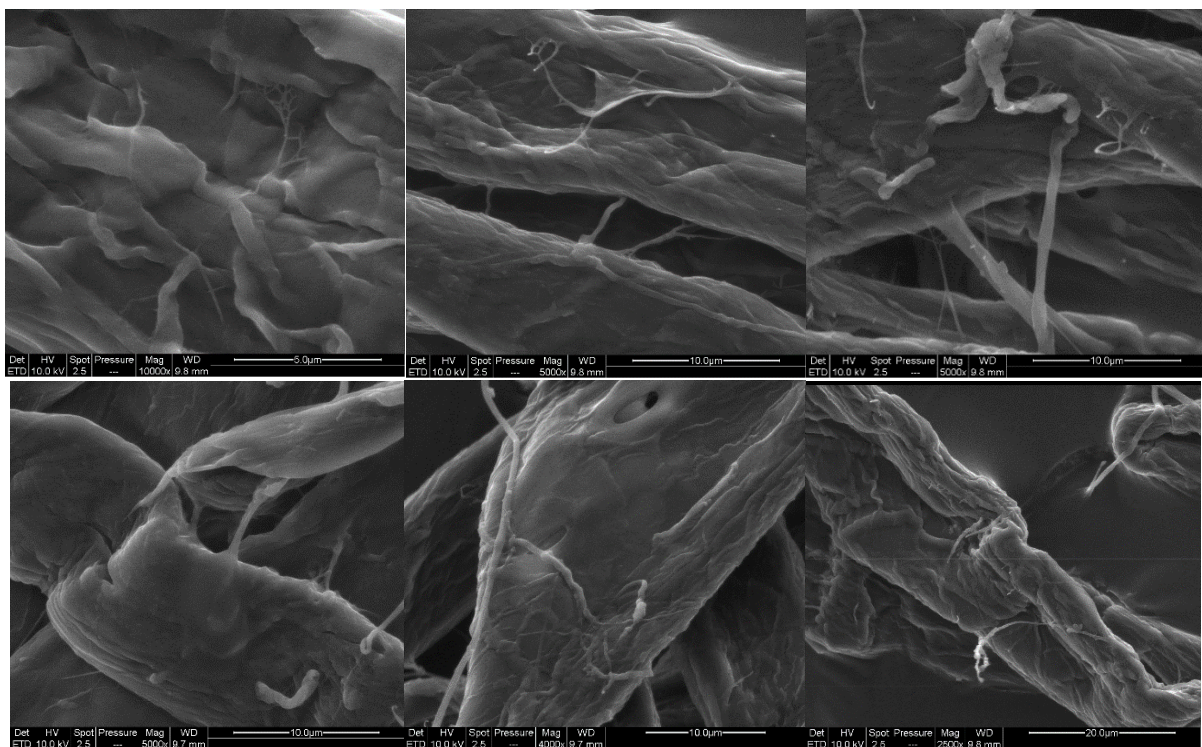


Figure 89- SEM analysis of mannanase-treated hardwood pulp for 72 hours.

3-6-2-2- Softwood

The softwood pulp that has been treated with mannanase for 2 hours showed slight changes in morphology (figure 90).

The treatment of softwood with mannanase for 72 hours shows a real difference in morphology of the fibers (figure 91) compared to the starting pulp. These treated fibers show the signs of big fibrillations, segregation of fibrils from the surface of the fibers, the appearance of many fibrils in the surface of the fibers, the peeling effects, the flakes, many cracks and in some parts of course the surfaces who are smoother.

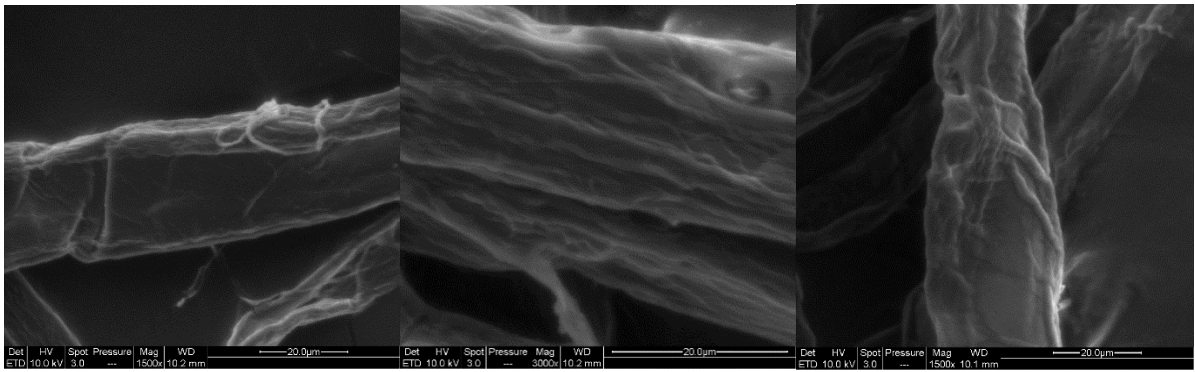


Figure 90- SEM analysis of mannanase-treated softwood pulp for 2 hours.



Figure 91- SEM analysis of mannanase-treated softwood pulp for 72 hours.

3-7- General conclusion of the chapter

Hardwood and softwood never-dried fully bleached kraft pulp were treated with different treatment: CCE, hemicellulases and combination of these two treatments.

Chapter 3 Study of the extraction of hemicelluloses from bleached kraft pulps

The CCE treatment was more efficient on the softwood pulp than on the hardwood pulp, as it removed 26% of hemicelluloses from the hardwood compared to 36% from the softwood pulp. CCE removed only 20% of the hardwood xylan, whereas it removed 55% of them in the softwood pulp. Inversely, glucomannans from the hardwood pulp were completely removed whereas only 7% of them were extracted from the softwood pulp. The difficulty to remove xylan from hardwood compared to softwood was confirmed when using the xylanase treatment.

With CCE treatment the DP values of both starting pulps showed a slight increase suggesting the dissolution of the lower-molecular-weight hemicelluloses and no effect on cellulose. It was confirmed by studying the molecular weight distribution of the pulps with the lowering the hemicellulose shoulder

The enzymatic treatments through different arrangements of xylanase and mannanase studied the efficiency of these two enzymes in the solubilization of hemicelluloses. The kinetic study of performance of each enzyme on starting pulps showed limitation in extraction yield of enzymes to pull out the hemicelluloses from the fiber structures: a plateau was reached after 48 hours and there was no significant effect of enzyme dosage, rising up the hypothesis of inaccessibility of substrates to enzymes. By releasing just the xylans, xylanase showed itself selective in hydrolyzing the xylan chains, while mannanase treatment resulted in releasing both xylans and mannans suggesting either that the enzyme solution did not contain pure mannanase, or that the dissolution of mannans facilitated the extraction of xylans. The best results obtained for the extraction of hemicelluloses from pulp were: 14.8% extraction yield for xylanase on hardwood pulp, 22.3% for xylanase on softwood pulp, 1.1% for mannanase on hardwood pulp and 7.9% for mannanase on softwood pulp. The extraction of hemicelluloses seemed easier from softwood than from hardwood pulp.

The study of the effect of pulp consistency of hemicelluloses extraction by enzymes showed better results for 5% compared to 10%, reflecting the importance of media homogeneity. However, the pulp consistency of 10% was preferred due to its industrial compatibility. Furthermore, due to the problematical effect of presence of buffer for further analysis of the hydrolysates, the manipulations without buffer were run and good results were obtained, although in the case of softwood pulps, the presence of buffer, in particular for long incubation times, was more effective.

Chapter 3 Study of the extraction of hemicelluloses from bleached kraft pulps

The xylanase treated pulps, showed a slight increase in DP, probably with the same logic as CCE treated pulps in the process of removing of small-chain hemicelluloses, which was confirmed by the study of distribution of molecular weight with the lowering the hemicellulose peak. However, in the case of mannanase treatment on softwood, the DP was slightly lowered. We have no explanation for that as no viscosity loss was observed when the hardwood pulp was treated with mannanase.

Combination of enzymes was studied. The use of both enzymes, either in a consecutive way or added together led to overall better xylan extraction from the softwood pulp, but no significant impact could be seen on the extraction of mannans. In the case of the hardwood pulp, the best result was obtained when xylanase and mannanase were added together, rather than in a consecutive way, which implies a strong synergistic effect in that case. The difference in arrangements supports probably the fact that the hemicelluloses have either different structures in the two wood species and/or do not show the same accessibility to enzymes.

Applying a CCE treatment after the enzymatic treatment was much more efficient than CCE alone or enzymatic treatment alone especially for long incubation times: the percentage of hemicelluloses in the softwood pulp dropped from 13.7% to 6.2%, and from 24.3% to 7.0% for the hardwood pulp. One explanation could be that the enzymatic treatment has decreased the DP of hemicelluloses making them more easily solubilized by the CCE treatment.

The study of the fiber morphology using scanning electron microscopy did not show overall significant changes.

Chapter 4 – Study of the effect of the enzymatic treatments on the structure of the extracted hemicellulosic oligomers

Introduction

In this chapter the hydrolysates obtained after the enzymatic treatments of the pulps, as well as those obtained after a combination of enzymatic treatments and CCE are studied through chromatography and mass spectroscopy analyses. Regarding the enzymatic treatment, it is studied in different arrangements of enzymes and on hardwood and softwood pulps.

In the chromatography analysis, the molecular size distributions of isolated components are studied and for the enzymatically extracted oligomers of hemicelluloses, as the main extracted components are short, they are characterized by two other chromatography columns to monitor more precisely the released oligomers.

Finally, the structure of isolated components is studied through MALDI-TOF.

4-1- Characterization of hydrolysates extracted by enzymatic treatments

4-1-1- Molecular weight distribution

Gel Permeation Chromatography (GPC) of the isolated oligomers of hemicelluloses after different enzymatic treatments was done in order to study their molecular weight distributions. The freeze-dried samples were analyzed on a Biogel P2 (Biorad) column, with on-line light-scattering, UV absorbance, and refractive index detectors, at 50°C, with deionized H₂O at 0.5 ml min⁻¹ as eluent, coupled to a RI detector. Elution of the samples was monitored by light scattering, RI and UV instruments. The sample is introduced at an injector, then passes through the SEC column, a UV detector (UV), a light scattering detector (LS), a differential refractive-index instrument (dRI) and then finally to waste. Light scattering detectors allow the measurement of absolute molecular weight. The interaction of a molecule with a light beam causes light scattering with intensity directly proportional to its molecular weight.

The four hydrolysates, analyzed by GPC were collected after xylanase treatment on hardwood pulp for two incubation times (1 and 8 h) and after mannanase treatment of softwood pulp for two incubation times (2 and 24 h).

Regarding the hydrolysate from xylanase treatment of hardwood pulp, two main peaks were detected (figures 92 and 93, table 17). The results are reported based on an average of several repetitions. In the GPC diagrams, the red color corresponds to LS, the green color to UV and the blue color to dRI.

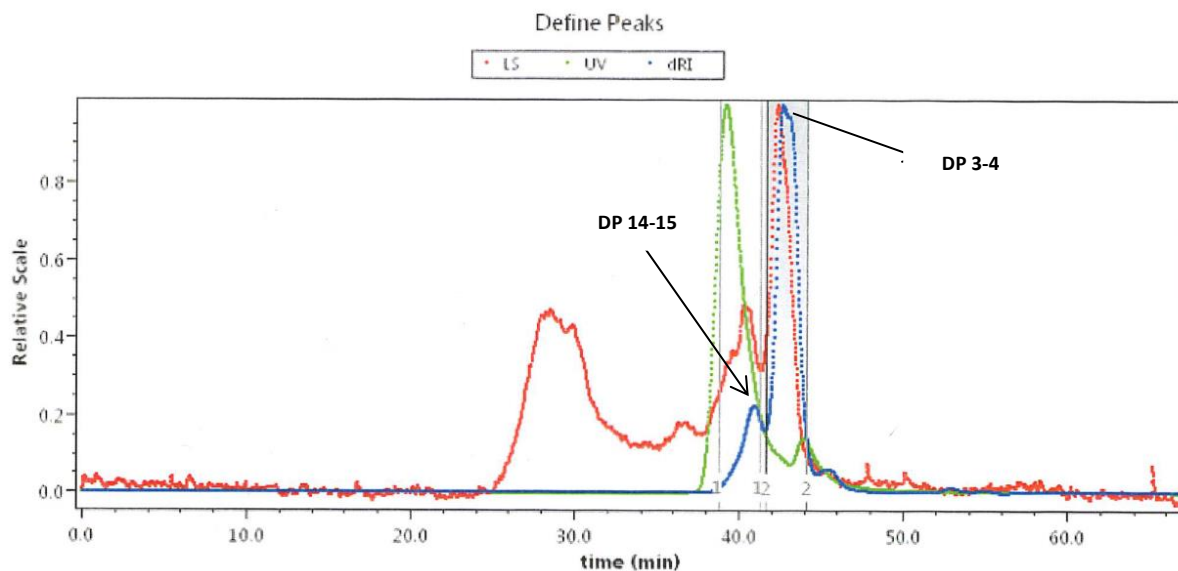


Figure 92- Molecular weight distribution of isolated oligomers of hemicelluloses from xylanase treatment of hardwood pulp during 1 hour.

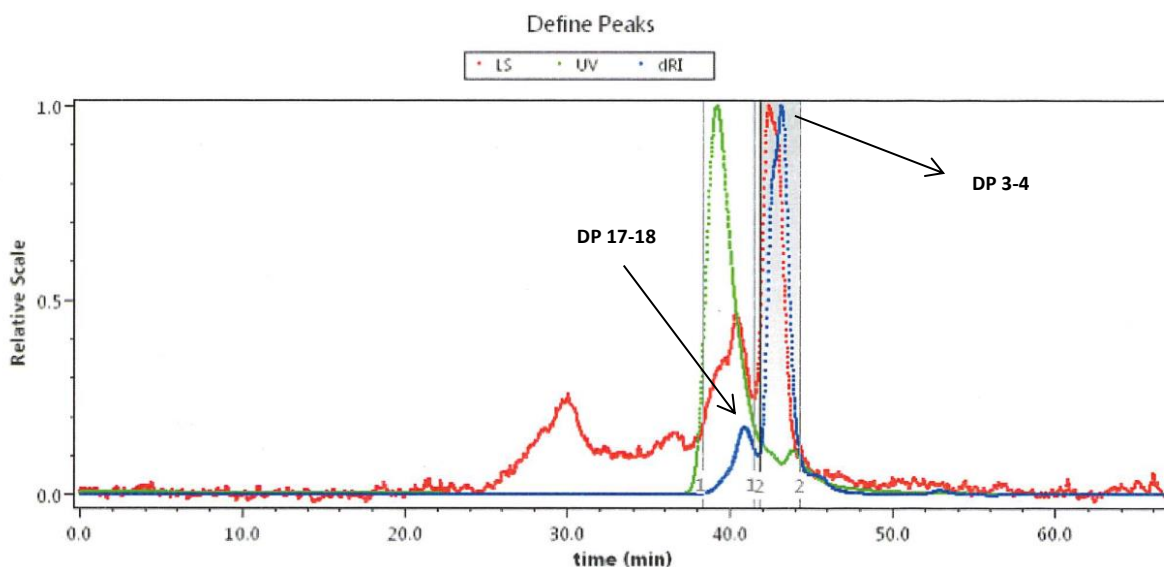


Figure 93- Molecular weight distribution of isolated oligomers of hemicelluloses from xylanase treatment of hardwood pulp during 8 hours.

For 1 hour of incubation time, the main family of oligomers has a DP of 3-4 and corresponds to 85% of the distribution, and the second family with a DP of 14-15 represents approximately 15% of the distribution. Therefore, most of the released oligomers have a small size.

For the incubation time of 8 hours, similarly, there are two main distributions corresponding to DP 3-4 (86% of mass distribution) and DP of 17-18 (14% of mass distribution). It seems that the average corresponding to the oligomers of higher molecular weight after 8 h of incubation is slightly bigger than that after 1 h, whereas the average DP for the smaller molecular weight oligomers is slightly smaller after 8 h of incubation compared to 1 hour.

Table 17- Molecular weight distributions of extracted xylans by xylanase treatments of hardwood pulp.

	Peak 1			Peak 2		
	DP ave.	%Mass Fraction	Polydispersity Index	DP ave.	%Mass Fraction	Polydispersity Index
Xylanase on Hardwood pulp 1 h	14-15	15.3 %	1.4	3-4 (3.9)	84.7 %	1.7
Xylanase on Hardwood pulp 8 h	17-18	14.2 %	1.8	3-4 (3.3)	85.8 %	1.6

This trend shows that xylanase starts hydrolyzing very efficiently from the very beginning of the treatment in a degree that after 1 h the main released oligomers in the media possess the average DP value of 3-4. After 8 h of treatment the average DP of the higher molecular weight oligomers increases and that means xylanase is active enough to hydrolyze the chains of hemicelluloses of higher molecular weight and meanwhile the average DP of the smaller molecular weight oligomers decreases, which means accumulation of smaller molecular weight oligomers as well. Thus after 8 h, although the overall trend is similar, there is an accumulation of both distributions, either higher molecular weights or smaller ones.

Furthermore, the value of polydispersity for all cases was in the range of 1.4-1.8. In total, it seems that the polydispersity index for 8 h is higher than that of 1 h, which can reflect the release of larger distributions of oligomers due to the longer duration of time and the production of various oligomers of hemicelluloses with varied DP from the original hemicelluloses chains. However, for the smaller oligomers the polydispersity index was rather similar for the two incubation times.

The two other hydrolysates analyzed through GPC were from the treatments of mannanase on softwood pulp (figures 94, 95 and Table 18).

Table 18- Molecular weight distributions of extracted mannans by mannanase treatments of softwood pulp.

	Peak 1			Peak 2		
	DP ave.	%Mass Fraction	Polydispersity Index	DP ave.	%Mass Fraction	Polydispersity Index
Mannanase on Softwood pulp 2h	2-3	9.1 %	1.9	< 1	90.9 %	1.2
Mannanase on Softwood pulp 24h	1-2	11.6 %	2.0	< 1	88.4 %	1.3

Interpretation of the results of mannanase on softwood is complicated. Indeed, as studied in chapter 3, when applying xylanase on hardwood, we have seen that only oligomers of xylans were released. But for softwood, it was seen that both mannans and xylans were extracted.

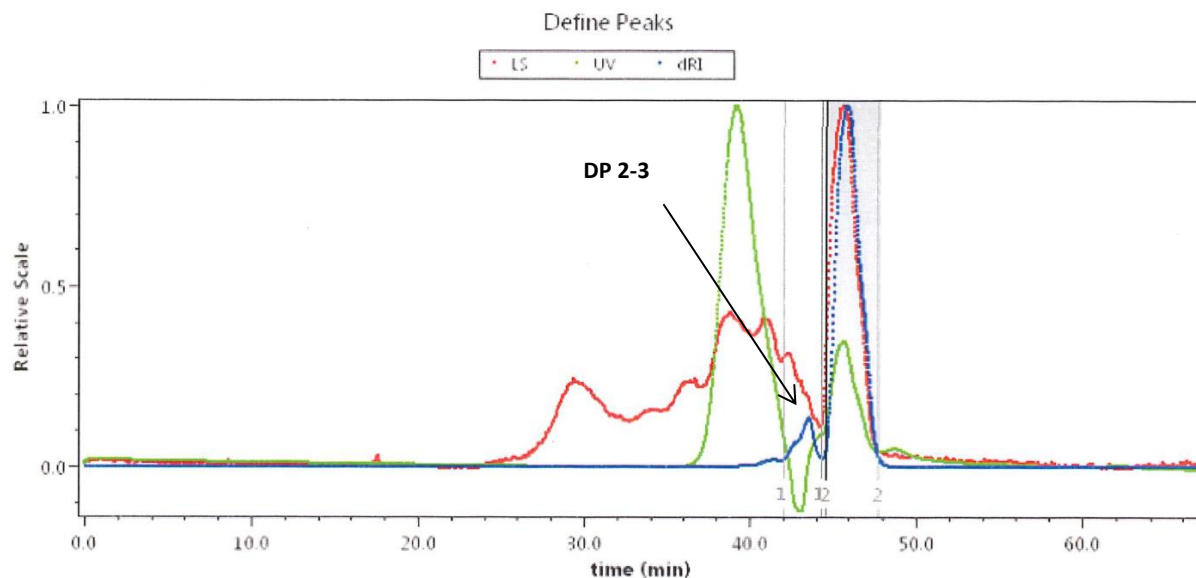


Figure 94- Molecular weight distribution of isolated oligomers of hemicelluloses from mannanase treatment of softwood pulp during 2 hours.

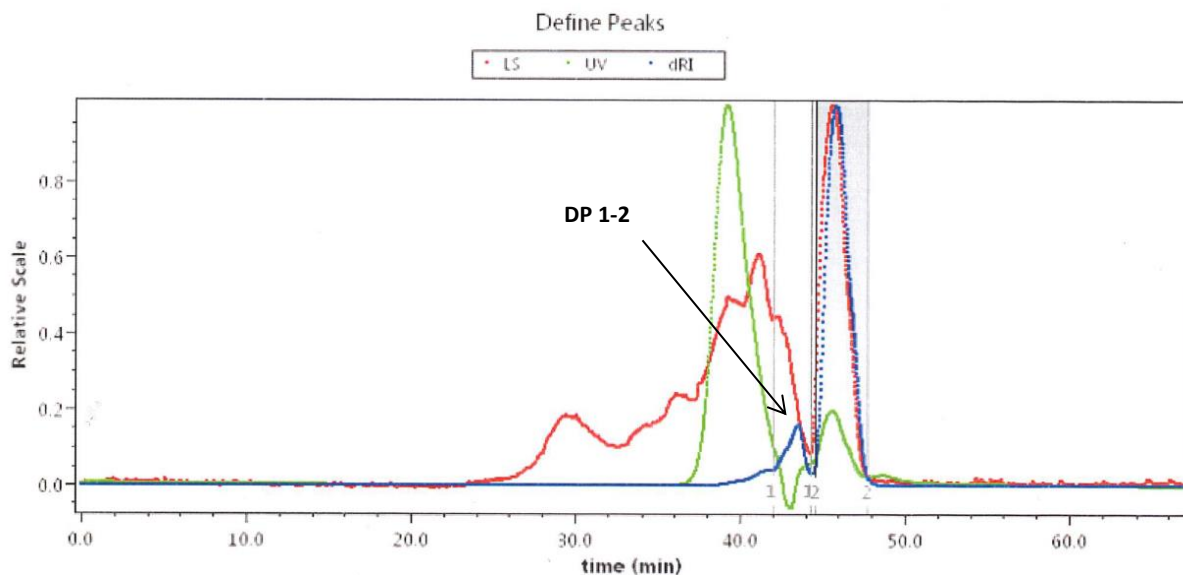


Figure 95- Molecular weight distribution of isolated oligomers of hemicelluloses from mannanase treatment of softwood pulp during 24 hours.

