Saimia University of Applied Sciences
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EXTRACTION OF HEMICELLULOSES BY ACID CATALYZED HYDROLYSIS

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ABSTRACT

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The techniques of chemical hydrolysis can be employed in order to separate polysaccharides from wood in a form of monosaccharides. Various acids can be used as the catalyst such as mineral acids and organic acids at certain operational conditions, i.e. temperature, pressure and time.

The aim of this study was to investigate effects of different hydrolysis techniques on the two types of raw material, birch chips and sawdust mixture of softwood. The obtained hydrolyzate were analyzed, e.g. pH measurement, measurement of dry solids content, and monosaccharides identification by the capillary electrophoresis method.

The results revealed that sulfuric acid catalyzed hydrolysis was more effective in a comparison with other two methods. Thus it is the most prospective approach for sugars extraction among tested methods. Moreover, hydrolyzates received from SW sawdust treatment contained more products than other hydrolyzates samples, but the hydrolyzate from treated birch chips revealed a high concentration of xylose.

Xylose-rich liquors of acid hydrolysis of birch chips can be used in xylitol production after required cleaning procedures, while glucose-rich hydrolyzates from treated SW sawdust can be utilized in biofuel production.

Key words: Biorefinery, Hydrolysis, Capillary Electrophoresis, Softwood Sawdust, Birch, Mineral Acid, Organic Acid.

CONTENTS

1 INTRODUCTION

The conversion of biomass into chemicals and energy is a key to sustainable use of natural resources. The wood biomass represents the most abundant and available biomass source, containing at least four valuable components: cellulose, lignin, hemicelluloses and extractives. In the scope of Biorefinery concept, wood can be fractionally converted into compounds which would be further separated and used for chemical and/or biochemical conversion. The hydrolysis techniques can be employed in order to separate polysaccharides from wood in a form of monosaccharides. (Garotte et al. 2001).

The mechanism of chemical hydrolysis is based on the catalytic cleavage of glycosidic bonds in polysaccharides. Various acids can be used as the catalyst, but the most common is sulfuric acid. However, several organic acids such as acetic and formic acids can also be used as catalysts. Moreover, hot water autohydrolysis can be applied as pre-treatment to extract valuable hemicelluloses from the wood prior to pulping. The obtained sugars can be used in various applications. The ethanol is obtained from hexoses by fermentation. Moreover, nowadays it is possible to convert pentoses to ethanol due to new techniques. In Finland, xylose is commercibally utilized for production of sweetening agent, xylitol. (Gellerstedt et. al. 2009, p. 175).

The objective of the work was to study the effect of hydrolysis on two different raw materials: birch chips and sawdust mixture of softwoods. Several hydrolysis methods were used to extract major monosaccharides. The selected hydrolysis techniques were: hot water treatment, sulfuric acid catalyzed hydrolysis and acetic acid catalyzed hydrolysis. The obtained hydrolyzate samples were subjected for analysis. Following parameters have been measured: pH values of hydrolyzate samples and concentration of dissolved solids. Monosaccharides were defined and quantified by means of capillary electrophoresis analysis. As the result, the efficient hydrolysis process was proposed and several important conclusions about hydrolyzate samples were made.

2 WOOD CHARACTERISATION

Wood is of biological origin and has a very complex structure morphologically as well as chemically. Wood species can be divided into two groups: hardwoods and softwoods. Softwoods are gymnosperm trees, while hardwoods are angiosperm trees. Also, density of most hardwoods is higher than density of softwoods. (Stenius 2000, p. 1; Pettersen 1984, p.57).

Although the chemical composition of wood varies quantitatively among tree species, it is possible to generalize content of a tree. The overall chemical composition of wood is shown in the figure 2.1.

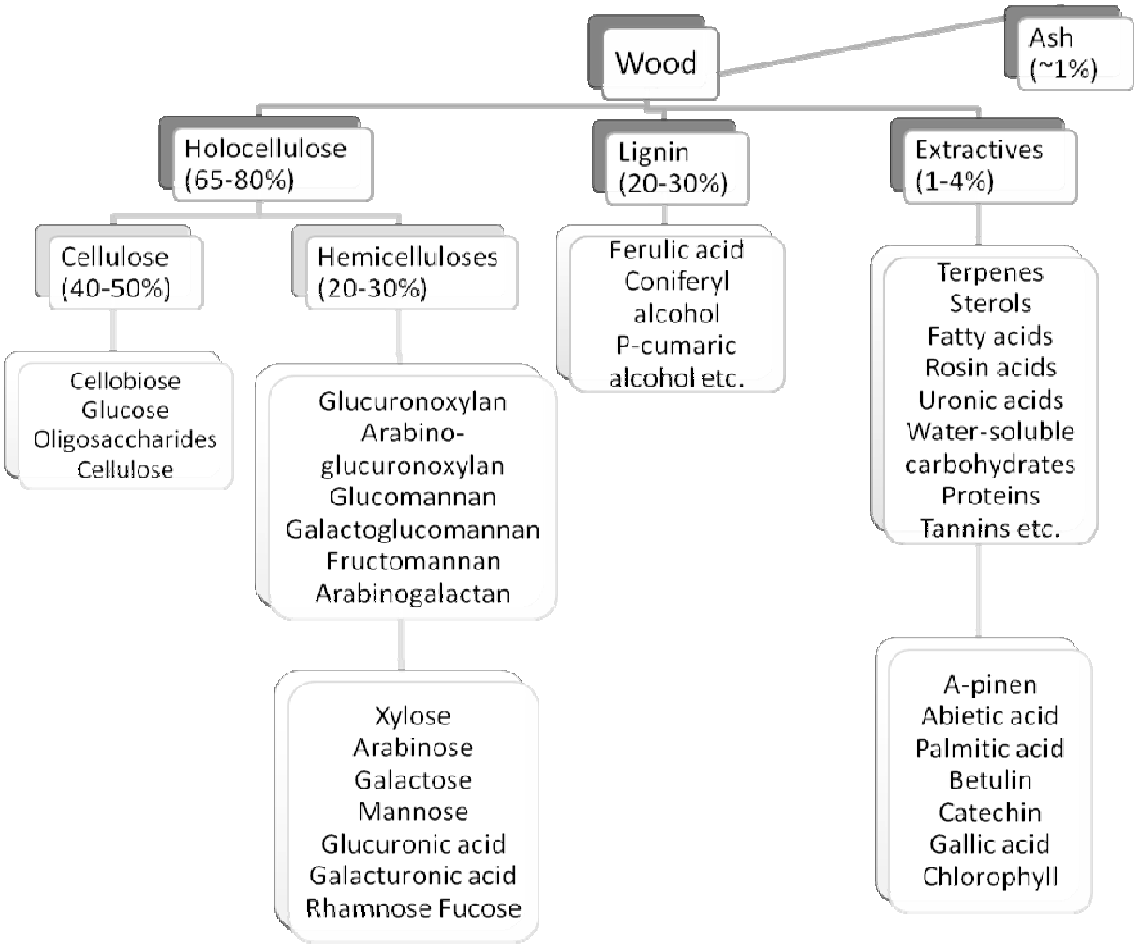

Figure 2.1 Scheme of chemical composition of the wood (Azarov et al. 1999, p. 184)

As can been seen from the figure 2.1, major part of wood consists of organic matter, which is about 99%, and only 1% of the wood is represented by mineral

compounds. The mineral compounds can be obtained after incineration of wood in the oven at temperatures 600-800 ^{o}C. Holocellulose, carbohydrate part of wood, is composed of cellulose and hemicelluloses. The cellulose content of both hardwood and softwood is approximately same 40%-45%, whereas content of other components may differ (Stenius 2000, p. 28). Softwood and hardwood differ in hemicelluloses´ composition; hemicelluloses of softwoods are mostly presented by hexosanes (hexatomic carbohydrates), while hemicelluloses of hardwoods are presented mostly by pentosans (pentatomic carbohydrates) (Azarov et al. 1999, p. 184). Generally, softwood species contain more lignin and less hemicelluloses than hardwoods. The lignin content of hardwoods is around 20%-25%, while amount of lignin in softwoods is around 25%-30% of the wood dry solids (Stenius 2000, p.28). Furthermore, hardwoods have more extractives than softwoods, in temperate tree species the extractives content is between 5% and 10% (Kellomäki 2009, p. 151).

The table 2.1 shows information about commonly used wood species in pulp and paper industry, where values are given in the percentages of dry wood weight.

Table 2.1 Chemical comparison of various wood species (Sixta 2006, p. 23)

Species	Common name	Extractives	Lignin	Cellulose	Hemicelluloses		Other polysaccharides
					Glucomannan	Glucuronoxylan	
Softwood							
Pinus radiata	Monterey pine	1.8	27.2	37.4	20.4	8.5	4.3
Picea abies	Norway spruce	1.7	27.4	41.7	16.3	8.6	3.4
Picea glauca	White spruce	2.1	27.5	39.5	17.2	10.4	3.0
Hardwood							
Betula verrucosa	Silver birch	3.2	22.0	41.0	2.3	27.5	2.6
Betula papyrifera	Paper birch	2.6	21.4	39.4	1.4	29.7	3.4
Eucalyptus globulus	Blue gum	1.3	21.9	51.3	1.4	19.9	3.9

Extraction with CH_2Cl_2, followed by C_2H_5OH.

As can be seen from the table 2.1, the most commercial wood species such as birch and spruce are rich in celluloses as well as in hemicelluloses, but have relatively small content of lignin. The extractives content of the stemwood is relatively low, and it significantly depends on the period of the harvesting and age of the tree. (Gellerstedt et al. 2009, p. 149).

Furthermore, wood contains small amounts of inorganic components, which play an important role in tree well-being. The amounts of those minerals differ according to the tree species and environment (Kellomäki, 2009). The table 2.2 shows the average concentration of important minerals in the dry mass of wood and bark.

Table 2.2 Concentration of some mineral elements in the stemwood and stem bark of five major tree species in Finland (Kellomäki 2009, p. 154)

Tree component	Concentration of primary elements,%				Concentration of trace elements, ppm					
	P	K	Ca	Mg	Mn	Fe	Zn	S	B	Cu
Softwoods										
Stemwood	0.01	0.06	0.12	0.02	147	41	13	116	3	2
Stem bark	0.08	0.29	0.85	0.08	507	60	75	343	12	4
Hardwood										
Stemwood	0.02	0.08	0.08	0.02	34	20	16	90	2	2
Stem bark	0.09	0.37	0.85	0.07	190	191	131	341	17	13

As can be seen from the Table 2, bark of both hardwood and softwood contains more inorganic compounds than in stemwood of respective tree species.

2.1 Cellulose

The most abundant natural polymer in the world is cellulose, which is the main structural material of plants cell wall. It is insoluble in water and in most organic solvents. However, during treatment with concentrated acid and high temperature, cellulose can be degraded into glucose units, because it is

composed of *β*-D-glucopyranose units (Laine 2005, p. 15). The structure of cellulose molecule is shown in the figure 2.2.

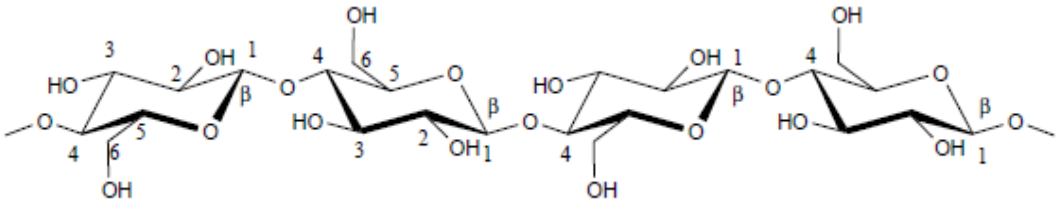

Figure 2.2 Chemical structure of cellulose (Laine 2005, p. 15)

As can be seen from the figure 2.2, cellulose is the linear homopolymer consisted of β-1,4-glycosidic linked D- glucopyranose units. Although structure of cellulose may look simple at first, the complicated supramolecular organization makes it a complex substance, which is able to form different types of hydrogen bonds within the same cellulose chain or between different chains (Pettersen 1984). The supramolecular structure of cellulose is presented by microfibrills which can form larger aggregates, fibrils. Cellulose in the microfibrils forms crystalline matrix, thus it is so difficult to dissolve cellulose. However, in microfibrils some parts of cellulose chain with amorphous structure are readily dissolved in hot water (Azarov et al. 1999).

2.2 Hemicelluloses

Hemicelluloses vary significantly among hardwoods and softwoods according to the type and content in the wood cell walls. Generally, hardwoods contain a high proportion of xylose units and more acetyl groups than softwoods. By contrast, softwoods have a high proportion of mannose units and more galactose units (Sixta 2006).