While assuming the release of C6 monomers, the bigger released oligomers contain 2-3 monomers, while for the smaller released molecules this value is less than 1. The value of less than one suggests release of other molecules than monosaccharide. As their molecular weights are close to acetic acid (the average for 2 h incubation time is 91.8 and for 24 h is 78.0 g/mol), there would be an accumulation of acetic acid and other impurities. The presence of acetic acid has been proven on the extracted hydrolysates of mannanase treatment of softwood pulp through HPLC analysis. If so, the acetic acid could come either from acetylase activity of enzyme (the presence of acetyl groups in the structure of remained hemicelluloses in the bleached pulp will be proven in the following analyses) or from the solution of enzyme.

The HPLC analysis of both enzyme solution and hydrolysates of mannanase-treated softwood pulp has shown that the quantity of acetic acid in mannanase solution is 97% and 95% of the quantity of released acetic acid in the hydrolysates of 2 and 48 h incubation time, respectively.

The mass fraction of the bigger released oligomers for 2 h of incubation time is 9.1% and for the smaller molecules it is 90.9%. These values confirm the fact that the released oligomers are in small quantities. Furthermore, the mannanase used only gives very small oligomers (DP 2-3 after 2 hours and 1-2 after 24 hours).

The poly-dispersity index for the first peak for both incubation times is rather high which suggests a broader range of released molecular weight in this peak even if the average molecular weight is between 1 and 3. Furthermore the release of monomers with the DP of 1 might suggest the mannosidase activity of solution of enzyme which is able to produce mannose. The polydispersity index for the second peak is very close to 1 which might confirm the release of smaller molecules with the same range of molecular weight.

The UV analyses of all these four samples presents an absorption exactly in the same retention time for all four samples. This observation suggests that there is the release of a component containing double bond bringing color to the samples. This phenomenon had been confirmed with naked eye after enzymatic treatment. The freeze-dried extracted oligomers had a pale yellow color which suggests the presence of the colored substances in the extracted components. This color could be due to the enzyme solutions. To check that hypothesis, the solutions of enzymes were analyzed through the same column (figures 96 and 97). The solution of each enzyme was inactivated in the same duration time and manner as the enzymatic treatment's. Then after the inactivated solutions were ultra-filtered through the membrane of 5 kD in order to separate the molecules of enzymes in order to avoid damaging the column. The collected solutions which have been passed through the membrane were freeze-dried and analyzed through GPC. The results of analyses have detected the peaks (the green one) in almost similar retention times than for xylanase and mannanase solution preparations.

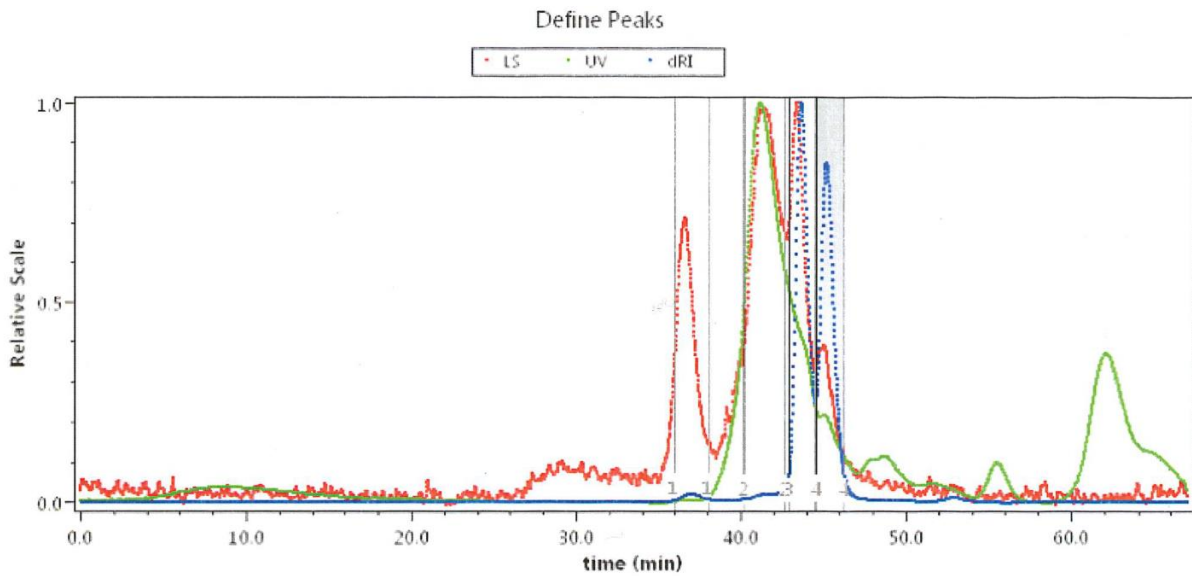


Figure 96- Molecular weight distribution of xylanase.

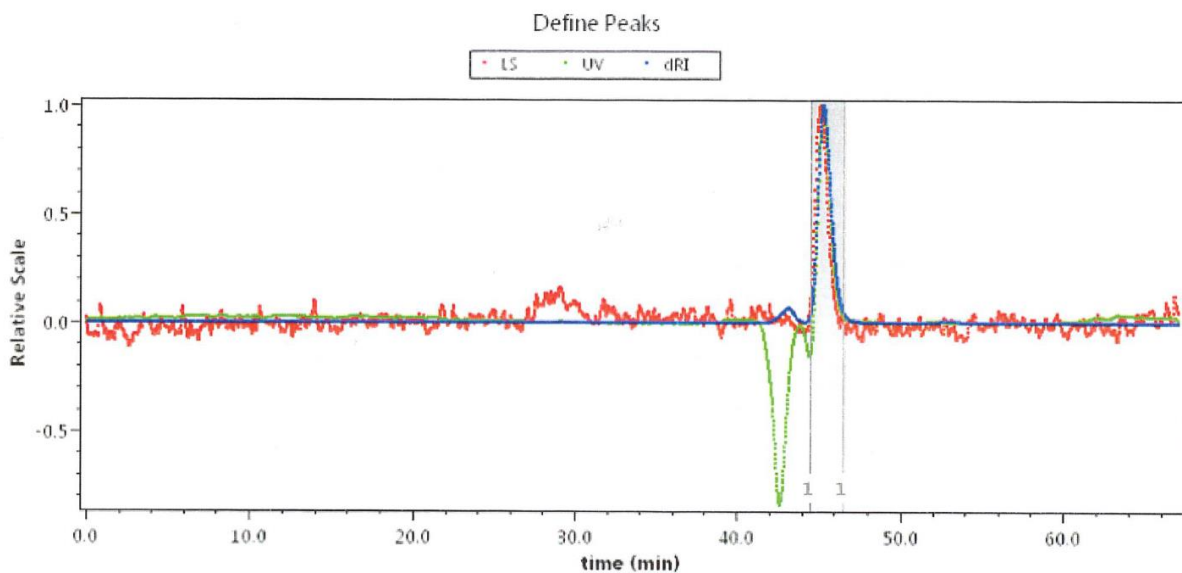


Figure 97- Molecular weight distribution of mannanase.

4-1-2- Monitoring the small released oligomers

Based on the results presented in the previous part, most of the released oligomers are small with an average DP of 3-4 in the case of xylanase treatment of hardwood pulp, and 1-3 in the case of mannanase treatment of softwood pulp. The objective of this part is to study these small oligomers more precisely with adapted size exclusion chromatography columns. Size exclusion chromatography was done with a LC system (Ultimate 3000 - ThermoFisher) coupled with Refractive index detector IOTA 2 (Precision instrument), a Superdex 200 10/300 GL column and Superdex Peptide 10/300 GL column (GE Healthcare) in series. The hydrolysates studied came from xylanase treatments of the hardwood pulp with incubation times of 2, 8

and 32 hours. The results presented in Figure 98 show that for the longer incubation time, accumulation of smaller chains occurred.

Another set of analysis of size exclusion chromatography with different columns, Superdex S30 \times 3, was done on hydrolysates of xylanase on hardwood and mannanase on softwood pulps (figures 99, 100 and 101). In some of these experiments (Figure 99) standard sugars (glucose and oligomers of glucose, as we did not have standard oligomers of xylose) were added. that the results seem to indicate that the hydrolysate of xylanase of hardwood pulp might contained xylobiose and xylotriose (figure 99). Furthermore, the results show two peaks that could correspond to mannose and mannobiose (M1 and M2 in figure 100) for the hydrolysate obtained from the mannanase treatment on softwood pulp. Figure 101 represents the combination of these two figures in one.

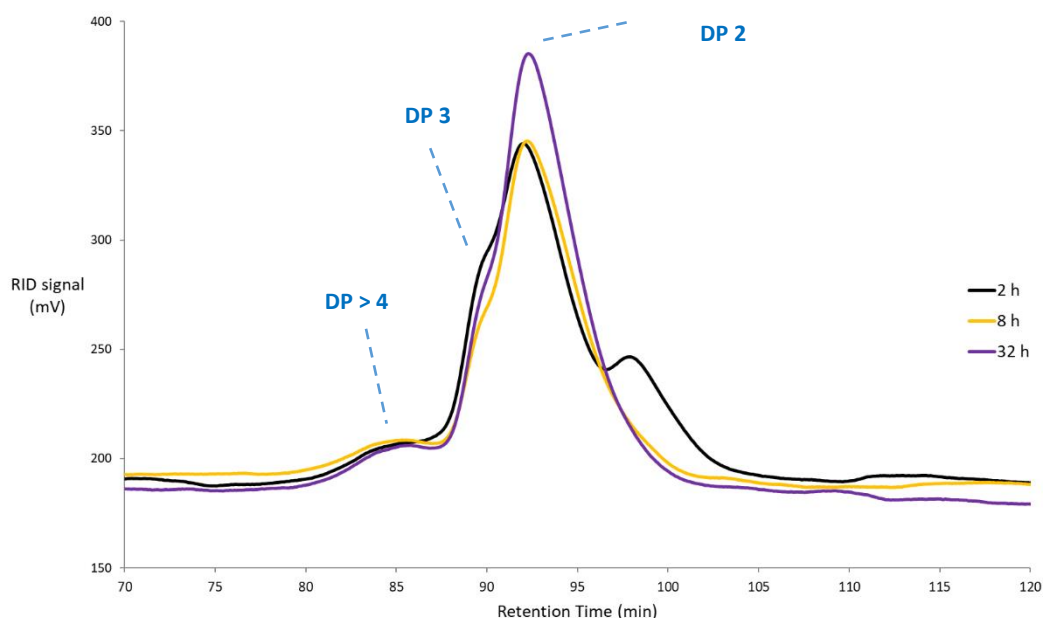


Figure 98- Size exclusion chromatography of extracted oligomers of xylan from xylanase treatment of hardwood pulp in three incubation times of 2, 8 and 32 hours.

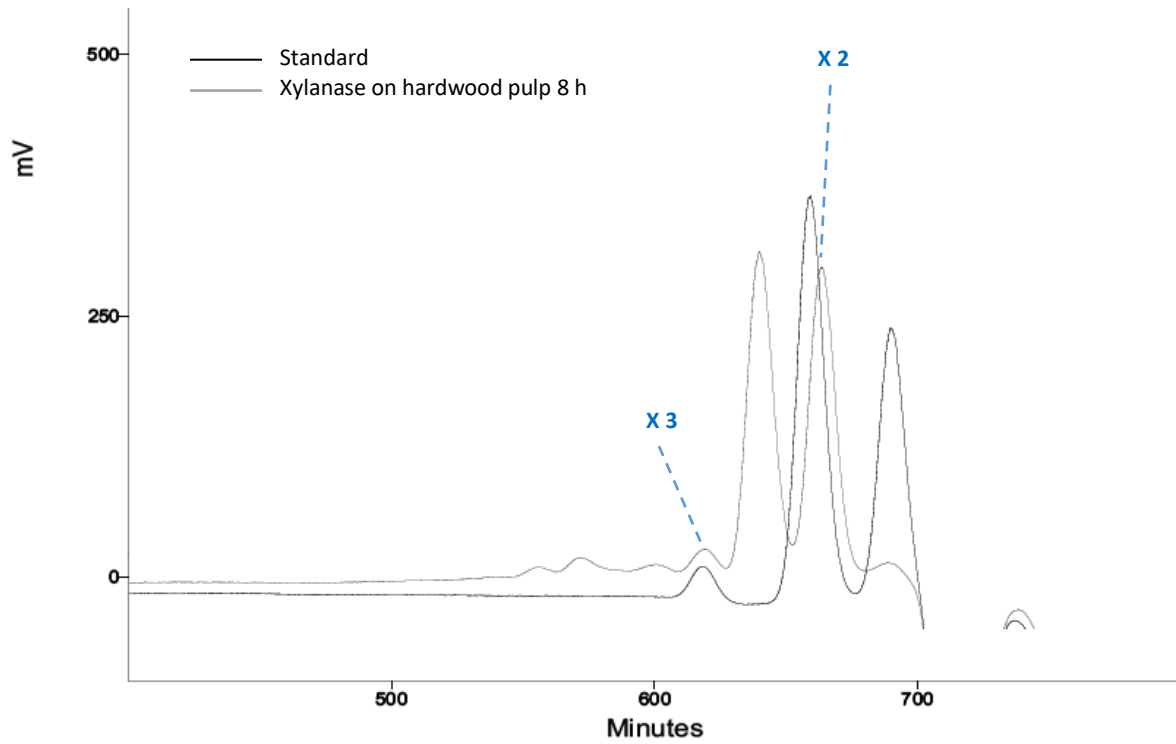


Figure 99- Size exclusion chromatography of extracted oligomers of xylan from xylanase treatment of hardwood pulp during 8 hours (X n: oligomers of xylan with the DP value of n). Comparison with standard mono and oligosaccharides from glucose.

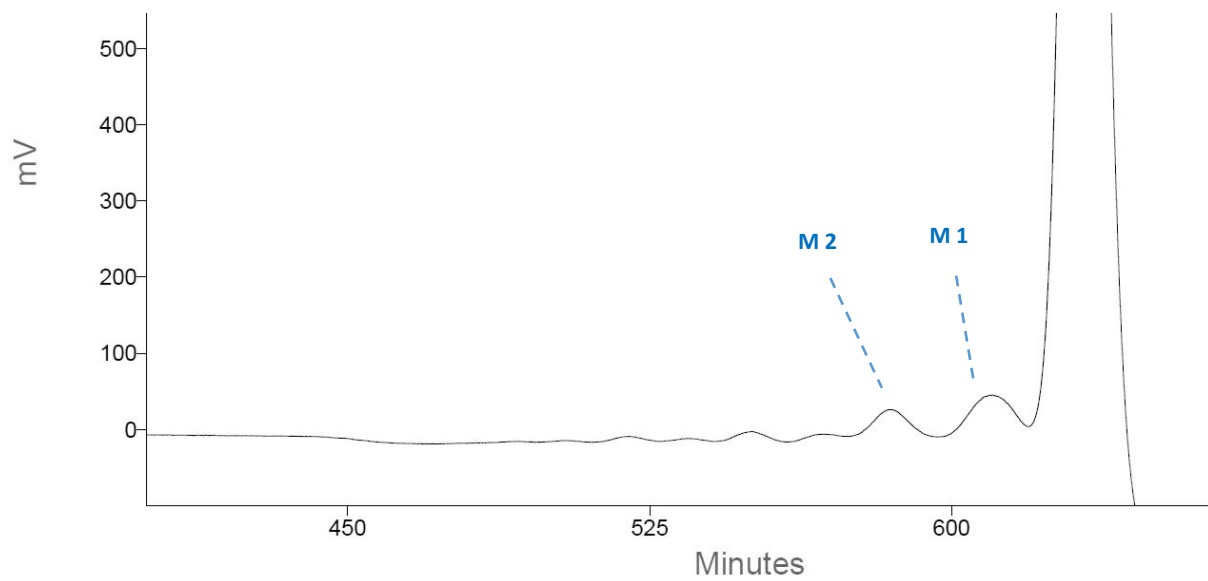


Figure 100- Size exclusion chromatography of extracted oligomers of mannan from mannanase treatment of softwood pulp during 24 hours (M n: oligomers of mannan).

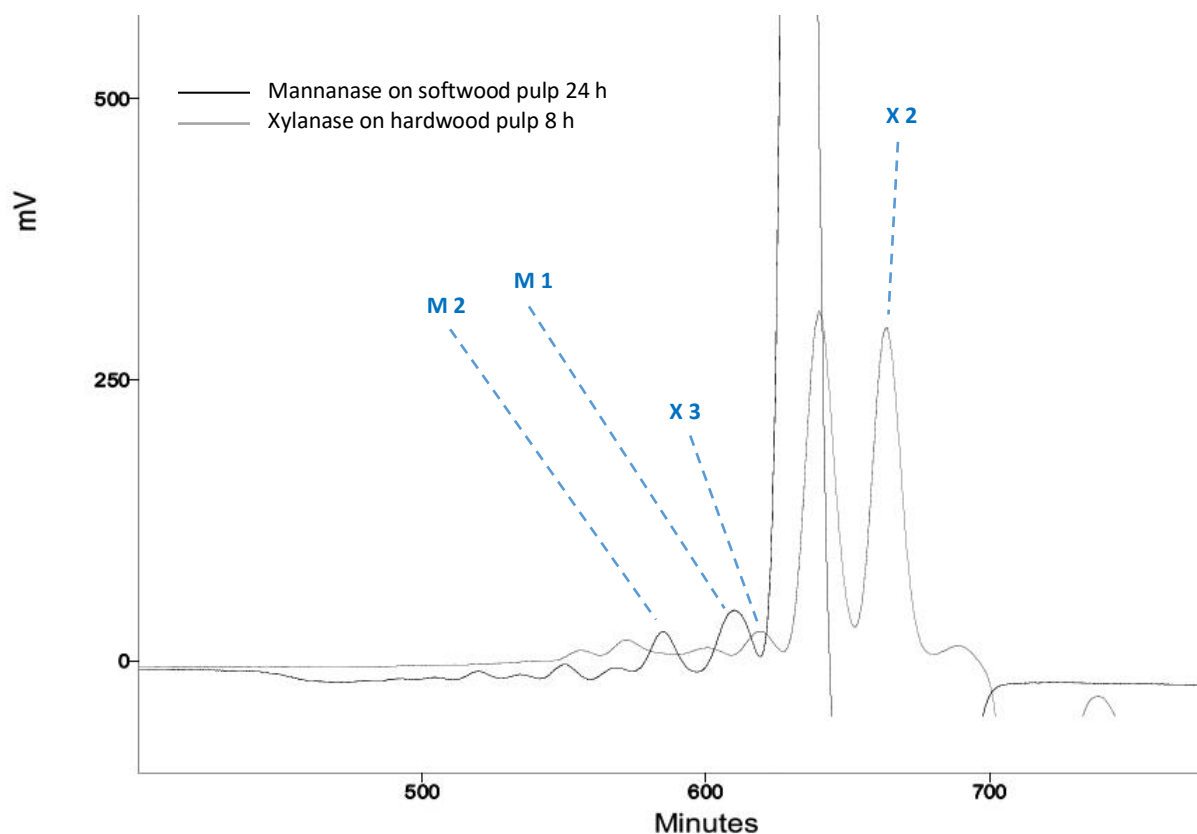


Figure 101- Size exclusion chromatography of extracted oligomers of xylan from xylanase treatment of hardwood pulp during 8 hours and of extracted oligomers of mannan from mannanase treatment of softwood pulp during 24 hours (M n: oligomers of mannan, X n: oligomers of xylan).

As a conclusion of this part, different chromatography analyses have been done on the enzymatically-isolated oligomers of hemicelluloses in order to study their molecular weight distributions. The study of extracted oligomers by the xylanase treatment of hardwood pulp showed the release of two main groups of oligomers; the dominant one with the DP value of 3-4 and the other in the range of 14-18, in which the former constituted around 85% of the total release. The results for the extracted oligomers by the mannanase treatment of softwood pulp showed the release of very small oligomers of 1-3 values of DP.

4-1-3- Molecular structure

The released oligomers of hemicelluloses have been analysed by MALDI-TOF mass spectroscopy in order to study the structure of isolated oligomers and their similarities and differences in different treatments. Among the hydrolysates studied by this method, some are discussed here: xylanase on hardwood with incubation times of 30 min, 1 h, 2, 8 and 32 h, xylanase on softwood pulp with reaction times of 2, 8 and 32 h and mannanase on softwood pulp with incubation times of 2 and 24 h.

MALDI-TOF mass spectroscopy has been done with two different modes to assure the detection of most ranges of released oligomers. While in reflectron mode the precision is the main priority, in the linear mode the detection of higher-molecular weight components is of importance. In a matrix of dihydroxy benzoic acid (DHB), the samples were diluted to 50 mg/ml (this value changed if necessary for a better detection) in water in order to being analysed with DHB/TA30 or in DMSO in order to being analysed with DHB/DMSO.

As the released oligomers were expected to be composed of C5 and C6 sugars, with or without side groups (acetyl, methyl glucuronic or hexenuronic acid groups), a library has been created based on that. Table 19 gives the list of abbreviations used.