“Hemicelluloses are relatively easily hydrolyzed by acids to their monomeric components consisting of D-glucose, D-mannose, D-xylose, L-arabinose, and small amounts of L-rhamnose,” (Sjöström 1981, p. 60). The quantities of these monosaccharides are shown in the table 2.3.

Table 2.3 Nonglucosic units of the hemicelluloses in various woods (Sixta 2006, p. 29)

Species	Mannose [%]	Xylose [%]	Galac-tose [%]	Arabi-nose [%]	Uronic Acid [%]	Rham-nose [%]	Acety-lated [%]
Softwoods							
Balsam fir	10	5.2	1.0	1.1	4.8	-	1.4
European larch	11.5	5.1	6.1	2.0	2.2	-	-
Norway spruce	13.6	5.6	2.8	1.2	1.8	0.3	-
Black spruce	9.4	6.0	2.0	1.5	5.1	-	1.3
Scots pine	12.4	7.6	1.9	1.5	5.0	-	1.6
Canadian hemlock	10.6	3.3	1.8	1.0	4.7	-	1.4
Hardwoods							
Red maple	3.3	18.1	1.0	1.0	4.9	-	3.6
Silver birch	3.2	24.9	0.7	0.4	3.6	0.6	-
European beech	0.9	19.0	1.4	0.7	4.8	0.5	-
Aspen	3.5	21.2	1.1	0.9	3.7	-	3.9

As can be seen from the table 2.3, hardwoods have higher amount of xylose, which is over 18%, and the largest amount of 24.9% xylose can be found in silver birch. By contrast, softwoods have a relatively small amount of xylose, for example Scots pine contains 7.6% and Norway spruce 5.6%. Also, it can be concluded that softwoods contain more mannose than hardwoods. Norway spruce contains 13.6% while aspen has only 3.5% of mannose.

2.2.1 Softwood Hemicelluloses

Galactoglucomannans are main hemicelluloses in softwoods, they have a linear backbone chain of (1→4)-linked β-D-glucopyranose with attached β-D-mannopyranose units (Sjöström 1981, p. 60). The galactoglucomannan structure is shown in the figure *2*.3.

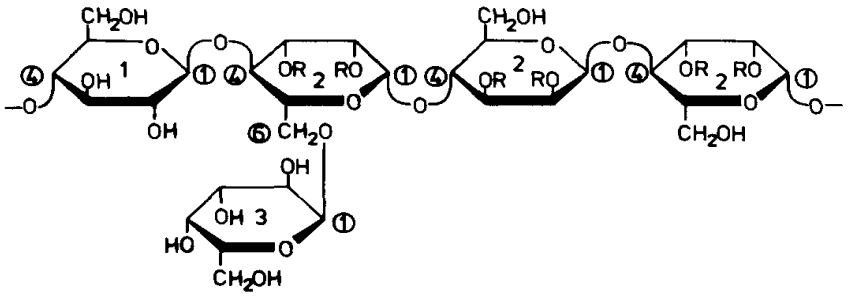

Figure 2.3 Chemical structure of galactoglucomannan (Sjöström, 1981, p. 61)

Galactoglucomannans may vary according to their galactose content. The galactoglucomannan have low galactose content with galactose:glucose:mannose ratio of about 0.1:1:4, whereas in the galactose-rich polysaccharides the corresponding ratio is 1:1:3. The fraction with low galactose content is referred to as glucomannan. An important structural feature is that the C-2 and C-3 positions in mannose and glucose units are partially substituted by acetyl groups, on the average one group per 3-4 hexose units. (Sjöström 1981, p. 60-61).

Softwoods contain 5%-10% of arabinoglucuronoxylan, which consists mainly of (1-4)-linked β-D-xylopyranose units with branches of α-(1-2) linked pyranoid 4-*O*-methyl-D-glucuronic acid, and α-(1-3)-attached L-arabinose units. The arabinose side chains can be easily hydrolyzed by acids due to their furanosidic structure which is less resistant to hydrolysis. (Sjöström 1981, p.61). The structure of arabinoglucuronoxylan is illustrated in the figure *2*.4.

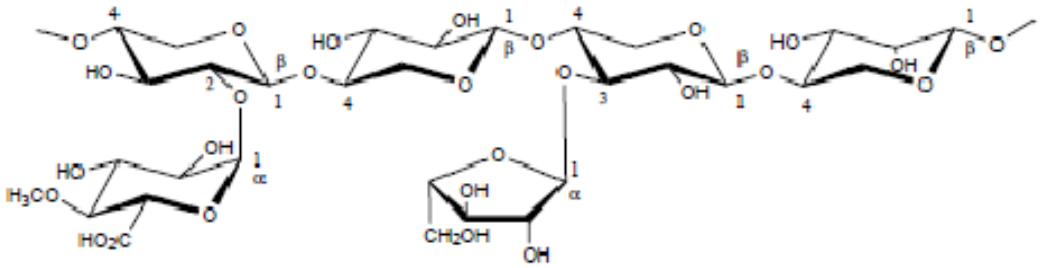

Figure 2.4 Structure of arabinoglucuronoxylan (Laine 2005, p. 16)

Arabinogalactan content is less than 1% in most softwoods species. However, some species have more arabinogalactan, for example Siberian larch contains around 10-25% of arabinogalctan by mass. (Gellerstedt et al. 2009, p. 114). Arabinogalactan consists of D-galactopyranose main units attached by β-(1→3)-bonds which form the main branch and side branches which are made of β-(1→6)-linked D-galactose units, L-arabinose units (Laine 2005). The structure of arabinogalactan can be seen in the figure 2.5.

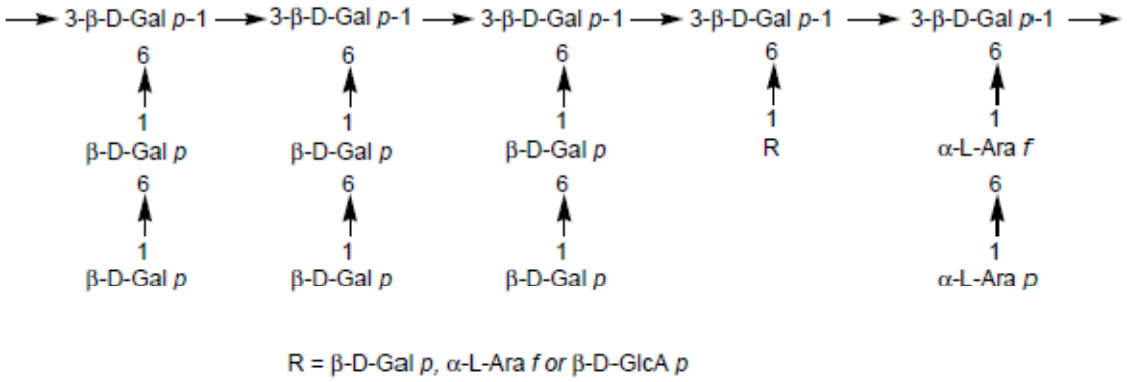

Figure 2.5 Structure of arabinogalactan of larches (Laine 2005, p. 17)

As it is shown in the figure 2.5, arabinogalactan represents a carbohydrate composed of arabinose and galactose monosaccharides. Since larch arabinogalactan is water-soluble, it can be extracted from untreated heartwood by hot water (Gellerstedt et al. 2009, p. 114).