Tableau 19- The abbreviation of different side groups.

Unit	abbreviation
Xylose	X
Mannose	M
Acetyl group	Ac
4-O-methylglucuronic acid	MeGlcA
Hexenuronic acid	Hex

4.1.3.1. Structure of the oligomers extracted from the hardwood pulp by the xylanase treatment

The Maldi TOF MS spectra obtained for the hydrolysate corresponding to the xylanase treatment for one hour is presented on figures 102 and 103. As expected from the analyses of molecular mass distribution presented in the previous part, the most abundant family of molecules are the small ones (between DP 2 and 4). On Figure 102 the presence of linear oligomers of xylan with DP from 2 to 11 can be seen.

The presence methylglucuronic acid group on xylan backbone has been observed from DP value of 2 and above. Figure 103 shows mass values corresponding to the formula of $nX+1MeGluA$ from DP 5 to 13.

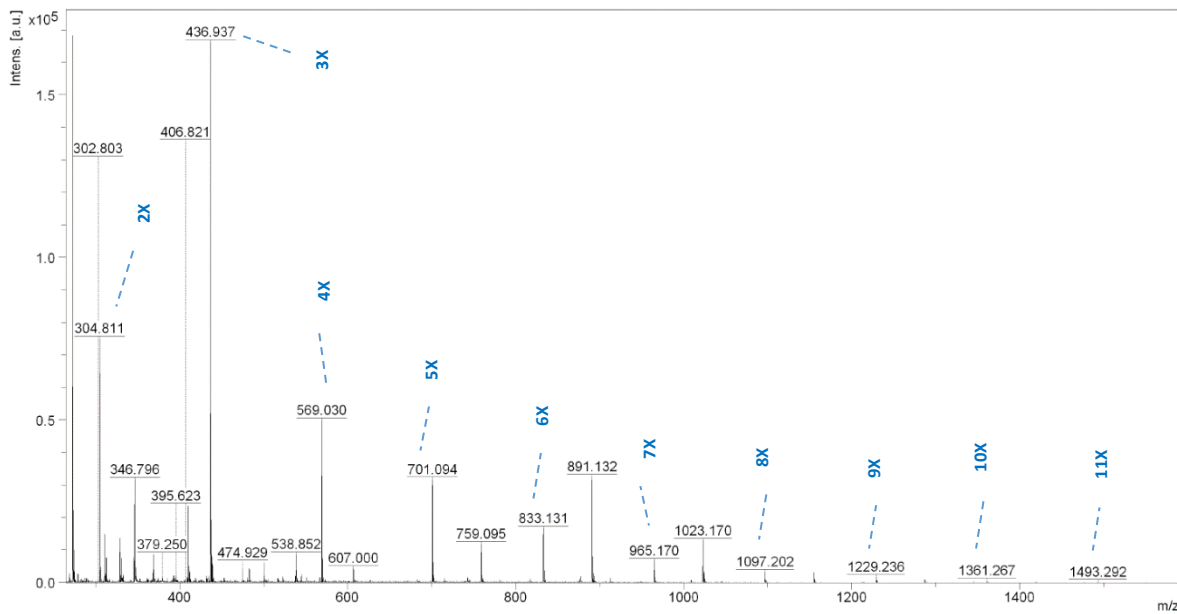


Figure 102- The spectrum of MALDI-TOF mass spectroscopy in reflectron positive mode for linear isolated oligomers of xylan from xylanase treatment of hardwood pulp during 1 hour (nX: oligomers of xylan with the DP value of n).

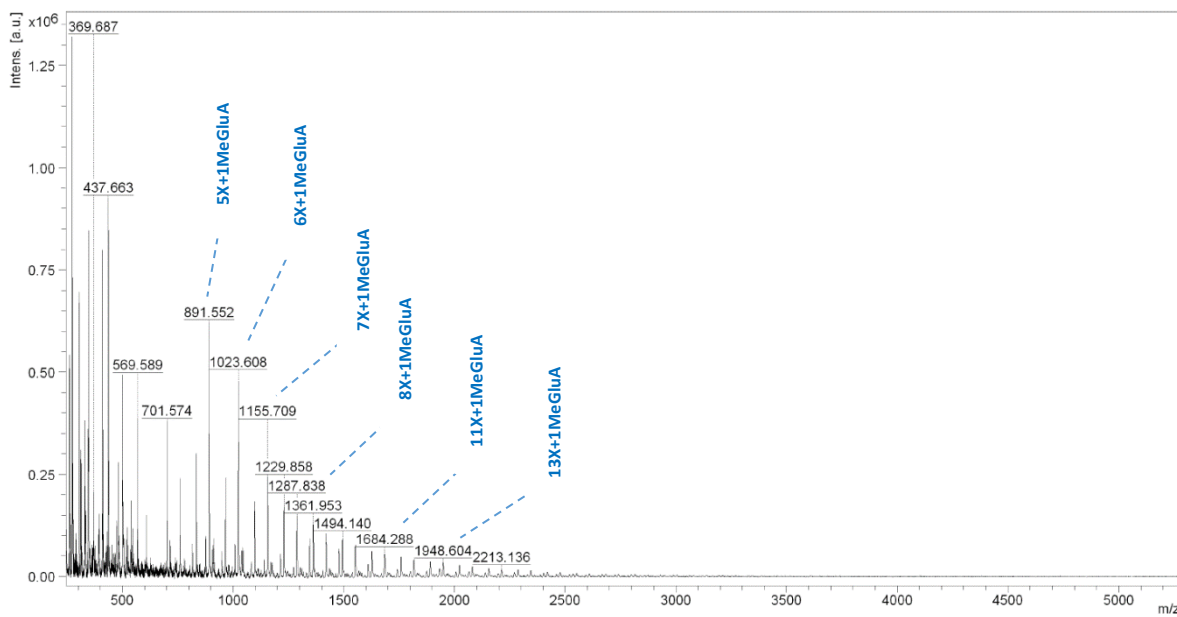


Figure 103- The spectrum of MALDI-TOF mass spectroscopy in linear positive mode for isolated oligomers of xylan with glucuronic acid side group from xylanase treatment of hardwood pulp during 1 hour (nX: oligomers of xylan with the DP value of n).

The presence of other combinations of side groups on the xylan backbone has also been detected. One of these combinations is 2X+1MeGluAc+1Ac and 2X+1MeGluA+1Ac (figure 104). In the chains with higher DP more complicated combinations have been observed in which the number of MeGluA and Ac groups are bigger and with different combinations.

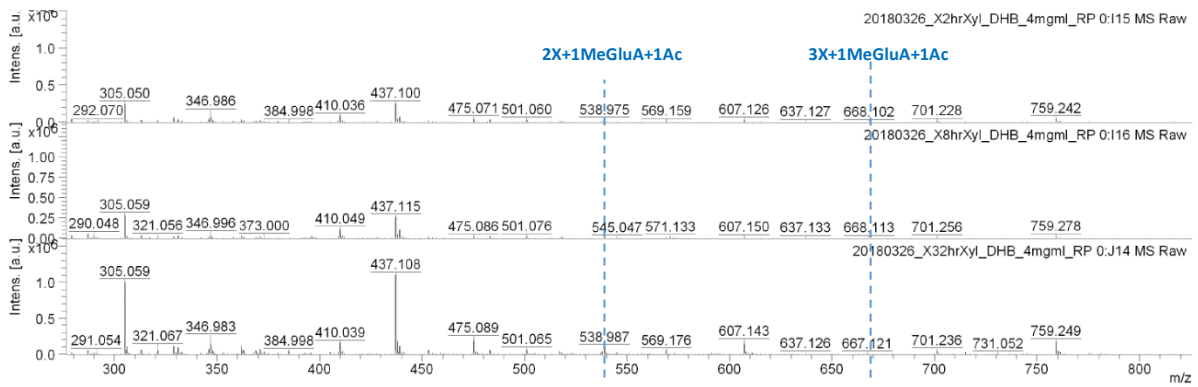


Figure 104- The spectrums of MALDI-TOF mass spectroscopy in reflectron positive mode for isolated oligomers of xylan from xylanase treatment of hardwood pulps in different incubation times of 2 h (top spectrum, 8 h and 32 h.

As an example of the presence of a regularity in the release of different combinations of oligomers, figure 105 shows that some of the released oligomers have the following formula: nX , $nX+1Hex$, $nX+1MeGluA$, $nX+1Hex+4Ac$ and $nX+2MeGluA+Ac$. According to that, similar combination of side groups attached to the oligomeric chain is observed in different lengths. Furthermore, the same oligomeric structures have been released in different incubation times.

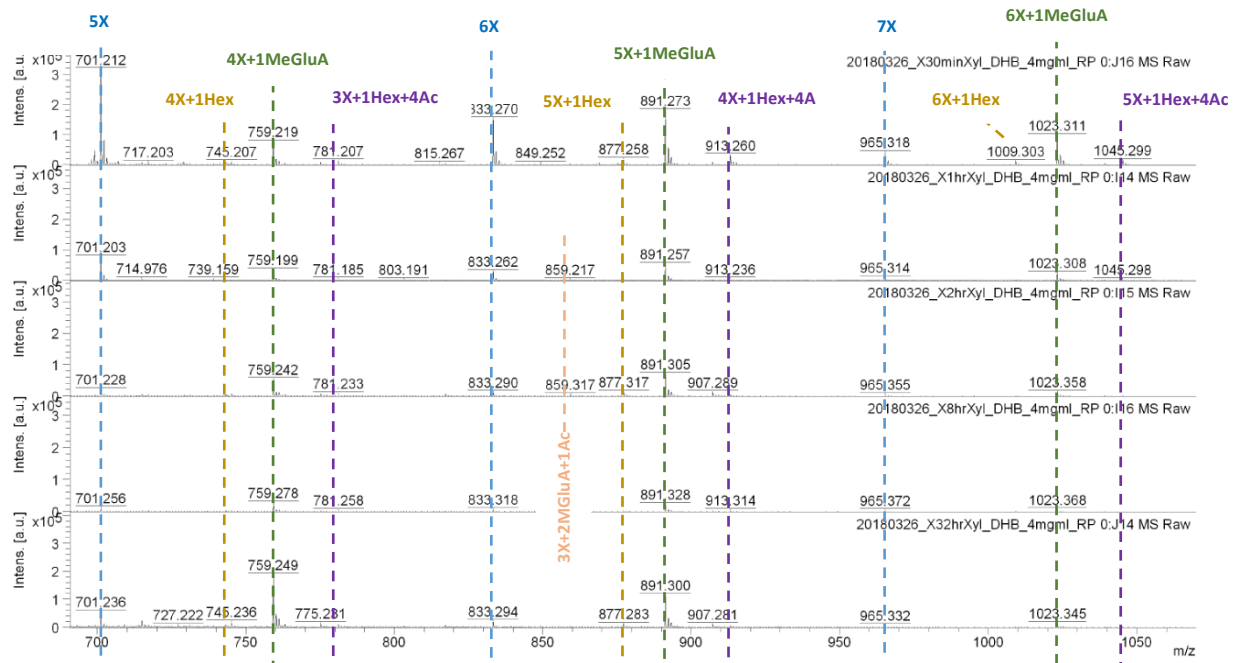


Figure 105- The spectrums of MALDI-TOF mass spectroscopy in reflectron positive mode for isolated oligomers of xylan from xylanase treatment of hardwood pulps in different incubation times of 30 min (top spectrum), 1 h, 2 h, 8 h and 32 h (nX : oligomers of xylan with the DP value of n).

Figure 106 gives an overview of the different size of oligomers detected, and of the presence of side groups, for the different incubation times. The xylan oligomers (without any side

groups) are present from DP2 to 24 for incubation times up to 8 hours. For 32 hours of incubation time only oligomers up to DP13 have been detected, which indicate that the released oligomers are gradually depolymerised by the enzymes

The acetylated oligomers are present in the hydrolysate with higher DPs, which is also the case for the xylan chains containing acidic groups, which can be explained by the fact that the acidic groups improve the solubility of xylans. The presence of such a variety of xylans with different side groups also indicate that the enzyme used is able to depolymerise not only pure xylans but also substituted ones.

In these enzymatic treatments using xylanase, no release of oligomers of mannans has been observed, which is consistent with the results obtained in the previous chapter.

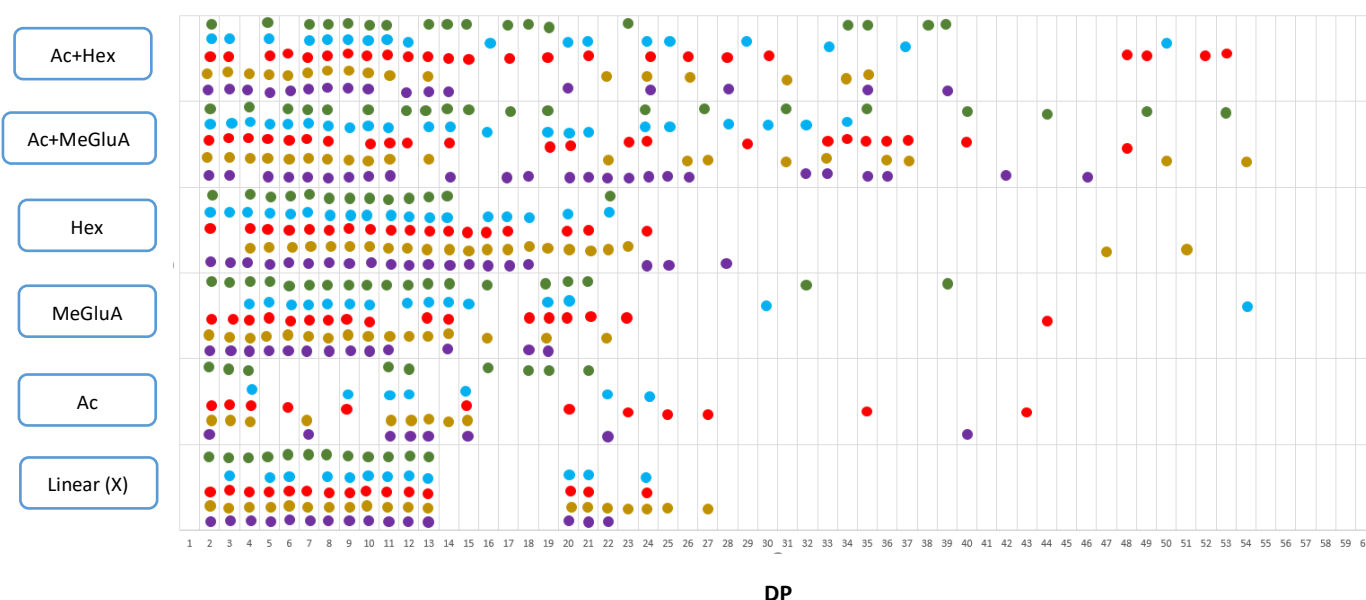


Figure 106- Structure of isolated oligomers of xylan after xylanase treatment of hardwood pulp, in different incubation times: purple: 30 min, yellow: 1 h, red: 2 h, blue: 8 h, green: 32 h. DP stands for the DP of the xylan backbone

Linear: xylan. Ac: presence of acetyl groups in xylans; MeGluA: presence of 4-O-methylglucuronic acid in xylans; Hex: presence of hexenuronic acid groups in xylans; Ac+ MeGluA: presence of both acetyl and 4-O-methylglucuronic acid groups in xylan; Ac+Hex: presence of both acetyl and hexenuronic acid groups in xylan.

In a study of xylanase hydrolysis of birch kraft xylans, HexA-xylobiose has been detected as HexA-containing product after hydrolysis [277]. In another study on hexenuronoxylan isolated from a hardwood kraft pulp treated by xylanase, MALDI-TOF analysis detected HexA-Xyl₂ and HexA-Xyl₃ in different quantities depending on the enzyme dosage and of pH [234]. In another study, after the hydrolysis of birch wood xylan with xylanase, the largest peaks were a cluster at masses corresponding to various products of xylotriose, furthermore there were smaller peaks corresponding to xylobiose, xylo-tetraose and a little xylopentaose. In addition, there

were peaks for xylo-tetraose up to xylo-heptaose with 4-O-methyl glucuronic acid attached [366].

4-1-3-2- Structure of the oligomers extracted from the softwood pulp by the xylanase treatment

The released oligomers of xylan in xylanase-treated softwood pulp detected by Maldi TOF MS have lower DP compared to those extracted from hardwood with the same enzyme (Figures 106 and 107), either for pure xylan oligomers or for substituted ones. Furthermore, no effect of incubation time on the type of oligosaccharide present in the hydrolysate could be seen. This may bring up the issue of accessibility of softwood's xylan to xylanase. It is probable that the accessibility of xylan in softwood to xylanase is more difficult than in hardwood. Another hypothesis would be the structure of substrates on which the enzyme act more specifically.

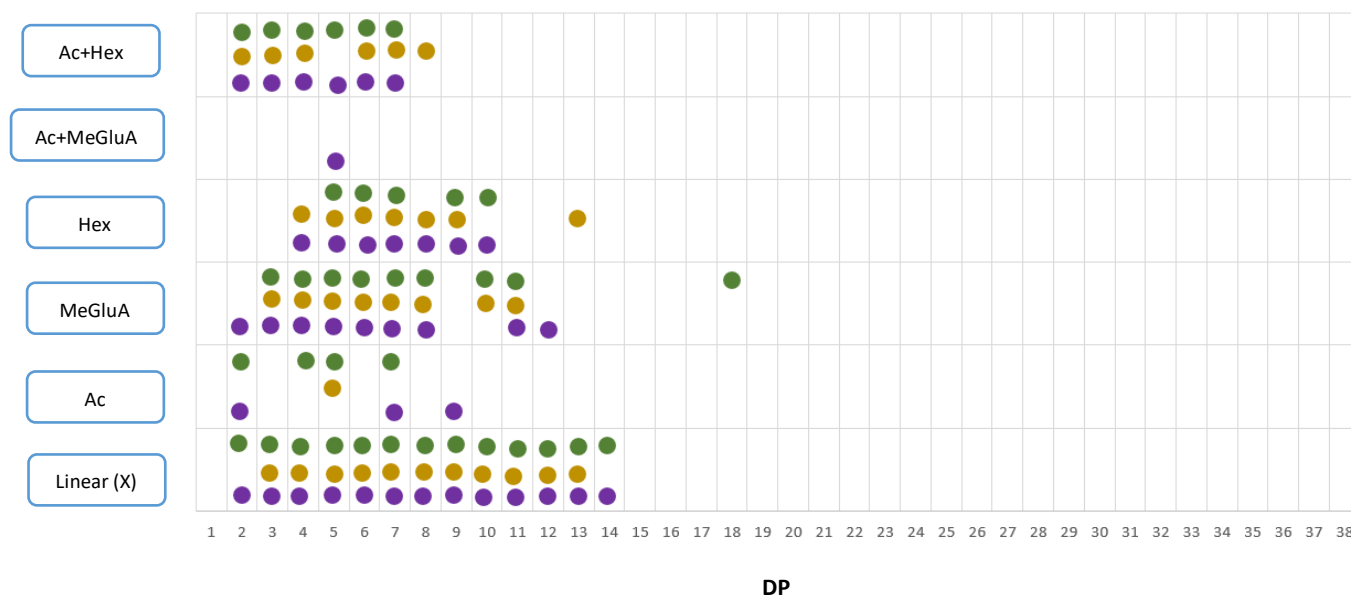


Figure 107- Structure of isolated oligomers of xylan due to xylanase treatment of softwood pulp, in different incubation times: purple 2 h, yellow 8 h, green 32 h.

Linear: xylan. Ac: presence of acetyl groups in xylans; MeGluA: presence of 4-O-methylglucuronic acid in xylans; Hex: presence of hexenuronic acid groups in xylans; Ac+ MeGluA: presence of both acetyl and 4-O-methylglucuronic acid groups in xylan; Ac+Hex: presence of both acetyl and hexenuronic acid groups in xylan.

The presence of different linear chain length with increasing trend of DP from 2 to 14, one by one, is observed on figure 108. Furthermore, regarding the release of non-linear oligomers of xylan, different combination of side groups attached to the main chain of xylan shows a regular trend (figure 109) ($nX + 1\text{Hex} + 4\text{Ac}$, $nX + 2\text{MeGluA}$, $nX + 1\text{Hex} - n$ in this figure is from 6 to 10).

Analysis of isolated oligomers of mannan by the xylanase treatment of softwood in different incubation times show that they were of small size and not very diverse (figure 110). It is

coherent with the previous results in analysis of sugar composition (chapter 3). It is more likely that the released molecules of mannan that can be seen are associated with the release of molecules of xylan.

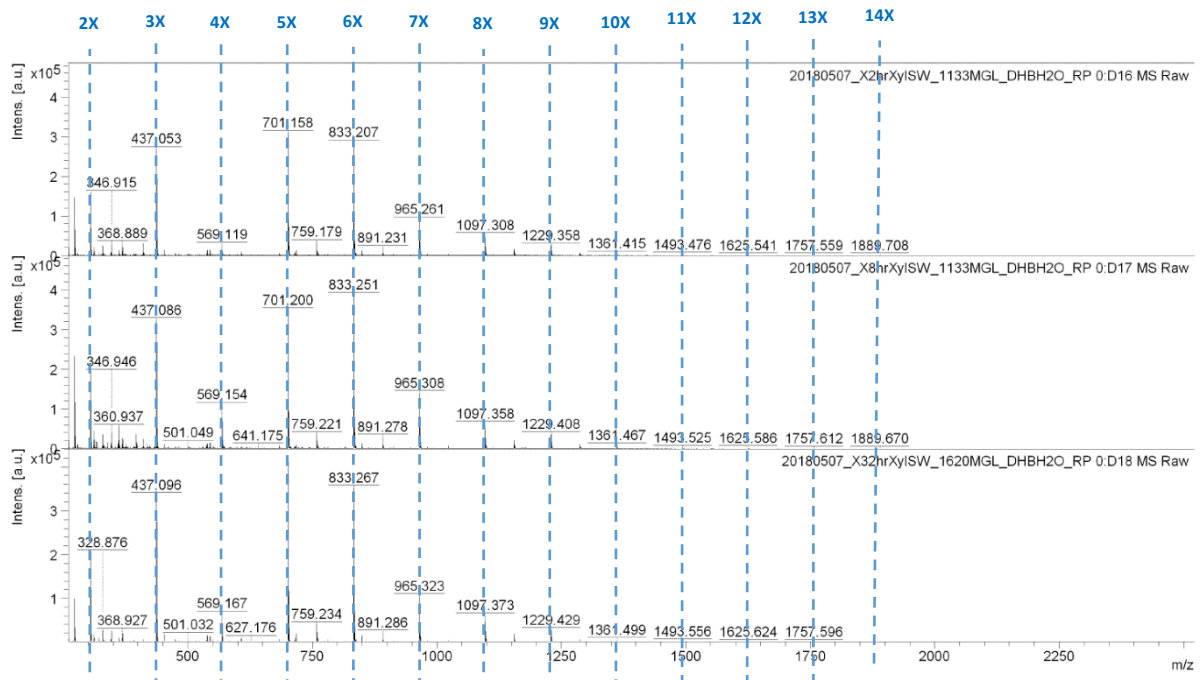


Figure 108- The spectrums of MALDI-TOF mass spectroscopy in reflectron positive mode for isolated oligomers of xylan from xylanase treatment of softwood pulps in different incubation times of 2 min, 8 h and 32 h (nX: oligomers of xylan with the DP value of n).

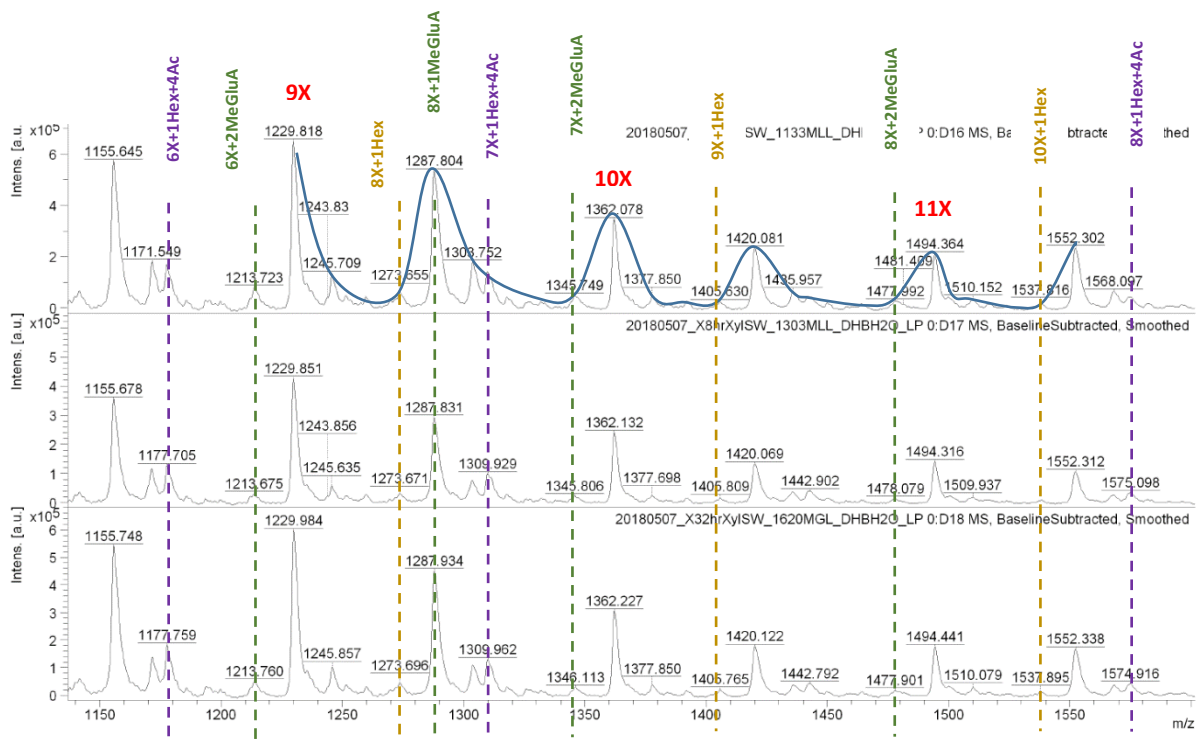


Figure 109- The spectrums of MALDI-TOF mass spectroscopy in linear positive mode for isolated oligomers of xylan from xylanase treatment of softwood pulps in different incubation times of 2 min, 8 h and 32 h (nX: oligomers of xylan with the DP value of n).

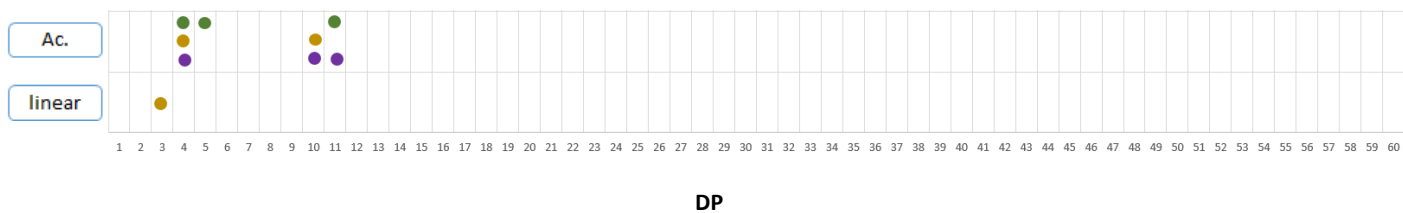


Figure 110- Structure of isolated oligomers of mannan due to xylanase treatment of softwood pulp, in different incubation times: purple 2 h, yellow 8 h, green 32 h.

4.1.3.3. Structure of the oligomers extracted from the softwood pulp by the mannanase treatment

Analysis of mannanase treatment on softwood in two different reaction times of 2 and 24 hours show the presence of both oligomers of xylan (figure 111) and mannans (figure 112), which is consistent with the results obtained in the previous chapter. Apart from linear chains, the chains containing side groups have become more diverse after 24 hours of incubation.

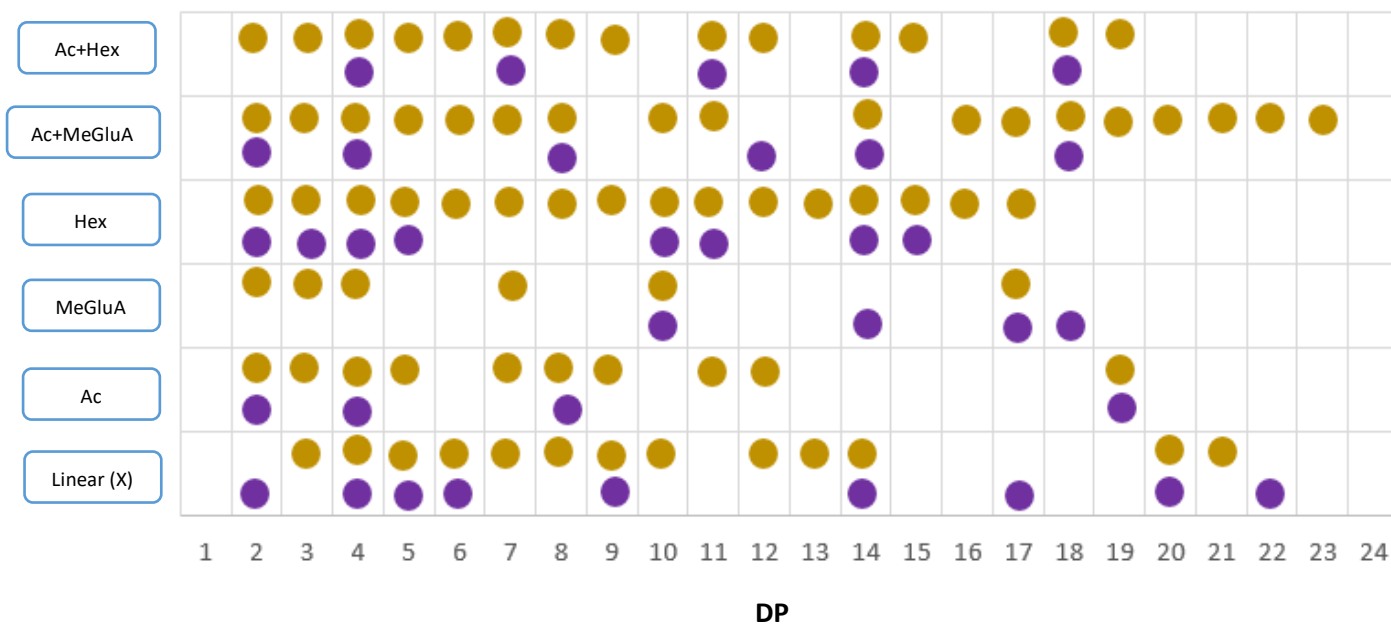
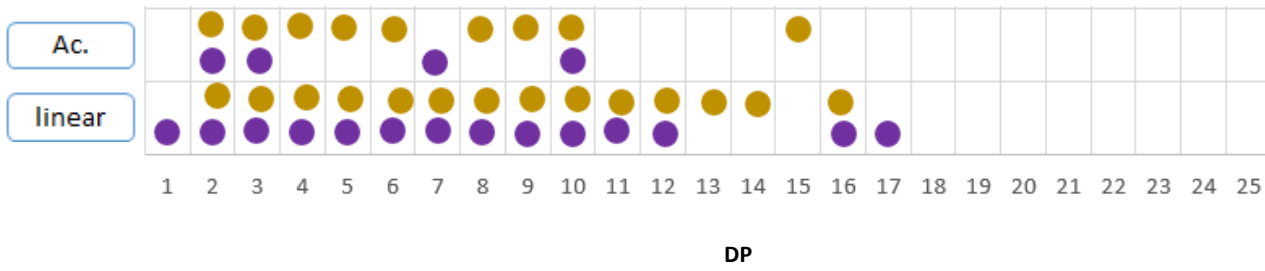


Figure 111- Structure of isolated oligomers of xylan due to mannanase treatment of softwood pulp, in different incubation times: purple 2 h, yellow 24 h.

Regarding oligomers of mannan, the oligomers detected are also of smaller size than those detected after xylanase treatment of hardwood pulp. The presence of the acetylated chains is also more diverse after 24 hours compared to two hours.



**Figure 112- Structure of isolated oligomers of mannan due to mannanase treatment of softwood pulp, in different incubation time: purple 2 h, yellow 24 h.
Linear: mannans; Ac: acetylated mannans**

Figures 113 and 114 show the released oligomers of mannans. Some of these peaks have the correspondent value besides, some do not have, especially for the oligomers of xylan, suggesting the weakness of the signal, although they exist in any case. Some of the non-linear oligomers, both for xylan and mannan, are shown in the figures as well as an example. The same phenomenon of regularity in releasing the non-linear chains is observed in these treatments as well.

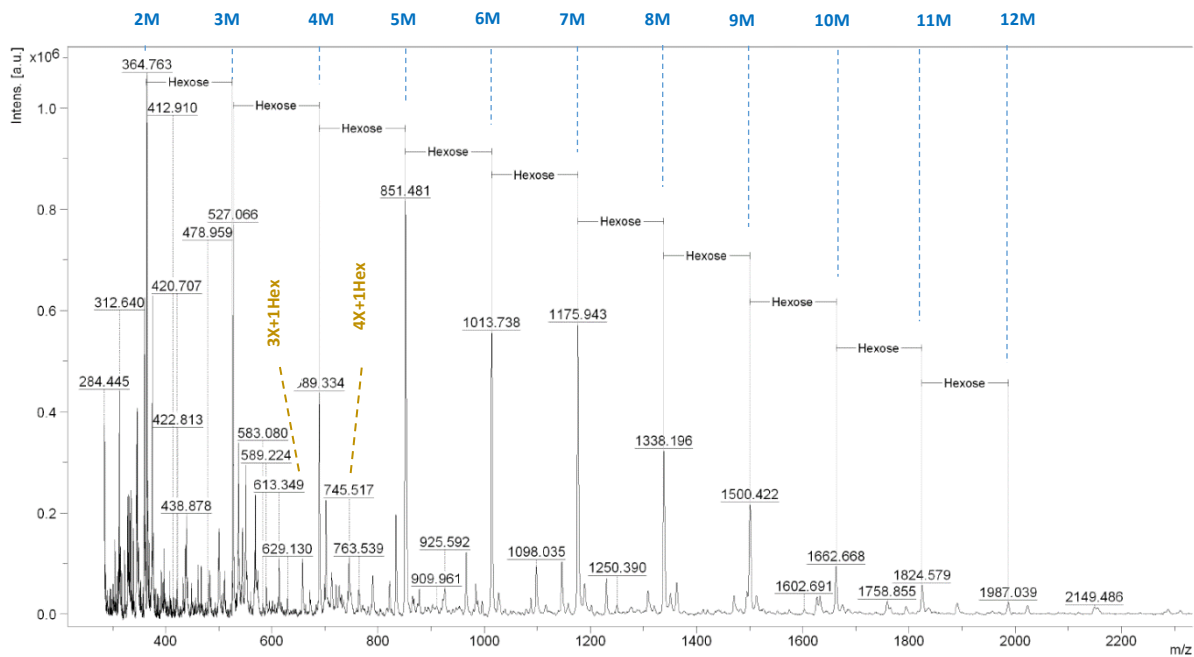


Figure 113- The spectrums of MALDI-TOF mass spectroscopy in linear positive mode for isolated oligomers of mannan from mannanase treatment of softwood pulps in two incubation times of 24 h (nM: oligomers of mannan with the DP value of n).

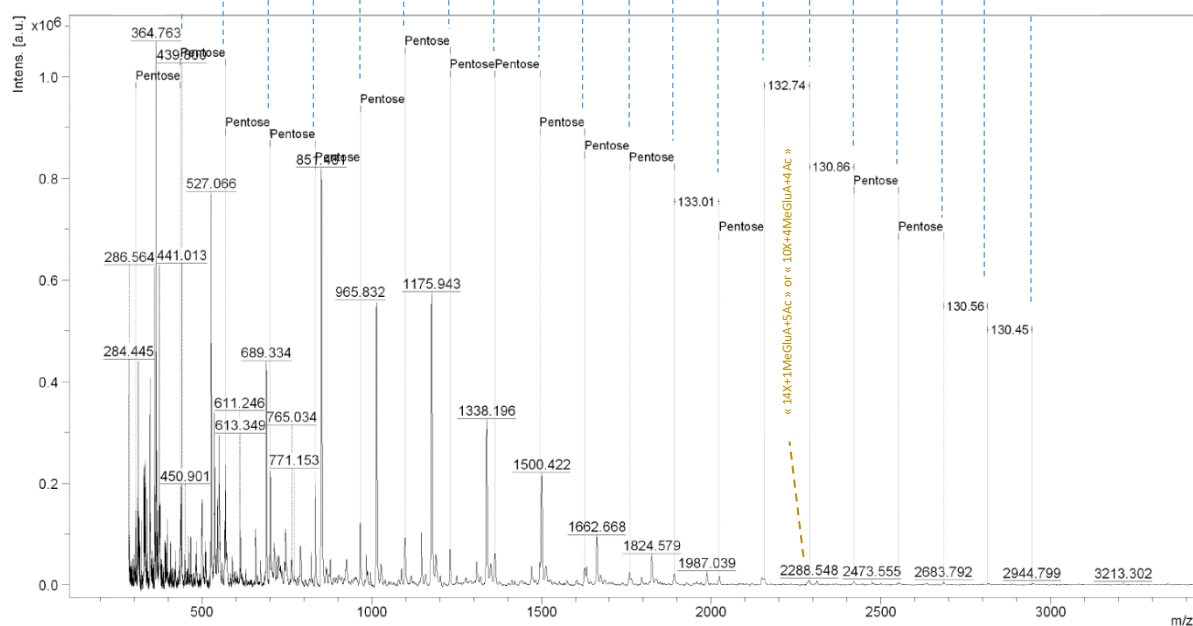


Figure 114- The spectrums of MALDI-TOF mass spectroscopy in linear positive mode for isolated oligomers of xylan from mannanase treatment of softwood pulps in incubation times of 24 h (nX: oligomers of xylan with the DP value of n).

Conclusion

The molecular weight distribution of isolated oligomers of hemicelluloses by xylanase and mannanase treatments of hardwood and softwood pulps showed the release of two main groups of oligomers; small size components and big size ones. Regarding xylanase treatment of hardwood, the smaller components, which represented 85% of the mass, had DP values of 3-4 for both incubation times and the bigger ones had DP values of 14-15 for 1 h incubation time and 17-18 for 8 h.

Regarding mannanase treatment on softwood, 10% of the mass released corresponded to average DP of 1-2, whereas 90% of the mass did not seem to be saccharides.

The mass spectroscopy analysis showed the release of a highly diverse component; from linear oligomers to non-linear with different combination of side-groups, especially for xylans.

4-2- Characterization of the oligomers extracted by CCE

Cold Caustic Extraction (CCE) has been done on air-dried grinded pulps with the NaOH solution of 11% and 10% pulp consistency for one and a half hours at ambient temperature. The collected liquids, after filtering, were then neutralized with a solution of HCl 32%. The suspension was centrifuged which separated two parts; one which was precipitated seated at the bottom of the vial (rather jelly) and the other the supernatant situated on the top in the form of a transparent liquid. These two parts were collected separately. Based on the sugar

analysis, the first part was rich in xylan and the second part was a mixture of xylan and mannan and the mannan was dominant.

4-2-1- Molecular weight distribution

Molecular weight distribution of extracted oligo or polysaccharides by CCE treatment of starting hardwood pulp and softwood pulps showed extraction of very high molecular weight components (figures 115, 116 and 117), which is certainly due to the fact that the some of the extracted polysaccharides formed aggregates. The smaller peaks were in the 300-500 DP range which is unexpected as hemicelluloses in wood are supposed to be smaller than DP 200. One explanation could be that the CCE treatment also extracted some short cellulose chains. Another possibility could be that lignin-hemicelluloses complexes have been extracted. Even though bleached pulps were used some traces of lignin can still be present. The presence of UV peak along with the blue one could be used as a clue.

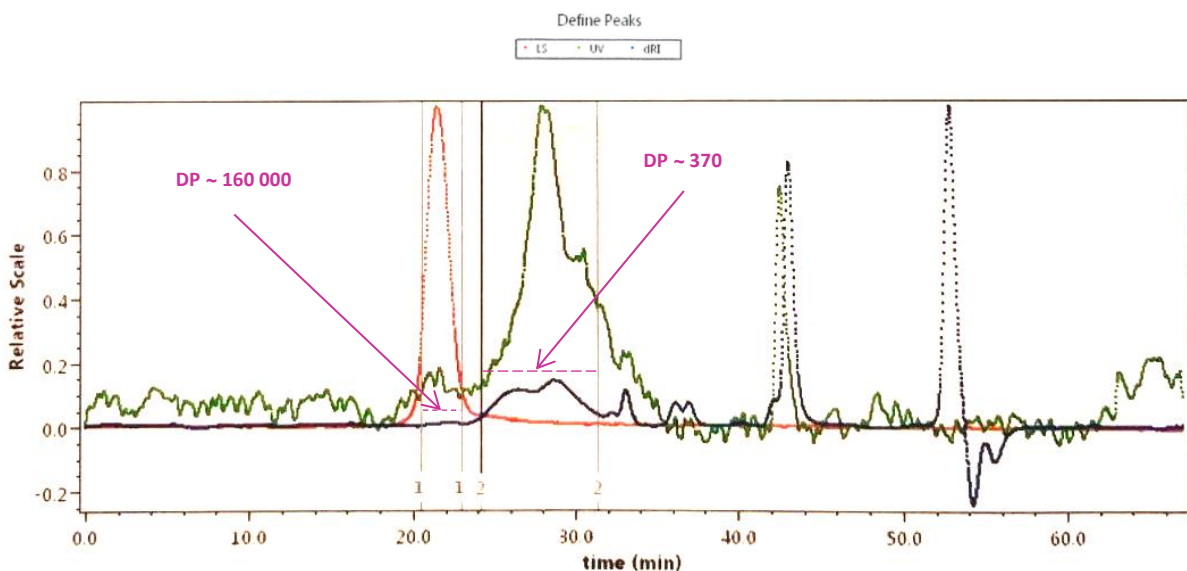


Figure 115- Molecular weight distribution of CCE extracted components (rich in oligomers of xylan comparing to the other types of hemicelluloses) from hardwood pulp.

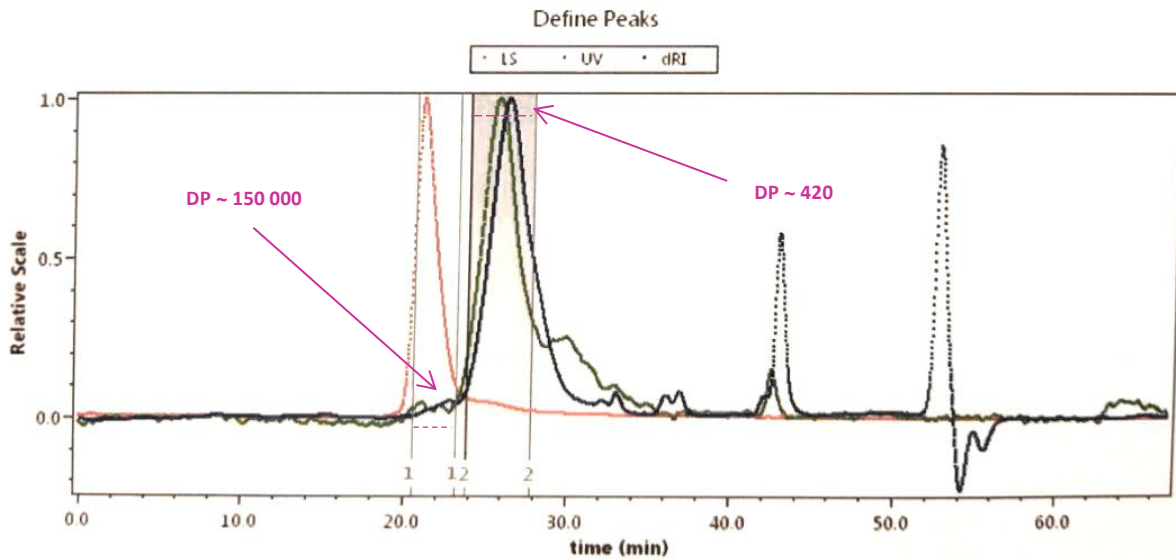


Figure 116- Molecular weight distribution of CCE extracted oligomers of xylan from softwood pulp.