Another minor compound of softwoods is xyloglucan. It can be found in a primary cell wall of higher plants, it is shown in the figure 2.6.

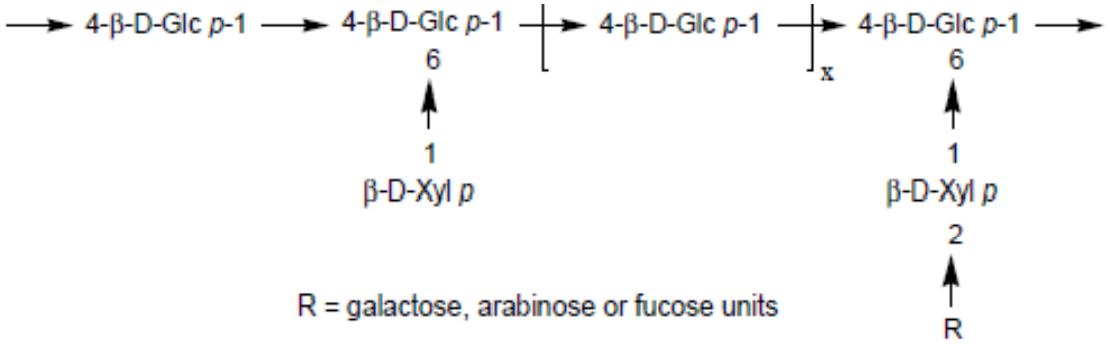

Figure 2.6 Structure of xyloglucan (Laine 2005, p. 17)

As can been seen from the figure 2.6, xyloglucan like cellulose has D-glucose monosaccharides attached by β-(1→4)-bonds, whereas side branches attached at the hydroxyl group of C6. Side branches are composed either of single xylose units or of galactose, arabinose or fructose units (1→2)-bonded to xylose.

2.2.2 Hardwood Hemicelluloses

The major polysaccharide of hemicelluloses in different hardwood species is *O*-acetyl-4-*O*-methylglucuruno-β-D-xylan, which is sometimes referred as glucuronoxylan (Sjöström 1981, p. 62, Gellerstedt et al. 2009, p. 108). Generally, the term xylan refers to the xylose-base hemicelluloses in both softwoods and hardwoods. Hardwood species contain 15%-30% of xylan (Sjöström 1981, p. 62). The structure of hardwood glucurunoxylan can be seen in the figure 2.7.

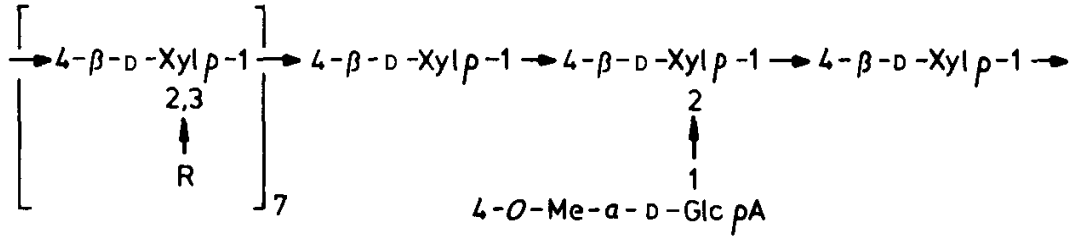

Figure 2.7 Abbreviated formula of glucuronoxylan. Sugar units: β-D-xylopyranose (xyl*p*), and 4-O-methyl-α-D-glucuronic acid (Glc*p*A). R is an acetyl group (CH_3CO) (Sjöström 1981, p. 63)

As shown in the figure 2.7, xylose consists of (1→4) linked β-D-xylopyranose units and acetyl groups are attached at C-2 or C-3 (Sjöström 1981, p. 62, Gellerstedt et al 2009, p. 109).

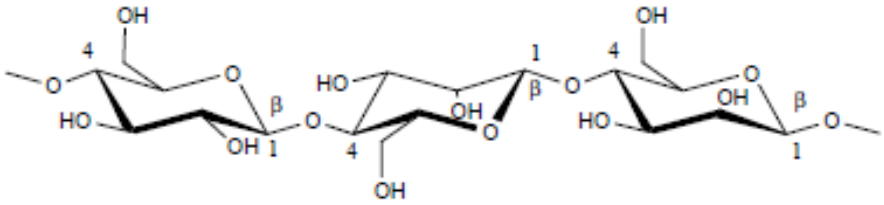

Figure 2.8 The structure of glucomannan (Laine 2005, p. 19)

The glucomannan content in hardwood is usually between 3% and 5%. Glucomannan is composed of β-(1→4)-linked D-mannopyranose backbone and D-glucupyranose units. The ratio between glucose and mannose is different among wood species, which can be between 1:2 and 1:1 respectively. (Sjöström 1981, p. 63, Gellerstedt et al. 2009, p. 113). The chemical structure of glucomannan can be seen in the figure 2.8.

2.3 Lignin

Lignin is aromatic polymer that binds together the cellulose microfibrils and hemicellulose fixating them towards each other (Gellerstedt et al. 2009, p. 121). The position of lignin within lignocellulosic matrix can be seen in the figure 2.9.