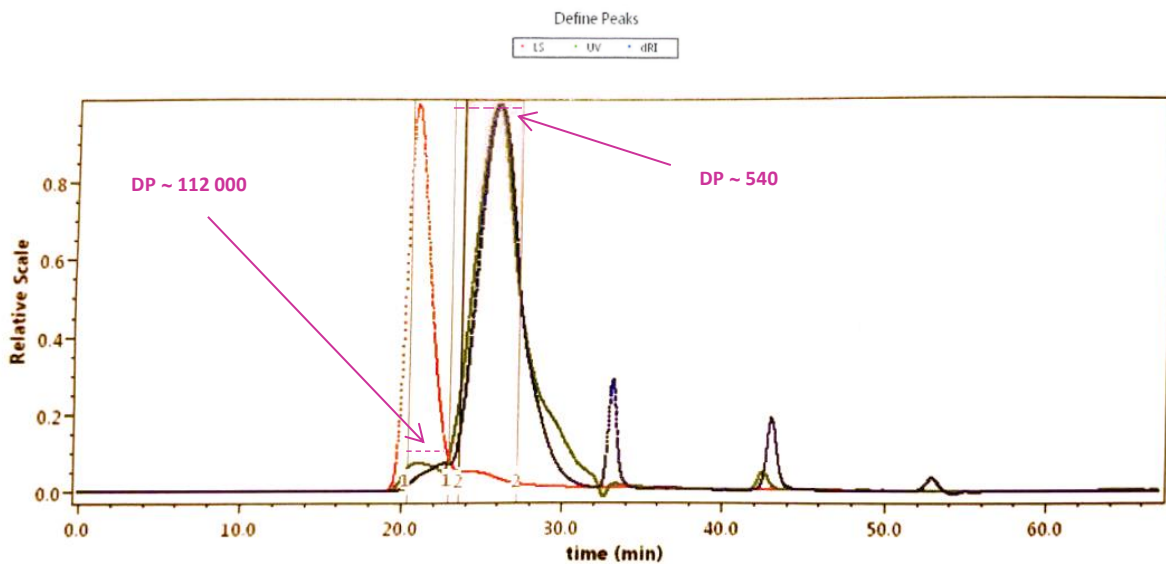


Figure 117- Molecular weight distribution of CCE extracted oligomers of mannan from softwood pulp.

4-2-2- Molecular structure

The released oligo and polysaccharides from CCE treatment of pulps were analysed by MALDI-TOF for the two different fractions that were obtained when doing a CCE treatment: one rich in xylan and the other rich in mannan. Therefore, four sets of samples were analysed: two for the hardwood pulp and two for the softwood pulp.

The xylan-rich fraction of CCE-extracts was first compared between the hardwood and the softwood pulp. Figure 118 compares the structure of softwood and hardwood xylans in this fraction, and Figure 119 compares the structure of softwood and hardwood mannans in the same fraction. The purple circle represents hardwood samples and the yellow ones belong to

the softwood. The horizontal axe represents the DP values of the components and the vertical axe represents the type of side-groups attached to the main chain: linear chain (no side-groups), Acetylated chain (Ac.), the chains with glucuronic acid side groups (Glu.), the chains with hexuronic acid side groups (Hex.), the combination of acetyl group and glucuronic groups (Ac. + Glu.) and the combination of acetyl group and hexuronic group (Ac. + Hex.).

Figure 118 shows that the DP values of released linear chains of xylan goes up to 12 for both wood species and the absence of longer linear chains could be due to their insolubility in the solvent used for the analysis. Molecules with higher DPs could be detected for substituted xylans.

Figure 119 show that the released mannans are either linear or acetylated.

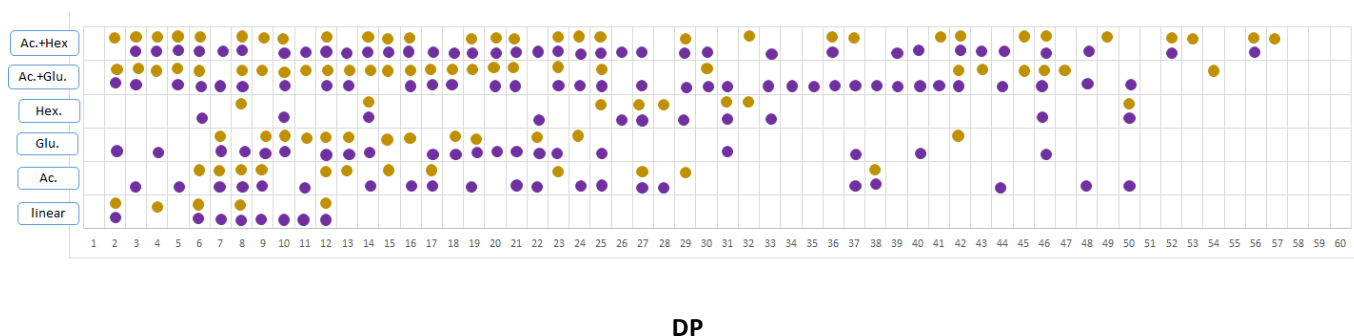


Figure 118- Structure of isolated oligomers of xylan by CCE-treatment of hardwood and softwood pulps, the portion rich in xylan (purple: hardwood, yellow: softwood).

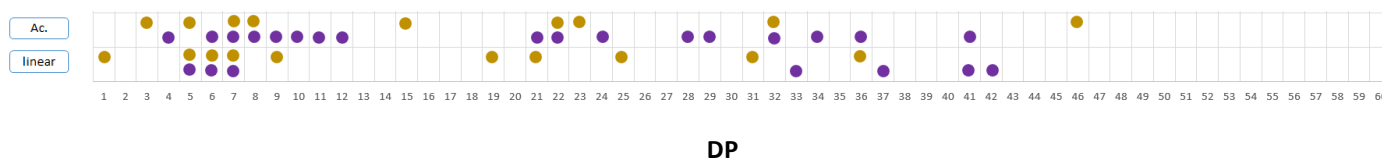


Figure 119- Structure of isolated oligomers of mannan by CCE-treatment of hardwood and softwood pulps, the portion rich in xylan (purple: hardwood, yellow: softwood).

Figure 120 represents the release of some linear oligomers of xylan and mannan in hardwood and some other non-linear oligomers of both xylan and mannan as an example. It is seen that even though this portion is rich in xylan, the presence of oligomers of hexoses has been detected, even in a very small quantity. In the very large view of the whole spectrum, the presence of non-linear chains is represented and for some peaks there are different possibilities of the molecular structure of the released component (figure 120 to 122).

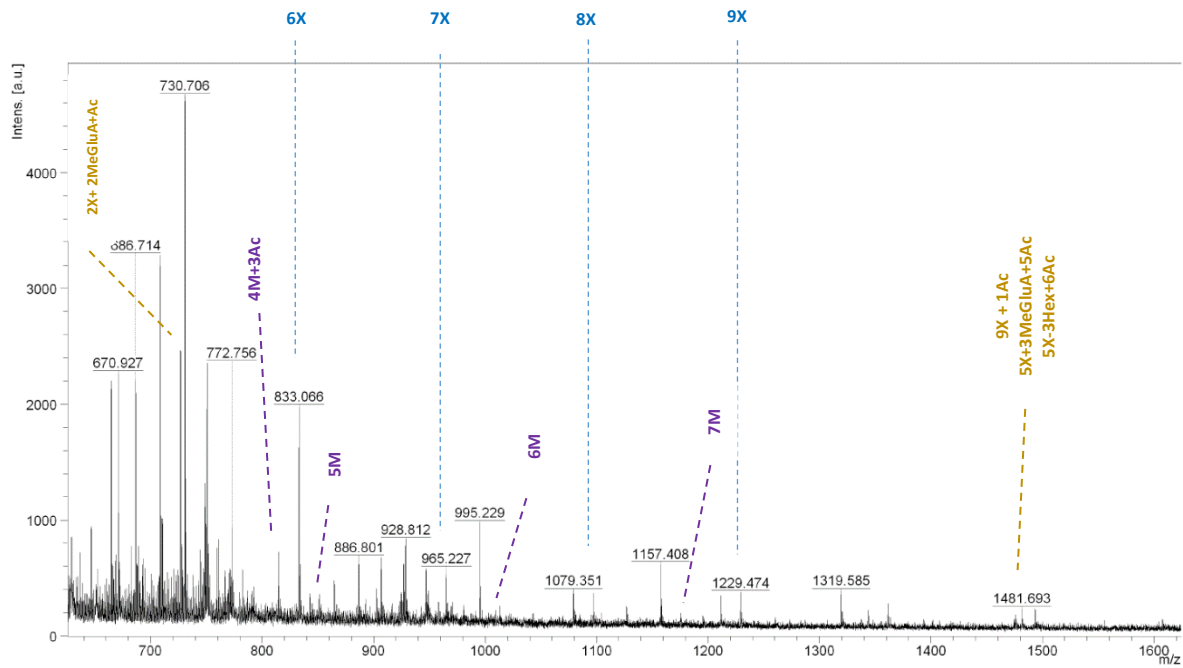


Figure 120- The spectrums of MALDI-TOF mass spectroscopy in reflectron positive mode for isolated oligomers of xylan and mannan through CCE treatment of hardwood pulps (nX: oligomers of xylan and nM oligomers of mannan with the DP value of n).

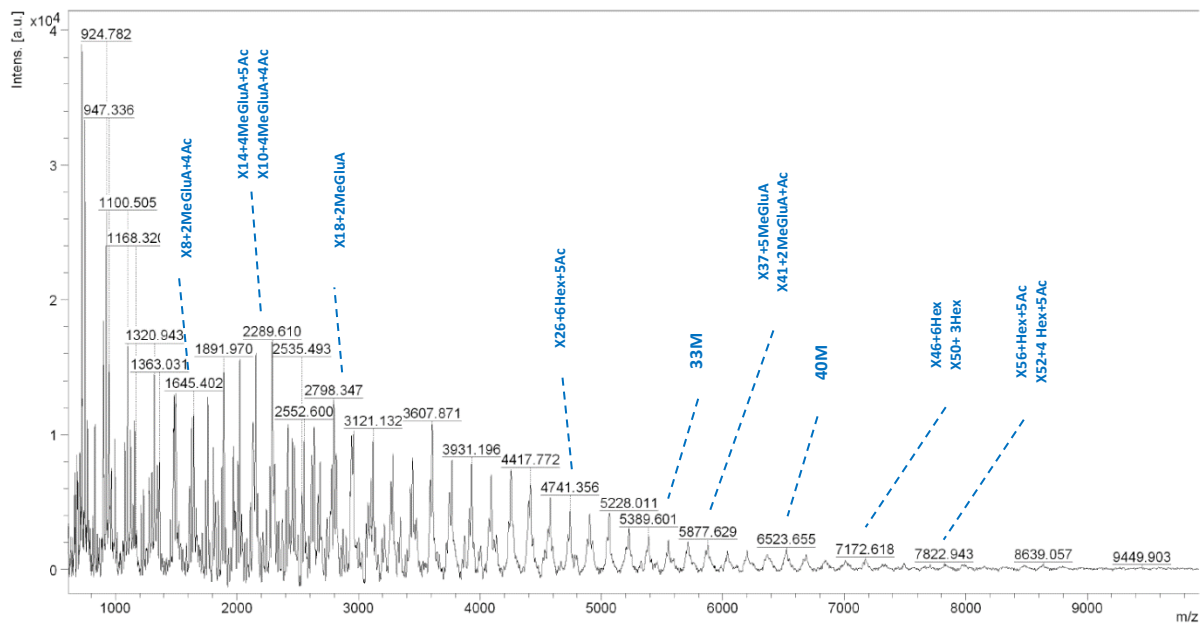


Figure 121- The spectrums of MALDI-TOF mass spectroscopy in linear positive mode for isolated oligomers of xylan and mannan through CCE treatment of hardwood pulps (nX: oligomers of xylan and nM oligomers of mannan with the DP value of n).

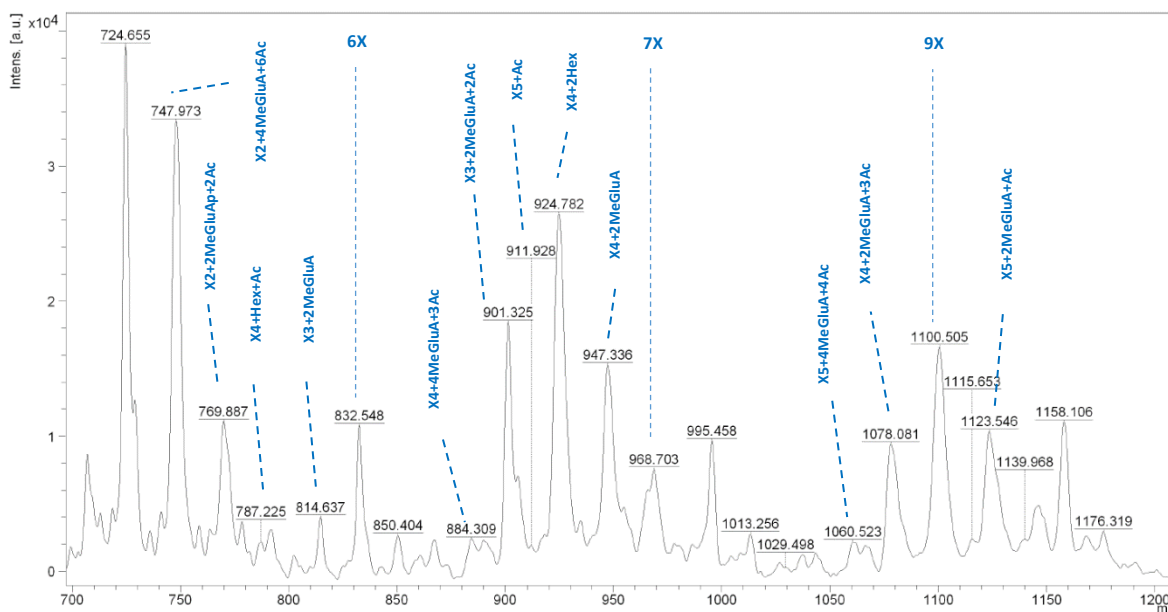


Figure 122- The spectrums of MALDI-TOF mass spectroscopy in linear positive mode for isolated oligomers of xylan and mannan through CCE treatment of hardwood pulps (nX: oligomers of xylan with the DP value of n).

The mannan rich fraction of CCE-extracts was then compared between the hardwood and the softwood pulp. Figure 123 compares the structure of softwood and hardwood xylans in this fraction, and Figure 124 compares the structure of softwood and hardwood mannans in the same fraction. Even though the quantitative sugar analysis (chapter 3) did not show significant quantities of xylans in the mannan rich fraction of CCE extracts, the MaldiTof analysis showed that they could be detected in a great variety of structures.

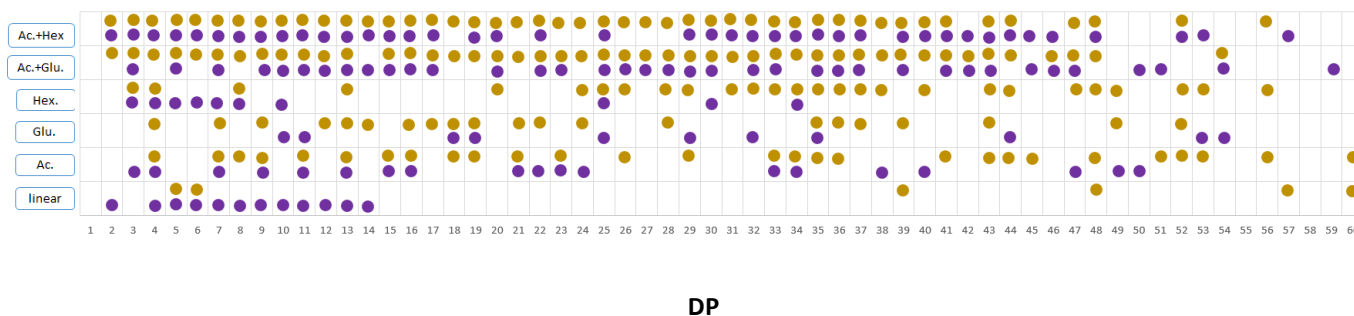


Figure 123- Structure of isolated oligomers of xylan through CCE-treatment of hardwood and softwood pulps, the portion rich in mannan (purple: hardwood, yellow: softwood).

Regarding the release of C6 oligomers which are dominant if this mannan rich fraction, linear and acetylated oligomers were detected (Fig 124).

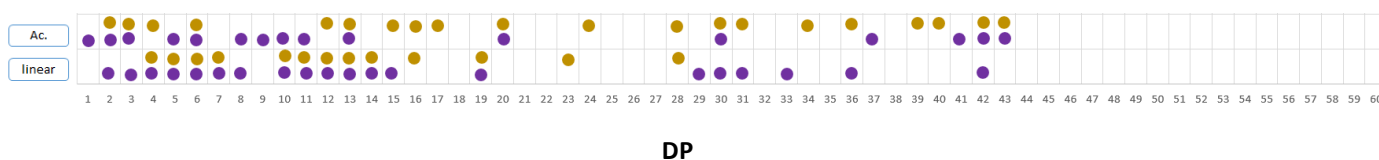


Figure 124- Structure of isolated oligomers of mannan through CCE-treatment of hardwood and softwood pulps, the portion rich in mannan (purple: hardwood, yellow: softwood).

Conclusion

This study has shown that the molecular structure of the xylans and mannans extracted by the CCE treatment is quite similar between hardwood and softwood pulps, which means that the cooking and bleaching processes used to prepare these pulps have levelled out the structures. Even though the alkaline cooking is supposed to hydrolyse the acetyl groups present in hemicelluloses in wood, there are still some left. The presence of methylglucuronic acid groups also suggest that not all of them were transformed into hexenuronic acid groups during cooking. Finally, even though the bleaching sequence used chlorine dioxide, prone to react with hexenuronic acid groups, there were still some left on xylans in the bleached pulps.

4-3- Molecular structure of the oligomers extracted by the sequential treatment of enzymes and CCE

The results presented in chapter 3 showed that applying an enzymatic treatment prior to CCE led to increased extraction of hemicelluloses. This part will study the structure of the release oligomers. The molecular weight distribution results were not exploitable, certainly because the polymers formed aggregates, but the hypothesis can be made that the enzymatic treatment led to either reduction of the size of pulp's hemicelluloses, and/or, modified their accessibility to caustic soda in the CCE treatment.

The hemicelluloses extracted from pulps by the sequential enzyme + CCE treatments were analysed by Maldi-TOF spectroscopy.

The results presented in Figures 126 to 129 show that the diversity of the isolated oligomers of hemicelluloses through the combination of enzymatic treatments and CCE is similar to the analysis of the CCE extracts without enzymatic pre-treatment, except that it seems that the values of the DP of the molecules detected are higher especially for the linear (non-substituted) xylan molecule (figures 126 versus fig 118).

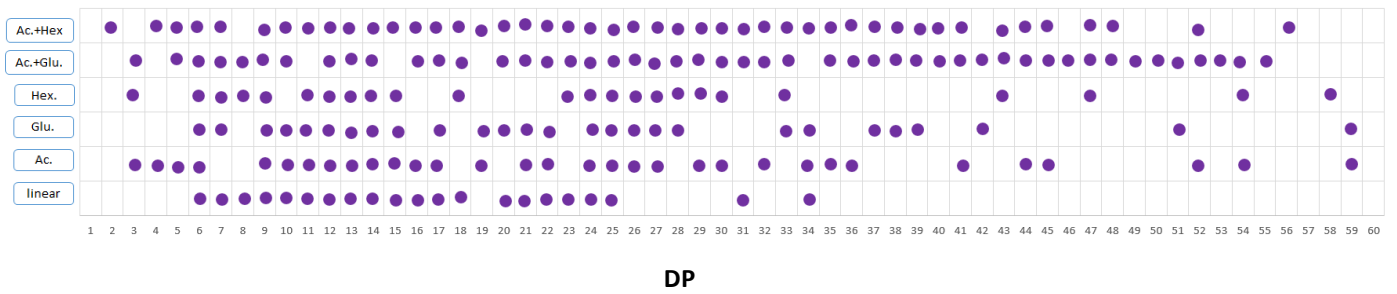


Figure 125- Structure of isolated oligomers of xylan through a combination of treatment with xylanase for 72 h and then CCE on hardwood pulp.

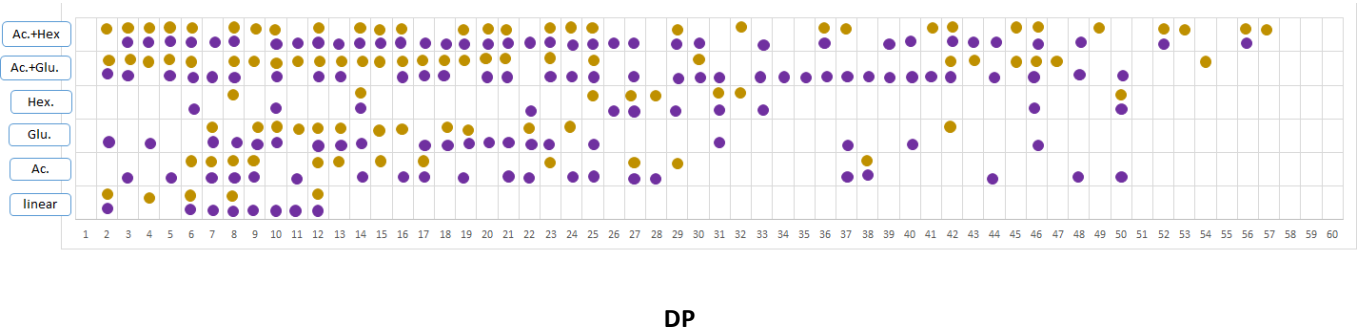


Figure 126- Structure of isolated oligomers of xylan through CCE-treatment of hardwood and softwood pulps, the portion rich in xylan (purple: hardwood, yellow: softwood).

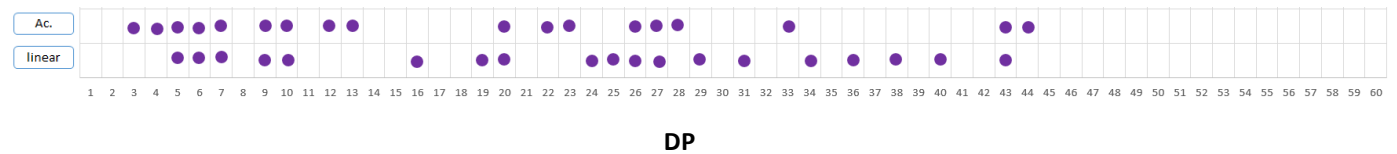


Figure 127- Structure of isolated oligomers of mannan through a combination of treatment with xylanase for 72 h and then CCE on hardwood pulp.

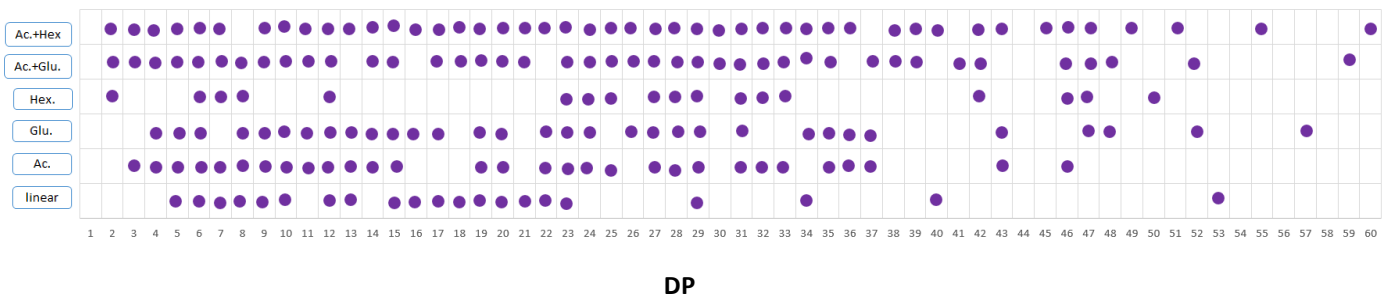


Figure 128- Structure of isolated oligomers of xylan through a combination of treatment with mannanase for 72 h and then CCE on softwood pulp.



Figure 129- Structure of isolated oligomers of mannan through a combination of treatment with mannanase for 72 h and then CCE on softwood pulp.

4.4. General conclusion of the chapter

The results presented in this chapter have shown that the xylanase treatment of hardwood pulp, led mainly (85% of the mass) to the extraction of short oligomers (average DP of 3-4), and to a minor extent (15% of the mass) to a second family of molecules with an average DP of 15-18. Regarding the effect of mannanase on softwood, which was not efficient as shown in chapter 3, 10% of the mass released corresponded to oligomers with average DP of 1-2, whereas 90% of the mass did not seem to be saccharides.

The mass spectroscopy analysis showed the release of highly diverse components; from linear oligomers to non-linear with different combination of side-groups (acetylene groups, glucuronic acid groups, methylglucuronic acid groups) on xylans. Acetylated mannans could also be found.

Regarding the hemicelluloses extracted from the pulps by the CCE treatment, this study has shown that the molecular structure of the xylans and mannans extracted is quite similar between hardwood and softwood pulps, which means that the cooking and bleaching processes used to prepare these pulps have levelled out the structures. Even though the alkaline cooking is supposed to hydrolyse the acetyl groups present in hemicelluloses in wood, there are still some left. The presence of methylglucuronic acid groups also suggest that not all of them were transformed into hexenuronic acid groups during cooking. Finally, even though the bleaching sequence used chlorine dioxide, prone to react with hexenuronic acid groups, there were still some left on xylans in the bleached pulps.

General conclusions and prospects

General conclusion and prospects

The main objective of this thesis was to investigate the performance of enzymatic treatments to remove hemicelluloses from bleached kraft hardwood and softwood pulps for possible further valorization of hemicelluloses.

Enzymatic treatment being an environmentally-friendly and selective method is indeed of interest for that purpose, and was at the heart of the PhD work. Cold caustic extraction (CCE) being used at industrial scale to remove hemicelluloses from pulp was used as a reference, and was also used in combination with the enzymatic processes studied.

The CCE treatment alone was more efficient on the softwood pulp than on the hardwood pulp, as it removed 26% of the hemicelluloses initially present from the hardwood compared to 36% from the softwood pulp. CCE removed only 20% of the hardwood xylan, whereas it removed 55% of them in the softwood pulp. Inversely, glucomannans from the hardwood pulp were completely removed whereas only 7% of them were extracted from the softwood pulp. The difficulty to remove xylan from hardwood compared to softwood was confirmed when using the xylanase treatment.

With CCE treatment the DP values of both starting pulps showed a slight increase suggesting the dissolution of the lower-molecular-weight hemicelluloses and no effect on cellulose. It was confirmed by studying the molecular weight distribution of the pulps with the lowering the hemicellulose shoulder

The enzymatic treatments were done by varying the quantity of enzymes, the duration and the consistency. Most of the experiments were done at 10% consistency, and showed that the release of hemicelluloses reached a plateau after 48 hours, whatever the charge in enzymes, hypothetically because of inaccessibility of substrates to enzymes. By releasing just xylans, xylanase showed itself selective in hydrolyzing the xylan chains, while mannanase treatment resulted in releasing both xylans and mannans suggesting either that the enzyme solution did not contain pure mannanase, or that the dissolution of mannans facilitated the extraction of xylans. The best results obtained for the extraction of hemicelluloses from pulp were: 14.8% when xylanase was applied on hardwood pulp, 22.3% for xylanase on softwood pulp, 1.1% for mannanase on hardwood pulp and 7.9% for mannanase on softwood pulp. The relative extraction of hemicelluloses seemed easier from softwood than from hardwood pulp.

The study of the effect of pulp consistency of hemicelluloses extraction by enzymes showed better results for 5% compared to 10%, reflecting the importance of media homogeneity.

The xylanase treated pulps, showed a slight increase in DP, probably with the same logic as CCE treated pulps in the process of removing of small-chain hemicelluloses, which was confirmed by the study of distribution of molecular weight with the lowering the hemicellulose peak. However, in the case of mannanase treatment on softwood, the DP was slightly lowered. We have no explanation for that as no viscosity loss was observed when the hardwood pulp was treated with mannanase.

Combination of enzymes was studied. The use of both enzymes, either in a consecutive way or added together led to overall better xylan extraction from the softwood pulp, but no significant impact could be seen on the extraction of mannans. In the case of the hardwood pulp, the best result was obtained when xylanase and mannanase were added together, rather than in a consecutive way, which implies a strong synergistic effect in that case. The difference in arrangements supports probably the fact that the hemicelluloses have either different structures in the two wood species and/or do not show the same accessibility to enzymes.

Applying a CCE treatment after the enzymatic treatment was much more efficient than CCE alone or enzymatic treatment alone especially for long incubation times: the percentage of hemicelluloses in the softwood pulp dropped from 13.7% to 6.2%, and from 24.3% to 7.0% for the hardwood pulp. One explanation could be that the enzymatic treatment has decreased the DP of hemicelluloses making them more easily solubilized by the CCE treatment.

Regarding the characterization of the oligosaccharides released by the enzymatic treatments, it was shown that the xylanase treatment of hardwood pulp, led mainly (85% of the mass) to the extraction of short oligomers (average DP of 3-4), and to a minor extent (15% of the mass) to a second family of molecules with an average DP of 15-18. Regarding the effect of mannanase on softwood, the oligomers released had an average value of DP 1-3.

The mass spectroscopy analysis showed the release of highly diverse components; from linear oligomers to non-linear with different combination of side-groups (acetyl groups, glucuronic acid groups, methylglucuronic acid groups) on xylans. Acetylated mannans could also be found.

Regarding the hemicelluloses extracted from the pulps by the CCE treatment, this study has shown that the molecular structure of the xylans and mannans extracted is quite similar between hardwood and softwood pulps, which means that the cooking and bleaching processes used to prepare these pulps have levelled out the structures. Even though the alkaline cooking is supposed to hydrolyse the acetyl groups present in hemicelluloses in wood, there are still some left. The presence of methylglucuronic acid groups also suggest that not all of them were transformed into hexenuronic acid groups during cooking. Finally, even though the bleaching sequence used chlorine dioxide, prone to react with hexenuronic acid groups, there were still some left on xylans in the bleached pulps.

As a prospect, it is suggested applying other enzymes than hemicellulases, like endoglucanase, as pre- or post-treatment, in order to loosen the bonds and to investigate its effect on extracting the oligomers of hemicelluloses and meanwhile to increase the reactivity of cellulose in the following steps of production of dissolving pulp, if such production is desired. Furthermore, applying enzymes like acetylase or glucuronidase could be investigated if pure linear oligomers are targeted.

Combination of enzymatic treatment with mechanical treatment of the fibers to improve the accessibility of enzymes to hemicelluloses could also be suggested.

References

References

- [1] Anatoly A. Shatalov, Helena Pereira, Impact of hexenuronic acids on xylanase-aided bio-bleaching of chemical pulps, *Bioresource Technology*, 2009, 100, p. 3069-3075
- [2] Xuejiao Lina, Zhengmei Wua, Chenyuan Zhang, et al., Enzymatic pulping of lignocellulosic biomass, *Industrial Crops & Products*, 2018, 120, p. 16-24
- [3] Gary Chinga-Carrasco, Per Olav Johnsen, Karin Øyaas, Structural quantification of wood fibre surfaces—Morphological effects of pulping and enzymatic treatment, *Micron*, 2010, 41, p. 648-659
- [4] Amélie Tribota, Ghenima Amerb, Maarouf Abdou Alio, Wood-lignin: Supply, extraction processes and use as bio-based material, *European Polymer Journal*, 2019, 112, p. 228-240
- [5] Francis W. M. R. SCHWARZE, Wood decay under the microscope, *fungus biology reviews*, 2007, 21, p. 133-170
- [6] Thomas Nilsson, Roger Rowell, Historical wood - structure and properties, *Journal of Cultural Heritage*, 2012, 13S, p. S5-S9
- [7] Herbert Sixta, Gabriele Schild, A New Generation Kraft Process, *Lenzinger Berichte*, 2009, 87, p. 26-37
- [8] Washington State Air Toxic Sources and Emission Estimation Methods, Chemical Pulp Mills
- [9] EPA (United states Environmental Protection Agency), Chapter 10: Wood Products Industry, *AP-42: Compilation of Air Emissions*, Fifth Edition, Volume I, 1990
- [10] European Environment Agency, Chapter 2: Pulp and Paper, *EMEP/EEA emission inventory guidebook*, 2009
- [11] Verena Gehmayr, Gabriele Schild, Herbert Sixta, A precise study on the feasibility of enzyme treatments of a kraft pulp for viscose application, *Cellulose*, 2011, 18, p. 479-491
- [12] Dionex Corporation, Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD), *Technical Note 20*, 2001
- [13] Khattri, Nidhi Zia, Heidi Gunnarson, et al., Chapter: Pulp and Paper Mills, *Pollution prevention and abatement handbook: toward cleaner production*, 1998, p. 395-400
- [14] B. Saake, *Cellulosederivate und Celluloseregenerate*, Vorlesungsfolien Holzwirtschaft Universität, Hamburg, 2013
- [15] Honghi Tran, Esa K. Vakkilainen, *The kraft chemical recovery process*, p. 1-8
- [16] Avdhes K. Gangwar, N. Tejo Prakash, Ranjana Prakash, Applicability of microbial xylanases in paper pulp bleaching: A review, *bioresources*, 2014, 9 (2), p. 3733-3754
- [17] John H. Gibbons, Chapter 2: The Pulp and Paper Making Processes, *Technologies for Reducing Dioxin in the Manufacture of Bleached Wood Pulp*, 1989, p. 17-26
- [18] Johanna Buchert, Maija Tenkanen, Anne Kantelinen, et al., Application of Xylanases in the Pulp and Paper Industry, *Bioresource Technology*, 1994, 50, p. 65-72
- [19] Rafael Vicuna, Elisa Oyarzun, Miguel Osses, Assessment of various commercial enzymes in the bleaching of radiata pine kraft pulps, *Journal of Biotechnology*, 1995, 40, p. 163-168

- [20] Ole Kirk, Torben Vedel Borchert, Claus Crone Fuglsang, Industrial enzyme applications, *Current Opinion in Biotechnology*, 2002, 13, p. 345-351
- [21] Jan B van Beilen, Zhi Li, Enzyme technology: an overview, *Current Opinion in Biotechnology*, 2002, 13, p. 338-344
- [22] J. Pérez, J. Munoz-Dorado, T. de la Rubia, et al., Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview, *International Microbiology*, 2002, 5, p. 53-63.
- [23] C. Ratledge, Chapter 8: Biodegradation of lignin and hemicelluloses, *Biochemistry of Microbial Degradation*, 1994, p. 233-277
- [24] Pratima Bajpai, Biological Bleaching of Chemical Pulps, *Critical Reviews in Biotechnology*, 2004, 24 (1), p. 1-58
- [25] Konrad B Otte, Bernhard Hauer, Enzyme engineering in the context of novel pathways and products, *Current Opinion in Biotechnology*, 2015, 35, p. 16-22
- [26] Jung-Min Choi, Sang-Soo Han, Hak-Sung Kim, Industrial applications of enzyme biocatalysis: Current status and future aspects, *Biotechnology Advances*, 2015, 33, p. 1443-1454
- [27] K.-E. L. Eriksson, Biotechnology in the pulp and paper industry, *Wood Science and Technology*, 1990, 24, p. 79-101
- [28] S. Subramaniyan, P. Prema, Biotechnology of Microbial Xylanases: Enzymology, Molecular Biology, and Application, *Critical Reviews in Biotechnology*, 2002, 22, 1, p. 33-64.
- [29] Rafael Vicuña, Fabiola Escobar, Miguel Osses, et al., Bleaching of eucalyptus Kraft pulp with commercial xylanases, *Biotechnology Letters*, 1997, 19, 6, p. 575-578
- [30] Rafael Vicuna, Maria Cristina Yeber, Miguel Osses, Bleaching of radiata pine Kraft pulp with the EnZone process, *Journal of Biotechnology*, 1995, 42, p. 69-73
- [31] Qian Liu, Guanhua Xun, Yan Feng, The state-of-the-art strategies of protein engineering for enzyme stabilization, *Biotechnology advances*, 2018
- [32] Jianguo Li, Huichao Hua, Hailong Li, et al., Kinetics and mechanism of hemicelluloses removal from cellulosic fibers during the cold caustic extraction process, *Bioresource Technology*, 2017, 234, p. 61-66
- [33] Brennan Pecha, Manuel Garcia-Perez, Chapter 26: Pyrolysis of Lignocellulosic Biomass, *Bioenergy*, 2015, p. 413-442
- [34] J. Haarhoff, C.J. Moes, C. Cerff, Characterization and biobleaching effect of hemicellulases produced by thermophilic fungi, *Biotechnology Letters*, 1999, 21, p. 415-420
- [35] Preeti B. Subhedar, Parag R. Gogate, Intensification of Enzymatic Hydrolysis of Lignocellulose Using Ultrasound for Efficient Bioethanol Production: A Review, *Industrial & Engineering Chemistry Research*, 2013, 52, p. 11816-11828
- [36] <https://pubchem.ncbi.nlm.nih.gov>
- [37] Jonas Wetterling, Modelling of Hemicellulose Degradation during Softwood Kraft Pulping, Master thesis in Chemical and Biological Engineering, Gothenburg, Sweden, 2012, 77 p
- [38] Charles E. Wyman, Stephen R. Decker, John W. Brady et al., Hydrolysis of Cellulose and Hemicellulose, 2005, p. 1023-1062

- [39] Sverker Danielsson, Xylan Reactions in Kraft Cooking, Process and Product Considerations, Ph. D. thesis in Wood Chemistry and Pulp Technology, Stockholm, 2007, 84 p
- [40] Dan Johansson, Carbohydrate degradation and dissolution during Kraft cooking, Ph. D. thesis in Chemical Engineering, Karlstad, Sweden, 2008, 62 p
- [41] A.L. Torres, M.B. Roncero, J.F. Colom, Effect of a novel enzyme on fibre morphology during ECF bleaching of oxygen delignified Eucalyptus kraft pulps, *Bioresource Technology*, 2000, 74, p. 135-140
- [42] P.C. Pinto, D.V. Evtuguin, C. Pascoal Neto, Structure of hardwood glucuronoxylans: modifications and impact on pulp retention during wood kraft pulping, *Carbohydrate Polymers*, 2005, 60, p. 489-497
- [43] Qijun Meng, Shiyu Fu, Bingyun Li, et al., The Impact of Xylanase and Hot Acid Pretreatment on HexAs in Eucalyptus Kraft Pulp Bleaching, *Journal of Wood Chemistry and Technology*, 2016, 35, 4, p. 239-250
- [44] J. M. Fang, R. C. Sun, J. Tomkinson, Isolation and characterization of hemicelluloses and cellulose from rye straw by alkaline peroxide extraction, *Cellulose*, 2000, 7, p. 87-107
- [45] L. Christov, P. Biely, E. Kalogeris, et al., Effects of purified endo- β -1,4-xylanases of family 10 and 11 and acetyl xylan esterases on eucalypt sulfite dissolving pulp, *Journal of Biotechnology*, 2000, 83, p. 231-244
- [46] Herbert Sixta, *Handbook of pulp*, 2006
- [47] Monica Ek, Göran Gellerstedt, Gunnar Henriksson, Volume 2: Pulp and Paper Chemistry and Technology, *Pulping Chemistry and Technology*, 2009
- [48] Juergen Puls, Ron Janzon, and Bodo Saake, Comparative Removal of Hemicelluloses from Paper Pulps Using NITREN, CUEN, NAOH, AND KOH, *Lenzinger Berichte*, 2006, 86, p. 63-70
- [49] L. Rahkamo, L. Viikari, J. Buchert, et al., Enzymatic and alkaline treatments of hardwood dissolving pulp, *Cellulose*, 1998, 5, p. 79-88
- [50] Eero Kontturi, presentation: Hemicellulose: dissolution, solution properties and modification, *Advanced Biomaterial Chemistry and Technology*, Alto university of technology, 2012
- [51] Tony Collins, Charles Gerday, Georges Feller, Xylanases, xylanase families and extremophilic xylanases, *FEMS Microbiology Reviews*, 2005, 29, p. 3-23
- [52] Wikipedia, Principles of Biochemistry/Enzymes
- [53] G.M Gübitz, T. Lischnig, D. Stebbing, et al., Enzymatic removal of hemicellulose from dissolving pulps, *Biotechnology Letters*, 1997, 19, 5, p. 491-495
- [54] Satyajit Das, Dominique Lachenal, Nathalie Marlin, Production of pure cellulose from Kraft pulp by a totally chlorine-free process using catalyzed hydrogen peroxide, *Industrial Crops and Products*, 2013, 49, p. 844– 850
- [55] Dmitry V. Evtuguin, Jorge L. Tomas, Artur M.S. Silva, et al., Characterization of an acetylated heteroxylan from Eucalyptus globulus Labill, *Carbohydrate Research*, 2003, 338, p. 597-604
- [56] Fengel, D., Wegener, G., *Wood - Chemistry, Ultrastructure, Reactions*, 1989, Berlin
- [57] T. Kent Kirk, Dan Cullen, Chapter 9: Enzymology and molecular genetics of wood degradation by white rot fungi, *Wood Degradation by White-Rot Fungi*, 1998, Wiley, New York, p. 273-307