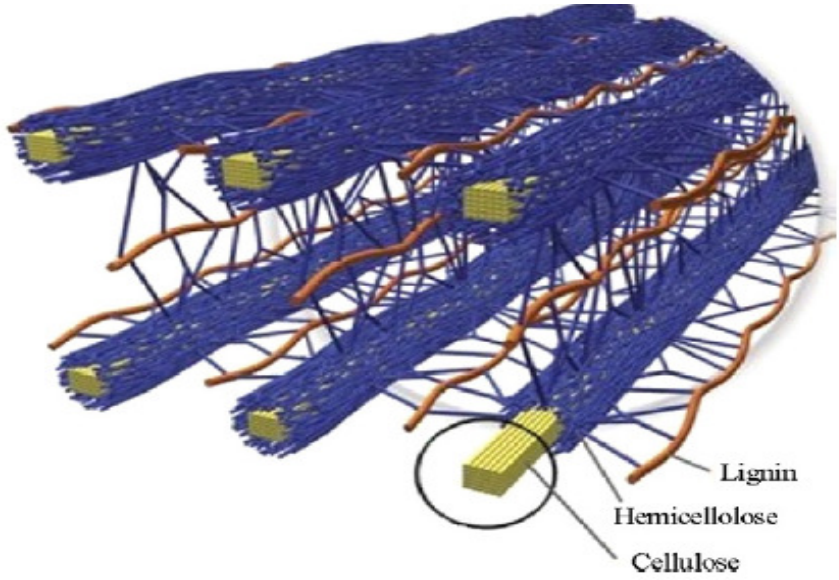

Figure 2.9 The allocation of the lignin within lignocellulosic matrix (Department of Energy Genomics, 2010)

As it is illustrated in the figure 2.9, cellulose acts as reinforcing fibers and lignin acts as “glue” between them (Gellerstedt et al. 2009, p. 121). Thus, it is difficult to extract lignin in its native form without breaking down the whole matrix and it is impossible to determine the molecular weight of lignin as well. Theoretically, it was suggested that wood lignin has degree of polymerization of several thousands, whereas in practice it might be even higher. (Gellerstedt et al. 2009).

Lignin macromolecules are composed of three different phenylpropanoid monomer units (monolignols) – predecessors of lignin. These units are bounded by various carbon-carbon and carbon-oxygen bonds (Doherty et al. 2011). Those phenylpropanoid monomer units are shown in the figure 2.10.

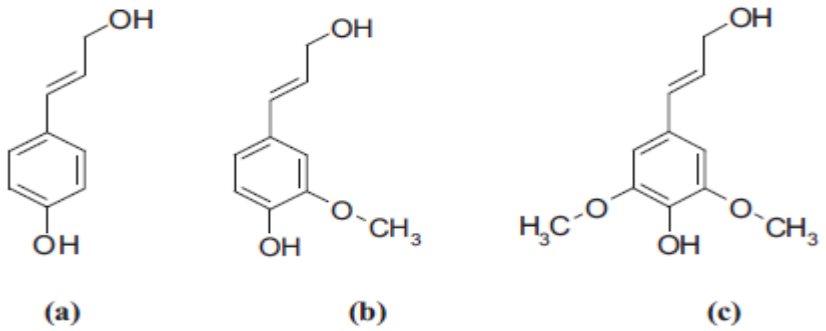

Figure 2.10 Monolignols, (a) p-Coumaryl alcohol (4-hydroxyl phenyl,H), (b) coniferyl alcohol (guaiacyl, G), (c) sinapyl alcohol (syringyl, S), (Doherty et al. 2011)

As can be seen in figure 2.10, the only difference between monolignols is the presence and allocation of methoxyl groups. The phenylpropanoid monomer units create a three-dimensional lignin net in wood. The “H” type monolignols are commonly encountered in the non-wood plants, while two other types (G and S) are found mostly in woody plants. Softwood lignin is more difficult to degrade than hardwood one as it contains more G units; while hardwood lignin is built of S units. Lignin has the most sophisticated structure among other wood compounds. However, the precise structure of native lignin is still unknown. (Azarov et al. 1999).

2.4 Extractives

The extractives are soluble compounds in neutral solvents such as water, diethyl ester, toluene, methanol, ethanol, acetone etc. (Stenius 2000, p. 43). The content of extractives and their quantity depend on the tree type and the environment affecting it (Kellomäki, 2009, p. 151). Generally, content of extractives is higher in bark, leaves and roots, than in stemwood. Chemically extractives are divided into hydrocarbons (mainly terpenes), alcohols, aldehydes and ketones, acids, resin acids, carbohydrates, phenol and nitric compounds (Azarov et al. 1999, Gellerstedt et al. 2009).

The classification according to the separation methods of extractives includes following groups: volatile oils, resins and water-soluble compounds. The schematic structure of this classification and corresponding products derived from the extractives can be seen in the figure 2.11.

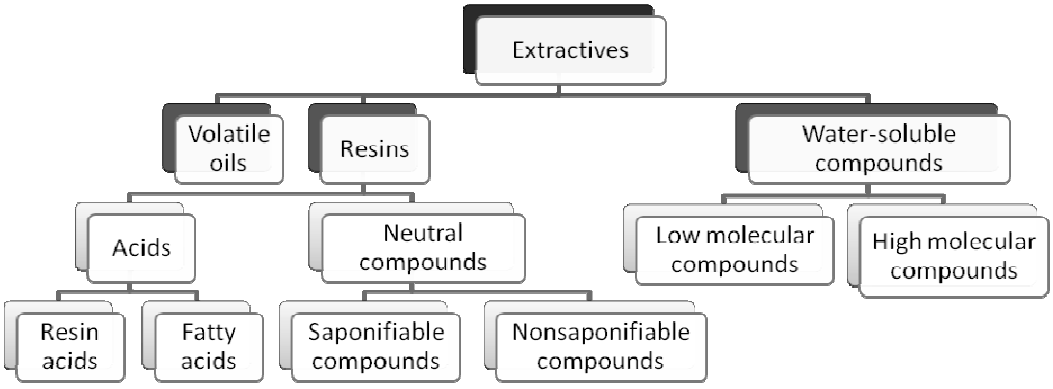

Figure 2.11 Scheme of extractives content (Azarov et al. 1999, p. 498)

Volatile oils are represented by high-volatile compounds, which can be separated by water distillation. They are mainly composed of monoterpenes and other volatile terpenes, terpenoids as well as of many different low molecular compounds. The volatile oils content of wood is relatively low and varies in range from 0.1% to 1%. (Sjöström 1981, p. 89; Azarov et al. 1999; Gellerstedt et al. 2009).

Resin acids can be extracted by organic solvents, but are not soluble in water. Resins are divided into free acids (resin and fatty) and neutral compounds. Neutral compounds are classified as saponifiable compounds (fats and waxes) and nonsaponifiable compounds (terpene alcohols, phytosterols, etc.).

Generally, the amount of resin acids in heartwood of hardwoods is 0.2% - 0.8%, whereas it is 2.4% - 4.8% in heartwood of pine. There are almost no resin acids in hardwood. (Gellerstedt et al. 2009).