- [58] M. Lawoko, R. Berggren, F. Berthold, et al., Changes in the lignin-carbohydrate complex in softwood Kraft pulp during Kraft and oxygen delignification, 2004, *Holzforschung*, 58, p. 603-610
- [59] L. Salmen, A.-M. Olsson, Interaction between hemicelluloses, lignin and cellulose: Structure property relationships, *Journal of Pulp and Paper Science*, 1998, 24, p. 99-103
- [60] Samriti Dhawan, Jagdeep Kaur, Microbial Mannanases: An Overview of Production and Applications, *Critical Reviews in Biotechnology*, 2007, 27, p. 197-216
- [61] M. Lawoko, Unveiling the structure and ultrastructure of lignin-carbohydrate complexes in softwoods, *International Journal of Biological Macromolecules*, 2013, 62, p. 705-713
- [62] Iuliana Spiridon, Ana Paula Duartet, Mohamed Naceur Belgacem, Enzymatic hydrolysis of pinus pinaster kraft pulp, *Appita Journal*, 2001
- [63] N. Terashima, M. Yoshida, J. Hafrén, et al., Proposed supramolecular structure of lignin in softwood tracheid compound middle lamella regions, 2012, *Holzforschung*, 66, p. 907-915
- [64] Koshijima T., Watanabe T., Association Between Lignin and Carbohydrates in Wood and Other Plant Tissues, *Springer-Verlag*, 2003, Heidelberg, Germany
- [65] Watanabe T, Koshijima T, Evidence for an ester linkage between lignin and glucuronic acid in lignin-carbohydrate complexes by DDQ-oxidation, *Agricultural and Biological Chemistry*, 1988, 52, p. 2953-2955
- [66] Joost van den Brink, Ronald P. de Vries, Fungal enzyme sets for plant polysaccharide degradation, *Applied Microbiology and Biotechnology*, 2011, 91, p. 1477-1492
- [67] Watanabe T, Ohnishi Y, Kaizu YS, et al., Binding site analysis of the ether linkages between lignin and hemicelluloses in lignin-carbohydrate complexes by DDQ-oxidation, *Agricultural and Biological Chemistry*, 1989, 53, p. 2233-2252
- [68] Badal C. Saha, Microbial Mannanases: Hemicellulose bioconversion, *Journal of Industrial Microbiology and Biotechnology*, 2003, 30, p. 279-291
- [69] Rubin Shmulsky, P. David Jones, *Forest Products and Wood Science: An Introduction*, Wiley-Blackwell, 2018
- [70] Reeve D.W., The kraft recovery cycle, Tappi Kraft recovery operations short courses, *Tappi Press*, 2002
- [71] L.P. Christov, M. Akhtar and B.A. Prior, Impact of Xylanase and Fungal Pretreatment on Alkali Solubility and Brightness of Dissolving Pulp, *Holzforschung*, 1996, 50, p. 579-582
- [72] Sjöström E., *Wood Chemistry: Fundamentals and Applications*, second edition, Academic Press, 1993, San Diego, California, USA
- [73] Franzon O., Samuelson O., Degradation of cellulose by alkali cooking, *Svensk Papperstidn*, 1957, 60, 23, p. 872-877
- [74] Young R. A., Sarkanen K. V., Johnson P. G., et al., Marine plant polymers, Part III, Kinetic analysis of the alkaline degradation of polysaccharides with specific reference to (1,3)- β -D-glucans, *Carbohydrate Research*, 1972, 21, p. 111-122
- [75] Lai Y. Z., Sarkanen K. V., Kinetic study on the alkaline degradation of amylose, *Journal of Polymer Science*, 1969, Part C, 28, p. 15-26

- [76] Sjöström E., The behavior of wood polysaccharides during alkaline pulping processes, *Tappi*, 1977, 60, 9, p. 151-154
- [77] Q. K. Beg, M. Kapoor, L. Mahajan, et al., Microbial xylanases and their industrial applications: a review, *Applied Microbiology and Biotechnology*, 2001, 56, p. 326-338
- [78] Veeresh Juturu, Jin Chuan Wu, Microbial xylanases: Engineering, production and industrial applications, *Biotechnology Advances*, 2012, 30, p. 1219-1227
- [79] Sartori J., Potthast A., Rosenau T., et al., Alkaline degradation of model compounds related to beech xylan, *Holzforschung*, 2004, 58, p. 588-596
- [80] Whistler R.L., BeMiller J.N., Alkaline degradation of polysaccharides, *Advances in carbohydrate chemistry*, 1958, 13, p. 289-329
- [81] Simonson R., The Hemicellulose in the Sulfate Pulping Process. Part 1. The Isolation of Hemicellulose Fractions from Pine Sulfate Cooking liquors, *Svensk Papperstidn*, 1963, 66, 20, p. 839-845
- [82] Corina Popovici, Mario Messier, Luc Thibault, Multiple benefits from the use of xylanase in a hardwood kraft mill at nexfor fraser paper in Thurso Quebec, *annual pulp and paper congress and exhibition*, 2002, Brasil
- [83] Cristina Valls, Oscar Gallardo, Teresa Vidal, Performance of new and commercial xylanases for ECF and TCF bleaching of eucalyptus kraft pulp, *Wood Science and Technology*, 2011, 45, p. 433-448
- [84] Aurell R., Hartler N., Kraft pulping of pine, Part 1. The changes in the composition of the wood residue during the cooking process, *Svensk Papperstidn*, 1965, 68, 3, p. 59-68
- [85] Hansson J., Hartler N., Alkaline degradation of Xylans from Birch and Pine, *Svensk Papperstidn*, 1968, 71, 9, p. 358-365
- [86] S. Gopi, P. Balakrishnan, D. Chandradhara, et al., General scenarios of cellulose and its use in the biomedical field, *Materials Today Chemistry*, 2019, 13, p. 59-78.
- [87] Digvijay Verma, T. Satyanarayana, Production of Cellulase-Free Xylanase by the Recombinant *Bacillus subtilis* and its Applicability in Paper Pulp Bleaching, *Biotechnology Progress*, 2013, Vol. 29, No. 6, p. 1441-1447
- [88] Lai Y. Z., Kinetics of base-catalyzed cleavage of glycosidic linkages, in: Ekman-Days, *International Symposium on Wood and Pulping Chemistry*, 1981, 2, p. 26-33
- [89] Yllner S., Enström B., Studies of the adsorption of xylan on cellulose fibres during the sulphate cook, Part 1. *Svensk Papperstid.*, 1956, 59, p. 229-32
- [90] Croon I., Enström B., The hemicelluloses in sulphate pulps from Scots pine, *Svensk Papperstid*, 1962, 65, p. 595-9
- [91] Hartler N., Lund A., Sorption of xylans on cotton, *Svensk Papperstid*, 1962, 65, p. 951-5
- [92] Yllner S., Ostberg K., Stockman L., A study of the removal of the constituents of pine wood in the sulphate process using a continuous liquor flow method, *Svensk Papperstid*, 1957, 60, p. 795-802
- [93] Jansson J., Palenius I., Aspects of the colour of kraft pulp and kraft lignin, *Paper Timber*, 1972, 54, p. 343-52
- [94] Jansson J., Palenius I., Stenkud B., et al., Differences in colour and strength of kraft pulps from batch and flow cooking, *Paper Timber*, 1975, 57, p. 387-90

- [95] Iversen T., Wannström S., Lignin-carbohydrate bonds in a residual lignin isolated from pine kraft pulp, *Holzforschung*, 1986, 40, p. 19-22
- [96] Ahlgren P. A., Yean W. Q., Goring D. A. I., Chlorite delignification of spruce wood. Comparison of the molecular weight of the lignin dissolved with the size pores in the cell wall. *Tappi Journal*, 1971, 54, p. 737-40
- [97] Scallan A. M., The accommodation of water within pulp fibres. In Proc. Fibre-Water Interactions in Paper Making, *Pergamon Press*, Oxford, 1977, p. 9-27
- [98] L. P. Christov, B. A. Prior, Repeated treatments with *Aureobasidium pullulans* hemicellulases and alkali enhance biobleaching of sulphite pulps, *Enzyme and Microbial Technology*, 1996, 18, p. 244-250
- [99] Vuorinen T., Fagerström P., Buchert J., et al., Selective hydrolysis of hexenuronic acid groups and its application in ECF and TCF bleaching of kraft pulps, *Journal of Pulp and Paper Science*, 1999, 25, p. 155-162
- [100] T. Kent Kirk and Thomas W. Jeffries, Chapter 1: Roles for Microbial Enzymes in Pulp and Paper Processing, *American Chemical Society*, 1996
- [101] Jiang Z.-H., Van Lierop B., Berry R., Hexenuronic acid groups in pulping and bleaching chemistry, *Tappi Journal*, 2000, 83, p. 167-175
- [102] Buchert J., Bergnor E., Lindblad G., et al., Significance of xylan and glucomannan in the brightness reversion of kraft pulps, *Tappi Journal*, 1997, 80, p. 165-171
- [103] Devenyns J., Chauveheid E., Uronic acids and metal control, *Nineth International Symposium on Wood and Pulp Chemistry*, Montreal, Canada, 1997, p. 1-4
- [104] Päivi Mäki-Arvela, Tapio Salmi, Bjarne Holmbom, et al., Synthesis of Sugars by Hydrolysis of Hemicelluloses- A Review, *Chemical Reviews*, 2011, 111, p. 5638-5666
- [105] Elsander A., Ek M., Gellerstedt G., Oxalic acid formation during ECF and TCF bleaching of kraft pulp, *Tappi Journal*, 2000, 83, p. 73-77
- [106] Li J., Gellerstedt G., The contribution to kappa number from hexenuronic acid groups in pulp xylan, *Carbohydrate Research*, 1997, 302, p. 213-218
- [107] Buchert J., Teleman A., Harjunpää, V., et al., Effect of cooking and bleaching on the structure of xylan in conventional pine kraft pulp, *Tappi Journal*, 1995, 78, 11, p. 125-130
- [108] P. Bajpai, N.K. Bhardwaj, P.K. Bajpai, The impact of xylanases on bleaching of eucalyptus kraft pulp, *Journal of Biotechnology*, 1994, 38, p. 1-6
- [109] Guay D.F., B.J.W. Cole, R.C. Fort, et al., Mechanisms of oxidative degradation of carbohydrates during oxygen delignification. II. Reaction of photochemically generated hydroxyl radicals with methyl beta-cellobioside, *Journal of Wood Chemistry and Technology*, 2001, 21, 1, p. 67-79
- [110] Pfister K., Sjöström E., Characterization of spent bleaching liquors. Part 5. Composition of material dissolved during oxygen-alkali delignification, *Paperi ja Puu*, 1979, 61, p. 525-528
- [111] Gellerstedt G., W.W. Al-Dajani, Some factors affecting the brightness and TCF-bleachability of kraft pulp, *Nordic Pulp and Paper Research Journal*, 2003, 18, 1, p. 56-62

- [112] Axelsson P., M. Ek, A. Teder, The influence of alkali charge and temperature in the kraft cook on the QPQP bleachability and the kappa number composition of birch pulp, *Nordic Pulp and Paper Research Journal.*, 2002, 17, 3, p. 206-212
- [113] L. P. Christov, B. A. Prior, Xylan Removal from Dissolving Pulp Using Enzymes of *Aureobasidium Pullulans*, *Biotechnology Letters*, 1993, 15, 12, p. 1269-1274
- [114] Baptista C., N. Belgacem, A.P. Duarte, The effect of surfactants on kraft pulping of *Pinus pinaster*, *Appita Journal*, 2004, 57, 1, p. 35-39
- [115] M. L. T. M. Polizeli, A. C. S. Rizzatti, R. Monti, et al., Xylanases from fungi: properties and industrial applications, *Applied Microbiology and Biotechnology*, 2005, 67, p. 577-591
- [116] Liisa Viikari, Anne Kantelinen, Jorma Sundquist, et al., Xylanases in bleaching: From an idea to the industry, *FEMS Microbiology Reviews*, 1994, 13, p. 335-350
- [117] Pan G. X., Bolton J. L., Leary G. J., Determination of ferulic and *p*-coumaric acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment, *Journal of Agricultural and Food Chemistry*, 1998, 46, p. 5283-5288
- [118] S.A. Rydholm, *Pulping Processes*, Interscience Publishers, John Wiley & Sons, New York, 1965
- [119] David I., Viviana K.P., Per Tomas L., et al., Combination of alkaline and enzymatic treatments as a process for upgrading sisal paper-grade pulp to dissolving-grade pulp, *Bioresource Technology*, 2010, 101, p. 7416-7423
- [120] Gehmayr V., Sixta H., Pulp properties and their influence on enzymatic degradability, *Biomacromolecules*, 2012, 13, p. 645-651
- [121] Li Y., Liu Y., Chen W., et al., Facile extraction of cellulose nanocrystals from wood using ethanol and peroxide solvothermal pretreatment followed by ultrasonic nanofibrillation, *Green Chemistry*, 2015, 18, p. 1010-1018
- [122] Panthapulakkal S., Kirk D., Sain M., Alkaline extraction of xylan from wood using microwave and conventional heating, *Journal of Applied Polymer Science*, 2015, 132
- [123] Panthapulakkal S., Pakharenko V., Sain M., Microwave assisted short-time alkaline extraction of birch xylan, *Journal of Polymers and the Environment*, 2013, 21, p. 917-929
- [124] Kocurek M.J., Ingruber O.V., Wong A., Sulfite Science and Technology, *Joint Textbook Committee of the Paper Industry*, Atlanta, 1985
- [125] Schild G., Sixta H., Sulfur-free dissolving pulps and their application for viscose and lyocell, *Cellulose*, 2011, 18, p. 1113-1128
- [126] Sixta H., Iakovlev M., Testova L., et al., Novel concepts of dissolving pulp production, *Cellulose*, 2013, 20, p. 1547-1561
- [127] Li H., Saeed A., Jahan M.S., et al, Hemicellulose removal from hardwood chips in the pre-hydrolysis step of the kraft-based dissolving pulp production process, *Journal of Wood Chemistry and Technology*, 2010, 30, p. 48-60
- [128] Wollboldt R.P., Zuckerstätter G., Weber H.K., et al., Accessibility, reactivity and supramolecular structure of E. globulus pulps with reduced xylan content, *Wood Science and Technology*, 2010, 44, p. 533-546

- [129] Dahlman O., Jacobs A., Sjöberg J., Molecular properties of hemicelluloses located in the surface and inner layers of hardwood and softwood pulps, *Cellulose*, 2003, 10, p. 325-334
- [130] Hakala T.K., Liitiä T., Suurnäkki A., Enzyme-aided alkaline extraction of oligosaccharides and polymeric xylan from hardwood kraft pulp, *Carbohydrate Polymers*, 2013, 93, p. 102-108
- [131] Cao S., Pu Y., Studer M., et al., Chemical transformations of *Populus trichocarpa* during dilute acid pretreatment, *RSC Advances*, 2012, 2, p. 10925-10936
- [132] Ikeda T., Tomimura Y., Magara K., et al., Sulfuric acid bleaching of kraft pulp III: Reactivity of kraft pulping-resistant structures under acidic conditions, *Journal of Wood Science*, 1999, 45, 5, p. 417-424
- [133] Vuorinen T., Fagerström P., Buchert J., et al., Selective hydrolysis of hexenuronic acid groups and its application in ECF and TCF bleaching of kraft pulps, *Journal of Pulp and Paper Science*, 1999, 25, 5, p. 155-162
- [134] Schrems M., Brandt A., Welton T., et al., Ionic liquids as media for biomass processing: opportunities and restrictions, *Holzforschung*, 2011, 65, p. 527-533
- [135] Welton T., Room-temperature ionic liquids. Solvents for synthesis and catalysis, *Chemical Review*, 1999, 99, p. 2071-2084
- [136] Honglu X., Tiejun S., Wood liquefaction by ionic liquids, *Holzforschung*, 2006, 60, p. 509-512
- [137] Maki-Arvela P., Anugwom I., Virtanen P., et al., Dissolution of lignocellulosic materials and its constituents using ionic liquids – A review, *Industrial Crops and Products*, 2010, 32, p. 175-201
- [138] Pinkert A., Marsh K.N., Pang S., et al., Ionic liquids and their interaction with cellulose, *Chemical Reviews*, 2009, 109, p. 6712-6728
- [139] Wang W., Zhu J., Wang X., et al., Dissolution behavior of chitin in ionic liquids, *Journal of Macromolecular Science, Part B*, 2010, 49, p. 528-541
- [140] Qu C., Kishimoto T., Hamada M., et al., Dissolution and acetylation of ball-milled lignocellulosic biomass in ionic liquids at room temperature: application to nuclear magnetic resonance analysis of cell-wall components, *Holzforschung*, 2013, 67, p. 25-32
- [141] Froschauer C., Hummel M., Iakovlev M., et al., Separation of hemicellulose and cellulose from wood pulp by means of ionic liquid/cosolvent systems, *Biomacromolecules*, 2013, 14, p. 1741-1750
- [142] Roselli A., Hummel M., Monshizadeh A., et al., Ionic liquid extraction method for upgrading eucalyptus kraft pulp to high purity dissolving pulp, *Cellulose*, 2014, 21, p. 3655-3666
- [143] Glegg R.E., Ingerick D., Parmerter R.R., et al., Acetylation of cellulose I and II. Studied by limiting viscosity and X-ray diffraction, *Journal of Polymer Science*, 1968, 6, p. 745-773
- [144] Janzon R., Puls J., Bohn A., et al., Upgrading of papergrade pulps to dissolving pulps by nitren extraction: yields, molecular and supramolecular structures of nitren extracted pulps, *Cellulose*, 2008, 15, p. 739-750
- [145] Laine C., Kempainen K., Kuutti L., et al., Extraction of xylan from wood pulp and brewer's spent grain, *Industrial Crops and Products*, 2015, 70, p. 231-237
- [146] Wallis A. F. A., Wearne R. H., Chemical cellulose from radiate pine kraft pulp, *Appita Journal*, 1990, 43, p. 355-357

- [147] Grönqvist S., Hakala T.K., Kamppuri T., et al., Fibre porosity development of dissolving pulp during mechanical and enzymatic processing, *Cellulose*, 2014, 21, p. 3667-3676
- [148] Hui L., Liu Z., Ni Y., Characterization of high-yield pulp (HYP) by the solute exclusion technique, *Bioresource Technology*, 2009, 100, p. 6630-6634
- [149] Tian C., Zheng L., Miao Q., et al., Improving the reactivity of kraft based dissolving pulp for viscose rayon production by mechanical treatments, *Cellulose*, 2014, 21, p. 3647-3654
- [150] Miao Q., Tian C., Chen L., et al., 2015. Combined mechanical and enzymatic treatments for improving the Fock reactivity of hardwood kraft based dissolving pulp, *Cellulose*, 22, p. 803–809
- [151] Viikari L., Ranua M., Kantelinen A., et al., Bleaching with enzymes, *Biotechnology in the pulp and paper industry, Proceedings of the 3rd International Conference of Biotechnology on Pulp and Paper Industry*, Stockholm, Sweden, 1986, p. 67-69
- [152] Paice M.G., Gurnagul N., Page D.H., et al., Mechanism of hemicellulose directed prebleaching of kraft pulp, *Enzyme and Microbial Technology*, 1992, 14, 4, p. 272–276
- [153] Wong K.K.Y., Jong E.D.D., Saddler J.N., et al., Mechanisms of xylanase aided bleaching of kraft pulp. Part 2: Target substrates, *Appita Journal*, 1997, 50, 6, p. 509-518
- [154] Paice M.G., Jurasek L., Removing Hemicellulose from Pulps by Specific Enzymic Hydrolysis, *Journal of Wood Chemistry and Technology*, 1984, 4, p. 187-198
- [155] Roberts J., McCarthy A., Flynn N.J., et al., Modification of paper properties by the pretreatment of pulp with *Saccharomonospora viridis* xylanase, *Enzyme and Microbial Technology*, 1990, 12, p. 210-213
- [156] Senior D.J., Mayers P.R., Miller D., et al., Selective solubilization of xylan in pulp using a purified xylanase from *Trichoderma harzianum*, 1988, *Biotechnology Letters*, 10, p. 907-12
- [157] Qijun Meng, Shiyu Fu, Bingyun Li, et al., The Impact of Xylanase and Hot Acid Pretreatment on HexAs in Eucalyptus Kraft Pulp Bleaching, *Journal of Wood Chemistry and Technology*, 2015, 35, p. 239-250
- [158] Roncero M.B., Torres A.L., Colom J.F., et al., Effect of xylanase on ozone bleaching kinetics and properties of eucalyptus kraft pulp, *Journal of Chemical Technology & Biotechnology*, 2003, 78, 10, p. 1023-1031
- [159] Valls C., Vidal T., Gallardo O., et al., Obtaining low-HexA-content cellulose from eucalypt fibres: Which glycosyl hydrolase family is more efficient? *Carbohydrate Polymers*, 2010, 80, 1, p. 154–160
- [160] E. Valcheva, I. Valchev, L. Yotova, Kinetics of enzyme action of Cartazyme NS-10 prior to bleaching of kraft pulp, *Biochemical Engineering Journal*, 2001, 7, p. 223-226
- [161] José H. B. de Araujo, Flavio F. de Moraes, Gisella M. Zanin, Bleaching of Kraft Pulp with Commercial Xylanases, *Applied Biochemistry and Biotechnology*, 1999, 77-79, p. 713-722
- [162] Sandra L. Nelson, *Xylanase prebleaching of kraft pulps derived from three softwood species*, Ph.D. in wood science, the university of British Columbia, 1995, 109 p
- [163] Valls C., Vidal T., Roncero M.B., Enzymatic strategies to improve removal of hexenuronic acids and lignin from cellulosic fibers, *Holzforschung*, 2014, 68, 2, p. 229-237
- [164] Rahkamo L., Siika-Aho M., Viikari L., et al., Effects of cellulases and hemicellulase on the alkaline solubility of dissolving pulps, *Holzforschung*, 1998, 52, p. 630-634