Water-soluble compounds can be extracted by cold or hot water. They consist of various phenol compounds (tannins, coloring compounds), carbohydrates, glycosides, and soluble salts (Azarov et al. 1999). The wood extractives contribute to wood properties such as color, odor, and decay resistance (Pettersen, 1984, p. 68).

2.5 Wood Chips and Sawdust

The wood chips are a commonly used form of raw material for pulping. Chips of good quality should have uniform size. Various types of chipper machines are available on the market nowadays, which makes it possible to choose correctly the appropriate equipment to the wood species. Chip size is a balance between mechanical fiber damage and liquor impregnation. The recommended chip sizes to be used in pulping are following: length x width x thickness = 15 x 20 x 3 mm, the illustration of chip dimension is shown in the figure 2.12. However, the size of chips can vary according to wood species and fiber length. The target of predefined chip size is to provide uniform penetration of cooking liquor through the chip. (Sixta 2006, p. 80).

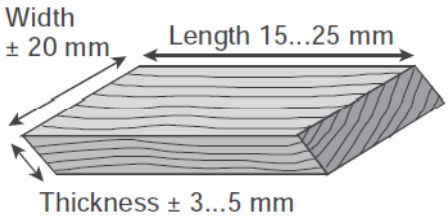

Figure 2.12 Recommended chip dimensions and size (Sixta 2006, p. 80)

The respective chip size is not preferable for hydrolysis, because the smaller chip size enables better penetration of solution, so that better dissolution of wood compounds. From this point of view, sawdust is more applicable raw material. (Testova 2006, p. 11).

Sawdust is a by-product of sawmills which is available in large amounts. Particles of sawdust are not as uniform as chips, however small size fraction is dominant. Saw dust may consist of a large proportion of heartwood. (Korpinen 2010). One way of sawdust utilization is the pulp production, which began during mid-1950s. Sawdust pulp is mixed with hardwood pulp to increase its initial strength, and also it is used as a raw material for production of laminating papers, since it provides good opacity and release properties (Knowpulp, VTT).

3 CHEMICAL HYDROLYSIS OF WOOD

The fractionation of lignocellulosic materials by various treatments is a modern approach in the scope of biorefinery. According to Garotte et al. (2001) wood biomass is a source of numerous chemical compounds that have a wide range of marketable appeal. Hydrolysis enables selective dissolution of carbohydrates, along with the formation of valuable byproducts such as furfural, HMF, acetic acid, levulinic acid etc.

3.1 The Mechanism of Cleavage of Glycosidic Bonds

The main principle of the chemical hydrolysis is the catalytic cleavage of the glycosidic bonds in polysaccharides catalyzed by dilute or concentrated mineral acids. Also organic acids can be used as a catalyst for the process. The mechanism of polysaccharides hydrolysis can be illustrated with an example of cellulose hydrolysis (Holkin 1989). The mechanism of the hydrolysis is shown in the figure 3.1.

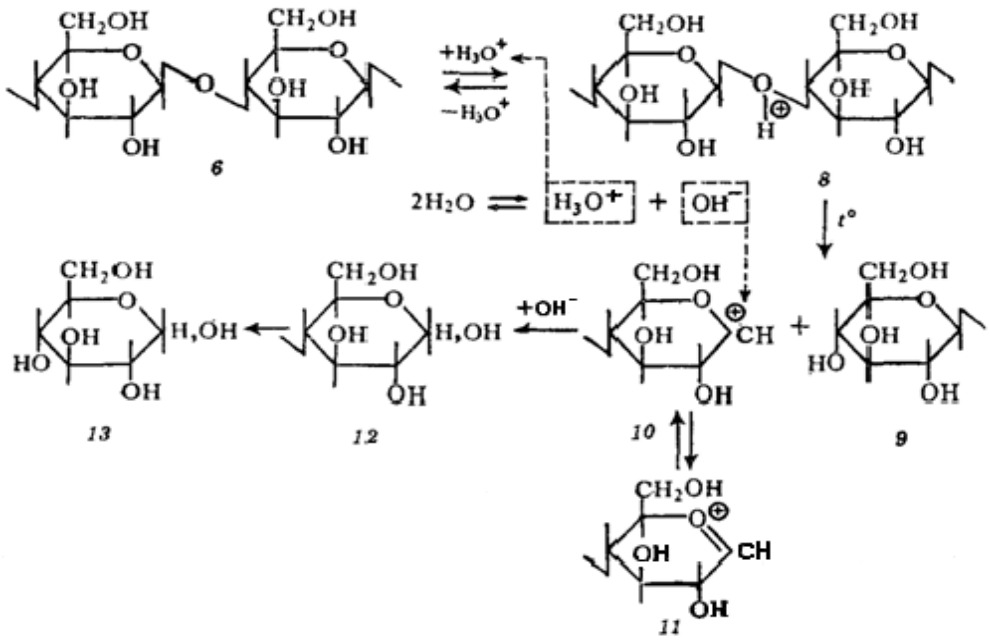

Figure 3.1 The mechanism of hydrolytic cleavage of glycosodic bonds (Holkin 1989, p. 53).

The protonation of the glycosidic oxygen atom, which contains a free pair of electrons, occurs during first stage of hydrolysis. As the result, oxonium macroion (8) is formed, which yields in activation of glycosidic bonds. In the protons conjugation reaction glycosidic bond poses nucliophilic character. The process is reversible, thus all of the active and inactive forms are in the thermodynamic equilibrium.

As the result of oxonium macroion dissociation, the macromoleculs of cellulose (9) and carbonium macroion (10) are formed, which are in equilibrium with oxonium ion (11). Due to the distribution of the positive charge between carbon and oxygen atoms, the system formed by these ions is called carboxonic ion. As the result of interaction of carbocation (10) with water the fragment of macromolecule is formed (12). Then, formed fragments of macromolecules are hydrolyzed further. The product of the full hydrolysis of cellulose is D-Glucose. The acid is playing a role of catalyst in this reaction, it is acting only with intermediate products and released unchanged after reaction.

The reaction of the cleavage of the oxonium ion (8) is determinative for the hydrolysis speed, due to lower speed of reaction in comparison with the process of the conjugation of water to the carbcation (10). (Holkin 1989).