- [165] William R. Kenealy, Thomas W. Jeffries, Rapid 2,2-bicinchoninic-based xylanase assay compatible with high throughput screening, *Biotechnology Letters*, 2003, 25, p. 1619-1623
- [166] Barbara R. Evans, Ruth Margalit, Jonathan Woodward, Enhanced Hydrolysis of Soluble Cellulosic Substrates by a Metallocellulase with Veratryl Alcohol-Oxidase Activity, *Applied Biochemistry and Biotechnology*, 1995, 51/52, p. 225-239
- [167] Saurabh Sudha Dhiman, Jitender Sharma, Bindu Battan, Industrial applications and future prospects of microbial xylanases: A review, *BioResources*, 2008, 3, 4, p. 1377-1402
- [168] Dr. P. Ghosh, Dr. Bijan Choudhury, Mr. Rahul Mantri, et al., Biobleaching: Enzymatic Colour Removal from Pulp, Indian Paper Manufacturers Association New Delhi, Birla Institute of Scientific Research Statue Circle, Jaipur, 2002
- [169] Keungarp Ryu, Youngon Kim, Adsorption of a xylanase purified from Pulpzyme HC onto alkali-lignin and crystalline cellulose, *Biotechnology Letters*, 1998, 20, 10, p. 987-990
- [170] Sandra L. Nelson, *Xylanase prebleaching of kraft pulps derived from three softwood species*, Th. Wood science, The university of British Columbia, May 1995, 109 p
- [171] Shen J., Kaur I., Baktash M.M., et al., A combined process of activated carbon adsorption, ion exchange resin treatment and membrane concentration for recovery of dissolved organics in pre-hydrolysis liquor of the kraft-based dissolving pulp production process, *Bioresource Technology*, 2013, 127, p. 59-65
- [172] Bajpai P., Bajpai P. K., Development of a process for the production of dissolving kraft pulp using xylanase enzyme, *Appita Journal*, 2001, 54, p. 381-384
- [173] Hyatt J. A., Fengel R. W., Edgar K. J., et al., Process for theco-production of dissolving-grade pulp and xylan, *United States Patent US6057438 A*, 2000
- [174] Jackson L. S., Heitmann J. A., Joyce T. W., Production of dissolving pulp from recovered paper using enzymes, *Tappi Journal*, 1998, 81, p. 171-178
- [175] Anatoly A. Shatalov, Helena Pereira, Effect of xylanases on peroxide bleachability of eucalypt (E. globulus) kraft pulp, *Biochemical Engineering Journal*, 2008, 40, p. 19-26
- [176] Olli-Pekka Lehtinen, *Modifying Wheat Bran for Food Applications - Effect of Wet Milling and Enzymatic Treatment*, thesis in Bio and Food Technology, 2012, 91 p
- [177] Bommarius A.S., Paye M.F., Stabilizing biocatalysts, *Chemical Society Reviews*, 2013, 42, 15, p. 6534-6565
- [178] Huisman GW, Liang J, Krebber A, Practical chiral alcohol manufacture using ketoreductases, *Current Opinion in Chemical Biology*, 2010, 14, p. 122-9
- [179] Reetz MT, Soni P, Fernandez L, et al., Increasing the stability of an enzyme toward hostile organic solvents by directed evolution based on iterative saturation mutagenesis using the B-FIT method, *Chem Commun*, 2010, 46, p. 8657-8
- [180] Adilson R. Gonçalves, Denise S. Ruzene, Regina Y. Moriya, et al., Pulping of Sugarcane Bagasse and Straw and Biobleaching of the Pulps: Conditions Parameters and Recycling of Enzymes, Presented at the 59th *Appita Conference*, Auckland, New Zealand, 16-19 May 2005
- [181] Lucia Franova, Vladimír Puchart, Peter Biely, b-Glucuronidase-coupled assays of glucuronoyl esterases, *Analytical Biochemistry*, 2016, 510, p. 114-119

- [182] Asish Mandal, Review on Microbial Xylanases and their Applications, *International Journal of Life Sciences*, 2015, 4, 3., p. 178-187
- [183] Vazquez-Figueroa E, Yeh V, Broering JM, et al., Thermostable variants constructed via the structure-guided consensus method also show increased stability in salts solutions and homogeneous aqueous-organic media, *Protein Engineering Design and Selection*, 2008, 21, p. 673–80
- [184] Vimalashanmugam, K, *Studies on xylanase enzyme production kinetics and modelling*, thesis in chemical engineering, Annamalai University, India, 2013, 422 p
- [185] Girish K Goswami, Seema Rawat, Microbial Xylanase and their applications - A review, *International Journal of Current Research and Academic Review*, 2015, 3, 6, p. 436-450
- [186] Rajendran Velmurugan, Aran Incharoensakdi, Chapter 18: Nanoparticles and Organic Matter: Process and Impact, *Nanomaterials in Plants, Algae, and Microorganisms*, Volume 1, 2018, P. 407-428
- [187] Bloom JD, Labthavikul ST, Otey CR, et al., Protein stability promotes evolvability, *Proceeding of the National Academy of Sciences*, 2006, 103, p. 5869-74
- [188] Krieger E, Koraimann G, Vriend G, Increasing the precision of comparative models with YASARA NOVA — a self-parameterizing force field, *Proteins*, 2002, 47, p. 393-402
- [189] Genencor International, *Product information, Multifect xylanase*
- [190] Schwab T, Skegro D, Mayans O, et al., A rationally designed monomeric variant of anthranilate phosphoribosyltransferase from *Sulfolobus solfataricus* is as active as the dimeric wild-type enzyme but less thermostable, *Journal of Molecular Biology*, 2008, 376, p. 506-16
- [191] L. Falcoz-Vigne, L. Heux, K. Mazeau, et al., Interaction of hemicelluloses and cellulose and their influence on the cellulose microfibrillation process, *European Fiber and Paper Research Organisations*, 17th - 19th November 2015, Brussels
- [192] Bommarius AS, Broering JM, Chaparro-Riggers JF, et al., High-throughput screening for enhanced protein stability, *Current Opinion in Biotechnology*, 2006, 17, p. 606-10
- [193] Illanes A, Cauerhff A, Wilson L, et al., Recent trends in biocatalysis engineering, *Bioresources Technol*, 2012, 115, p. 48-57
- [194] Kazlauskas R, Lutz S., Engineering enzymes by 'intelligent' design, *Current Opinion in Chemical Biology*, 2009, 13:1-2
- [195] Khare SD, Kipnis Y, Greisen PJ, et al., Computational redesign of a mononuclear zinc metalloenzyme for organophosphate hydrolysis, *Nature Chemical Biology*, 2012, 8, p. 294-300
- [196] Bhabha G, Lee J, Ekiert DC, et al., A dynamic knockout reveals that conformational fluctuations influence the chemical step of enzyme catalysis, *Science*, 2011, 332, p. 234-8
- [197] Joo JC, Pack SP, Kim YH, et al. Thermostabilization of *Bacillus circulans* xylanase: computational optimization of unstable residues based on thermal fluctuation analysis, *Journal of Biotechnology*, 2011,151, p. 56-65
- [198] Lee SC, Kim JH, Kim HS, Design and evolution of biocatalysts, *Current Organic Chemistry*, 2010, 14, p. 1894-901

- [199] Akoh CC, Chang SW, Lee GC, et al., Biocatalysis for the production of industrial products and functional foods from rice and other agricultural produce, *Journal of Agricultural and Food Chemistry*, 2008, 56, p. 10445-51
- [200] Queiroga AC, Pintado AM, Malcata FX, Novel microbial-mediated modifications of wool, *Enzyme and Microbial Technology*, 2007, 40, p. 1491-5
- [201] Yachmenev VG, Bertoniere NR, Blanchard EJ, Intensification of the bio-processing of cotton textiles by combined enzyme/ultrasound treatment, *Journal of Chemical Technology and Biotechnology*, 2002, 77, p. 559-67
- [202] Dincer A, Telefoncu A, Improving the stability of cellulase by immobilization on modified polyvinyl alcohol coated chitosan beads, *Journal of Molecular Catalysis B: Enzymatic*, 2007, 45, p. 10-4
- [203] Silva CJS, Prabakaran M, Gubitz G, et al., Treatment of wool fibres with subtilisin and subtilisin-PEG, *Enzyme and Microbial Technology*, 2005, 36, p. 917-22
- [204] David Gregg, John N. Saddle, A Techno-Economic Assessment of the Pretreatment and Fractionation Steps of a Biomass-to-Ethanol Process, *Applied Biochemistry and Biotechnology*, 1996, 57/58, p. 711-727
- [205] Patrick K., Enzyme technology improves efficiency, cost, safety of stickies removal program, *Paper Age*, 2004, p. 120:22-5
- [206] Demuner B., Junior N., Antunes A., Technology prospecting on enzymes for the pulp and paper industry, *Journal of Technology Management and Innovation*, 2011, 6, p. 148-158
- [207] Nie S., Zhang K., Lin X., et al., Enzymatic pretreatment for the improvements of dispersion and film properties of cellulose nanofibrils, *Carbohydrate Polymers*, 2018, 181, p. 1136-1142
- [208] Duan C., Wang X., Zhang Y., et al., Fractionation and cellulase treatment for enhancing the properties of kraft-based dissolving pulp, *Bioresource Technology*, 2017, 224, p. 439-444
- [209] Singh R., Bhardwaj N.K., Choudhury B., Cellulase-assisted refining optimization for saving electrical energy and pulp quality evaluation, *Journal of Scientific and Industrial Research*, 2015, 74, p. 471-475
- [210] Mohamed Naceur Belgacem, Alessandro Gandini, Chapter; Hemicelluloses: major sources, properties and applications, *Monomers, Polymers and Composites from Renewable Resources*, 2008, p. 289-304
- [211] Maan P., Dutt D., Reduction in pollution load in terms of AOX by using xylanase enzyme in bio-bleaching studies of *C. equisetifolia.*, *Environment and Ecology*, 2017, 35, p. 1159-1163
- [212] Christiane Laine, *Structures of Hemicelluloses and Pectins in Wood and Pulp*, Ph.D.: Chemical Technology, Espoo, Finland, 2005, 63 p
- [213] Nie S., Yao S., Qin C., et al., AOX Formation Mechanisms During the Chlorine Dioxide Bleaching of Bagasse Pulp, *BioResources*, 2014, 9, 3, p. 5604-5614
- [214] Gonçalves I., Silva C., Cavaco-Paulo A., Ultrasound enhanced laccase applications, *Green Chemistry*, 2015, 17, p. 1362-1374
- [215] Pei Y., Wang S., Qin C., et al., Optimization of laccase-aided chlorine dioxide bleaching of bagasse pulp, *Bioresources*, 2016, 11, p. 696-712

- [216] Wang Q., Jahan M.S., Liu S., et al., Lignin removal enhancement from prehydrolysis liquor of kraft-based dissolving pulp production by laccase-induced polymerization, *Bioresource Technology*, 2014, 164, p. 380-385
- [217] Kantelinen A., Hortling B., Sundquist J., et al., Proposed mechanism of the enzyme bleaching of kraft pulp with xylanases, *Holzforschung*, 1993, 47, p. 318-324
- [218] Engström A-C, Ek M, Henriksson G, Improved accessibility and reactivity of dissolving pulp for the viscose process: pretreatment with monocomponent endoglucanase, *Biomacromolecules*, 2006, 7, p. 2027-2031
- [219] Henriksson G, Christiernin M, Agnemo R, Monocomponent endoglucanase treatment increases the reactivity of softwood sulphite dissolving pulp, *Journal of Industrial Microbiology and Biotechnology*, 2005, 32, p. 211-214
- [220] Ibarra D, Köpcke V, Larsson PT, et al., Combination of alkaline and enzymatic treatments as a process for upgrading sisal paper-grade pulp to dissolving-grade pulp, *Bioresource Technology*, 2010, 101, p. 7416-7423
- [221] Köpcke V., Ibarra D., Ek M., Increasing accessibility and reactivity of paper grade pulp by enzymatic treatment for use as dissolving pulp, *Nordic Pulp and Paper Research Journal*, 2008, 23, p. 363-368
- [222] Luo M, Enzymatic treatment of pulp for lyocell manufacture, Weyerhaeuser-Company, *Patent PCT/US2008/086367*, 2009
- [223] Hinck JF, Casebier RL, Hamilton JK, Dissolving pulp manufacture. In: Kocurek MJ, Ingruber OV, Al-Wong PE (eds) *Sulfite science & technology*, 3rd eds., *Tappi*, CPPA, Atlanta, 1985, p. 213-243
- [224] Hiett LA, Dissolving cellulose: its present position and prospects for future development, *Tappi Journal*, 1985, 68, p. 42-48
- [225] Viikari L, Kantelinen A, Poutanen K, et al., Characterization of pulps treated with hemicellulolytic enzymes prior to bleaching, In: Kirk, K.T., Chang, H.-M (eds), *Biotechnology in Pulp and Paper Manufacture*, 1990, Butterworth-Heinemann, Boston, MA, p. 145-151
- [226] Annariikka Roselli, Sari Asikainen, Agnes Stepan, Comparison of pulp species in IONCELL-P: selective hemicellulose extraction method with ionic liquids, *Holzforschung*, 2016, 70, 4, p. 291-296
- [227] Christiane Lainea, Sari Asikainena, Riku Taljaa, et al., Simultaneous bench scale production of dissolving grade pulp and valuable hemicelluloses from softwood kraft pulp by ionic liquid extraction, *Carbohydrate Polymers*, 2016, 136, p. 402-408
- [228] Buchert J, Ranua M., Kantehnen A., et al., The role of two *Trichoderma reesei* xylanases in the bleaching of pine kraft pulp, *Applied Microbiology and Biotechnology*, 1992, 37, p. 825-829
- [229] Stone J.E., Scallan A.M., The effect of component removal upon the porous structure of a cell wall of wood, Part III. A comparison between the sulphite and kraft processes, *Pulp Paper Mag*, 1968, 6, p. 69-74
- [230] Wong K.Y., Deverell K.F., Mackie K.L., et al., The relationship between fiber porosity and cellulose digestibility in stream-exploded *Pinus radiata*, *Biotechnology and Bioengineering*, 1988, 31, p. 447-456
- [231] Suurnäkki A., Li T.-Q., Buchert J., et al., Effects of enzymatic removal of xylan and glucomannan on the pore size distribution of kraft fibres, *Holzforschung*, 1997, 51, p. 27-33

- [232] André Ferraz, Anderson Guerra, Régis Mendonça, Technological advances and mechanistic basis for fungal biopulping, *Enzyme and Microbial Technology*, 2008, 43, p. 178-185
- [233] Jianguo Li, Yishan Liu, Chao Duan, et al., Mechanical pretreatment improving hemicelluloses removal from cellulosic fibers during cold caustic extraction, *Bioresource Technology*, 2015, 192, p. 501-506
- [234] Stone J.E., Scallan A.M., Donefer E., et al., Digestibility as a simple function of a molecule of similar size to a cellulase enzyme. In: Hajny, G.J., Reese, R.T. (Eds.), *Cellulases and Their Applications*, American Chemical Society, Washington DC, 1969, p. 219-241
- [235] Grethlein H.E., The effect of pore size distribution on the rate of enzymatic hydrolysis of cellulosic substrates, *Biotechnology and Bioengineering*, 1985, 31, p. 447-456
- [236] Stone J.E., Scallan A.M., The effect of component removal upon the porous structure of a cell wall of wood, Part II. Swelling in water and the fiber saturation point, *Tappi*, 1967 50, p. 496-501
- [237] Jianguo Li, Hongjie Zhang, Chao Duan, et al., Enhancing hemicelluloses removal from a softwood sulfite pulp, *Bioresource Technology*, 2015, 192, p. 11-16
- [238] Srebotnik E., Messner K., Accessibility of sulfite pulp to lignin peroxidase and marker proteins with respect to enzymatic bleaching, *Journal of Biotechnology*, 1990, 13, p. 199-210
- [239] Christov LP, Prior BA, Role of hydrolysis conditions in the enzyme-mediated release of reducing sugars from sulphite pulp, *Biotechnology Letters*, 1995, 17, p. 821-826
- [240] Minor JL, Chemical linkage of polysaccharides to residual lignin in loblolly pine kraft pulps, *Journal of Wood Chemistry and Technology*, 1986, 6, p. 185-201
- [241] Gamerith G., Strutzenberger H., In: *Xylans and Xylanases*, J. Visser, et al., eds. 1992, Amsterdam: Elsevier, p. 339-348
- [242] Christov L. P., Prior B. A., Enzymatic prebleaching of sulphite pulps, *Applied Microbial and Biotechnology*, 1994, 42, p. 492-498
- [243] M.E. Brown, D. Dollimore, A.K. Galwey, *Reactions in the Solid State*, Elsevier, Amsterdam, 1980
- [244] Hyvonen L., Koivistoinen P., Voirol F., Food technological evaluation of xylitol, *Advances in Food Research*, 1982, 28, p. 373-403
- [245] Edgar W.M., Sugar substitutes, chewing gum and dental caries—a review, *British Dental Journal*, 1998, 184, p. 29-32
- [246] Makinen K.K., Dietary prevention of dental caries by xylitol—clinical effectiveness and safety, *Journal of Applied Nutrition*, 1992, 44, p. 16-28
- [247] Taherzadeh M.J., Eklund R., Gustafsson L., et al., Characterization and fermentation of dilute-acid hydrolyzates from wood, *Industrial & Engineering Chemistry Research*, 1997, 36, p. 4659-4665
- [248] Dekker R.F.H., Wallis A.F.A., Enzymic saccharification of sugarcane bagasse pretreated by autohydrolysis steam explosion, *Biotechnology and Bioengineering*, 1983, 25, p. 3027-3048
- [249] Torget R., Walter P., Himmel M., et al., Dilute-acid pretreatment of corn residues and short-rotation woody crops, *Applied Biochemistry and Biotechnology*, 1991, 28-29, p. 75-86
- [250] Kabel M.A., Carvalheiro F., Garrote G., et al., Hydrothermally treated xylan rich byproducts yield different classes of xylo-oligosaccharides, *Carbohydrate Polymers*, 2002, 50, p. 47-56

- [251] Aguilar R., Ramirez J.A., Garrote G., et al., Kinetic study of the acid hydrolysis of sugar cane bagasse, *Journal of Food Engineering*, 2002, 55, p. 309-318
- [252] Neureiter M., Danner H., Thomasser C., et al., Dilute-acid hydrolysis of sugarcane bagasse at varying conditions, *Applied Biochemistry and Biotechnology*, 2002, 98-100, p. 49-58
- [253] Grohmann K., Torget R., Himmel M., Optimization of dilute acid pretreatment of biomass, *Biotechnology and Bioengineering Symposium*, 1985, 15, p. 59-80
- [254] Magee R.J., Kosaric N., Bioconversion of hemicelluloses, *Advances in Biochemical Engineering Biotechnology*, 1985, 32, p. 61-93
- [255] Timell T.E., Wood hemicelluloses, *Advances in Carbohydrate Chemistry and Biochemistry*, 1965, 20, p. 409-483
- [256] Brown W, Wikstrom R., A viscosity-molecular weight relationship for cellulose in cadoxen, *European Polymer Journal*, 1965, 1, p. 1-10
- [257] Donohoe B.S., Vinzant T.B., Elander R.T., et al., Surface and ultrastructural characterization of raw and pretreated switchgrass, *Bioresource Technology*, 2011, 102, p. 11097-11104
- [258] Yarbrough J.M., Himmel M.E., Ding S.Y., Plant cell wall characterization using scanning probe microscopy techniques, *Biotechnology in Biofuels*, 2009, 2, p. 1754-6834
- [259] Vardakou M, Katapodis P, Topakas E, et al., Synergy between enzymes involved in the degradation of insoluble wheat flour arabinoxylan, *Innovative Food Science and Emerging Technology*, 2004, 5, p. 107-12
- [260] Gary Smook, Handbook for Pulp & Paper Technologists, *Tappi Press*, 2016, 447 p
- [261] Carlton W. Dencen Douglas W. Reeve., Pulp Bleaching: Principles and practice, *Tappi Press*, Atlanta, Georgia, 1996, 868 p
- [262] Antonio Blanco, Gustavo Blanco, *Medical Biochemistry*, Academic Press, 2017, 826 p
- [263] Pietro Tundo, Liang-Nian He, Ekaterina Lokteva, et al., *Chemistry Beyond Chlorine*, Springer International Publishing, Switzerland, 2016, 608 p
- [264] P. Bajpai, Realities and trends in enzymatic prebleaching of kraft pulp, In: *Biotreatment, Downstream Processing and Modelling*, Scheper Thomas (Ed.), Springer-Verlag Berlin Heidelberg, 1997
- [265] Elwood W. Cooper, Process for treating hardwood pulp with an enzyme mixture to reduce vessel element picking, *Patent US 5725732 A*, 1998
- [266] Tetsuo Koshijima, Takashi Watanabe, *Association Between Lignin and Carbohydrates in Wood and Other Plant Tissues*, Springer-Verlag Berlin Heidelberg, 2013, 329 p
- [267] Gary S. Sayler, John Sanseverino, Kimberly L. Davis, *Biotechnology in the Sustainable Environment*, Springer Science & Business Media, 2012, 389 p.
- [268] Dilip K. Arora, *Handbook of Fungal Biotechnology*, CRC Press, 2003, 600 p
- [269] Jeffrey Tolan, Brian Foody, Method for the use of enzymes in bleaching paper pulp, *Patent US 5591304 A*, 1997
- [270] Elwood W. Cooper, Process for treating paper machine stock containing bleached hardwood pulp with an enzyme mixture to reduce vessel element picking, *Patent US 5770012 A*, 1998

- [271] Dimitris Charalampopoulos, Robert A. Rastall, *Prebiotics and Probiotics Science and Technology*, Springer Science & Business Media, 2009, 1262 p
- [272] Willem Broekaert, Christophe Courtin, Jan Delcour, Method for making soluble arabinoxylans as co-product of fermentation of whole-grain cereals, *Patent WO 2008000050 A2*, 2008
- [273] Pratima Bajpai, *Xylanolytic Enzymes*, Academic Press, 2014, 120 p
- [274] Kusakabe I., Park G.G., Kumita N., et al., Specificity of p-mannanase from *Penicillium purpurogenum* for konjac glucomannan, *Agricultural and Biological Chemistry*, 1988, 52, p. 519-524
- [275] McCleary B.V., Matheson N.K., Action patterns and substrate-binding requirements of β -D-mannanase with mannosaccharides and mannan-type polysaccharides, *Carbohydrate Research*, 1983, 119, p. 191-219
- [276] McCleary B.V., Comparison of endolytic hydrolases that depolymerize 1,4- β -D-mannan, 1,5- α -L-arabinan, and 1,4- β -D-galactan, *Enzymes in Biomass Conversion (ACS Symposium series)*, American Chemical Society, Washington, DC, 1991, Ser 460, p. 437-449
- [277] A. Rydlund, O. Dahlman, Oligosaccharides obtained by enzymatic hydrolysis of birch kraft pulp xylan: analysis by capillary zone electrophoresis and mass spectrometry, *Carbohydrate Research*, 1997, 300, p. 95-102
- [278] Francesco Cherubini, Anders H. Strømman, Chapter 1 - Principles of Biorefining, *Biofuels, Alternative Feedstocks and Conversion Processes*, 2011, p. 3-24