3.2 Dissociation Constant

The catalyzing action of acids is not equal. Mineral acids are much more effective as catalysts of hydrolysis process, than for instance organic acids. The essence of this difference can be explained by the acid dissociation constant, K_a. This constant is a quantitative measure of acid strength in solution, which is calculated as follows:

$$K_a = \frac{[A^-][H^+]}{[HA]} \quad (1)$$

In this equilibria, A^- is the conjugate base of the acid HA, and H^+ is the proton (in this context means a hydrogen ion) according to Atkins and Paula (2005, pp. 179-181).

Due to large difference between constant values for different acids, it is common to use a logarithmic value - pK_a, as follows:

$$pK_a = -\log_{10} K_a \quad (2)$$

Equation of pK_a parameter is obtained from Atkins and de Paula, p. 180. The higher value of pK_a, the weaker acid is. Thus weak acids have pK_a in range of -2 to 12 in water solution. On the other hand, acids with pK_a lower than -2 are considered to be strong. For Instance, hydrochloric acid has pK_a of -7, while pK_a of acetic acid is just around 5. The acid dissociation constant shows concentration of H^+ ions in the solution, which is especially important for hydrolysis process since it is catalyzed by these ions. The higher concentration of H^+, the faster hydrolysis proceeds. Therefore strong mineral acids are used for hydrolysis more often than organic acids. (Atkins & Paula 2005, p. 181).

3.3 Hot Water Autohydrolysis

Autohydrolysis is a type of hydrolysis without presence of any catalyst. It is targeted at degradation of hemicelluloses as both cellulose and lignin remain in a solid phase. The depolymerization of hemicelluloses is catalyzed by hydronium ions from water and naturally generated compounds such as acetic,

uronic and phenolic acids. (Liu 2010). Also, cellulose decomposition and lignin repolymerization is avoided because of mild operational conditions and reaction selectivity. However, under severe conditions, condensation reactions between furfural and lignin may occur (Garotte et al. 2001). The hot water extraction of readily hydrolysable carbohydrates is beneficial for utilization and recovery of the hydrolyzate components. Moreover, environmental friendly recovery is achieved, because no caustic or sodium is added to treat the process streams. Therefore, there are no byproducts generated from sulfuric acid neutralization. (Liu 2010).

3.4 Concentrated-acid Hydrolysis

Concentrated acid hydrolysis is a quite old process, which enables production of higher amount of sugars (90% of theoretical glucose yield) in a comparison with dilute acid hydrolysis, where degradation of cellulose occurs in harsh environment. Moreover, concentrated acid hydrolysis can be operated at low temperatures. (Khan 2010, p. 8). Usually, the solution of 72% sulfuric acid is applied for 120 minutes at 20 ^{o}C, and then it is diluted to 1 M acid concentration and heated for 240 minutes at 100 ^{o}C (Hon 2001, p. 184). However, there are several disadvantages of the process, namely, the need of special non-corrosive construction materials and high energy consumption of acid recovery process (Jones 1984).

3.5 Dilute Acid Hydrolysis

The principle of this technique is to apply temperature and pressure in order to soften lignocellulosic providing better penetration of the acid, and then degrade carbohydrate part of wood into monosaccharides (Lavarack et al. 2002). During treatment various products are formed: monosaccharides (xylose, arabinose, mannose etc.), some sugar-dehydration products (furfural, hydroxylmethyl-furfural), while lignin and part of cellulose remain as solid residue (Garotte et al. 2001, p. 155). Research works on the dilute acid hydrolysis of different lignocellulosic materials have defined optimal process conditions: temperature

80-200^{o}C, sulfuric acid concentration 0.25–8 wt%, and reaction time 10-2000 min (Lavarack et al. 2002, Hon et al. 2001, p. 184, Garotte et al. 2001). Sulfuric acid is a commonly used acid due to low cost, non-volatileness and affordable corrosion strength (Mosier et al. 2005).

Despite all of the benefits of sulfuric acid hydrolysis, some limitations take place including high corrosion rates and expensive construction materials. Also, liquors have to be neutralized prior to fermentation of sugars, thus gypsum is formed. (Mosier et al. 2005). The large amounts of gypsum negatively influence the downstream process, and also results in a low-value byproduct stream (Yang et al., 2008). Thus, the treatment entails considerable expenses, which limits wide commercial implementation in comparison with other possible methods of hydrolysis (Hu et al. 2008).

3.6 Organic Acid Hydrolysis

The acetic acid shows good properties as organic solvent. It is acting as an agent for fractionation, enables degradation of hemicelluloses simultaneously with extensive delignification at high acid concentration. Furthermore, furfural can be produced in relatively large quantities according to the severity of the treatment conditions (Abad et al., 1996). The conditions suggested by Abad et al. (1996) were targeted on the furfural generation during the fractionation of eucalyptus wood with acetic acid-water-HCl solutions. For the following objective, wood samples were treated at 120-130^{o}C with 95% acetic acid solutions containing 0.2-0.4% HCl as catalyst. The HCl-catalyzed acetic acid hydrolysis is a similar process to sulfuric acid catalyzed treatment, which entails the same problems as occurrence of reactions leading to the consumption of sugars/furfural. By contrast, hydrolysis in acetic acid media has benefits: the short reaction times, the mild operational temperatures, lower catalyst concentration. (Abad et al. 1996).

The other alternative to sulphuric acid pretreatment can be suggested maleic and fumaric acids (Maarten et al., 2009, p. 126). Neither maleic nor fumaric acid

does not provide such reactions resulting in formation of large amounts of hemicelluloses degradation products (Maarten et. al., 2009, p. 126). Moreover, the quality of the byproduct streams improves significantly by applying organic acids, because it may be used for fertilizing soil, or applied in animal feed (Maarten et. al., 2009, p. 126).

The treatment conditions for the organosolvent hydrolysis of wheat straw were suggested by Maarten et al. as follows: 50 mM water solution of maleic/fumaric acids was applied to 7.34 g of dry matter, which resulted in 10% (w/w) of dry straw solids loading. The impregnation time for mixture was 24 hours at room temperature, after that mixture was treated with temperature of 130, 150, and 170 ^{o}C for 30 min. (Maarten et. al., 2009, p. 127).

4 EXPERIMENTAL PART

4.1 Equipment

The extraction of hemicelluloses was carried out in a laboratory at Saimia University of Applied Sciences, Imatra. The set of equipment consisted of vessel autoclave and ethylene glycol bath. These pieces of equipment are shown in the figures 4.1 and 4.2.

Figure 4.1 Laboratory autoclave used for experiments

The autoclave in the figure 4.1 is a cylindrical rotating vessel made of stainless steel, while rotation provides mixing of reaction medium. The total volume of vessel is around 10 litters. The rotation and heating degree of the vessel can be adjusted: rotation is in on/off mode, temperature is controlled by relative scale from one to ten. The autoclave has a range of working temperatures between 20^{o}C and 180^{o}C, and working pressure is approximately from 2 atm to 15 atm. A lid is equipped with temperature and pressure meters, and gas outlet is fastened to an autoclave with nuts which makes it leakproof.

The second part of experiments was performed in ethylene glycol bath, which can be seen in the figure 4.2. The eight reservoirs with volume of approximately 200 ml were placed inside the glycol bath, then the cover was closed and they were sunk into the ethylene glycol. During treatment the reservoirs were constantly rotated. The treatment temperature range is set on the control panel, while rotation can only be adjusted as on/off.

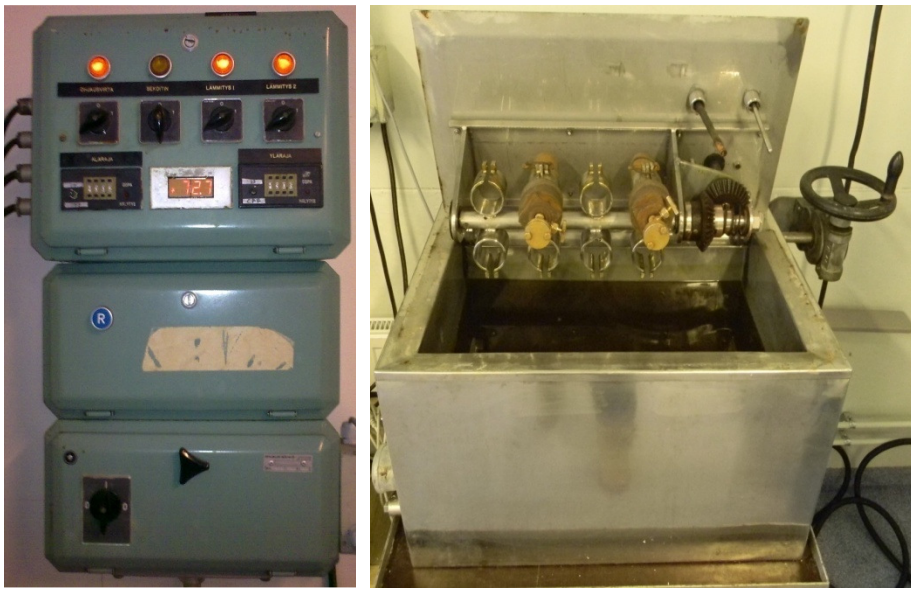

Figure 4.2 On the left hand side, control panel is illustrated and glycol bath is on the right hand side.

Both of equipment enables to follow required treatment conditions and obtain products of desired quality. However, the amounts of production are different, the autoclave enables to produce large quantities of hydrolyzate (around 10 liters) and treated lignocellulosic material, whereas glycol baths provides small sample quantities. The benefit of glycol bath is that up to eight different samples can be produced at one run, because eight samples of different materials and solvents can be placed in reservoirs. The cooling down time is different for equipment, because it takes more time to naturally decrease the temperature of glycol bath.

4.2 Materials and Methods

The raw materials for the experiments were birch chips, and sawdust mixture of mainly pine and other softwoods obtained from Imatra sawmill. The chips were screened prior to hydrolysis. The chips screen and obtained fraction of chips are shown in the figure 4.3.

Figure 4.3 Gyratory screen and selected fraction of chips

The smallest fractions of chips were taken for experiments, the sizes of which were in the range from 5 x 10 x 3 mm to 15 x 20 x 3 mm (length x width x thickness). Afterwards, the dryness of each wood type was measured according to ASTM D4442-07, which is 49% and 50.8% for sawdust mixture and birch chips respectively. The chips and sawdust were stored at room temperature.

The chips with determined dry matter content were weighed and loaded to the autoclave. Then the amount of solvent was calculated considering liquid-to-wood ratio 5:1. In case of acidic solution, the amount of concentrated 97% sulfuric acid was calculated according to its final concentration in water solution of 1.5%. The weighed sample of sulfuric acid was added to water. After that, pH of the water solution prior to treatment was measured by Crison pH meter. Then solution of acid was added to the wood in digester. The lid was tightly closed and heating up the vessel was started. It took around 40 minutes to heat autoclave up to 100^{o}C. When the temperature was around 120^{o}C, the count of treatment time was started. The conditions of treatments for each experiment are shown in the table 4.1.

Table 4.1 The methods of hemicelluloses extraction for 2 hours at 150 ^{o}C

Wood species	Water	Sulfuric acid solution	Acetic acid solution
Birch chips	—	1.5%	1.5%
SW sawdust	—	1.5%	1.5%

Sawdust and chips were treated two hours, then pressure was equalized and temperature decreased to 90^{o}C. Unhydrolyzed residual was separated from the liquor and stored in cool storage room at 8^{o}C. Remained liquor was subjected to further chemical analyses.

4.3 Capillary Electrophoresis

The analysis of hydrolyzate samples were carried out in the laboratory of analytical chemistry at Lappeenranta University of Technology. Separations of the carbohydrate standards and acid hydrolysis samples were performed with a P/ACE MDQ (manufactured by Beckman-Coulter Inc.) capillary electrophoresis instrument equipped with a photodiode array UV detector at wavelength 270 nm. Fused silica capillaries of 50 micrometers I.D. and length 51.5/60 cm (effective length/ total length) were used in the experiments. The samples were injected at a pressure of 0.5 psi (34.47 mbar) and the injection time was optimized to 8.0 seconds. Another apparatus, 3D CE (Agilent), was used for the experiments, the capillary I.D. of which is 50 micrometers and injection pressure and temperature are 50.0 mbar for 8.0 seconds. Both apparatus have a separation voltage of 17 kV. Also an electrolyte solution consisted of 130 mM NaOH and 36 mM $Na_2HPO_4 \cdot 2\ H_2O$, pH of the electrolyte solution had a value of 12.6. Before each measurement the capillaries were conditioned by rinsing with 0.1 M sodium hydroxide, Milli-Q water, and electrolyte solution.

5 RESULTS

Several analytic techniques were used in order to characterize the liquors such as pH measurement, measurement of dry solids content, identification of monosaccharides by capillary electrophoresis.

5.1 pH Measurement

The pH measurement was done for all hydrolyzates received during experimental work. The results are illustrated in the figures 5.1 and 5.2, pH of liquors was measured before and after hydrolysis by revolving autoclave and glycol bath respectively.

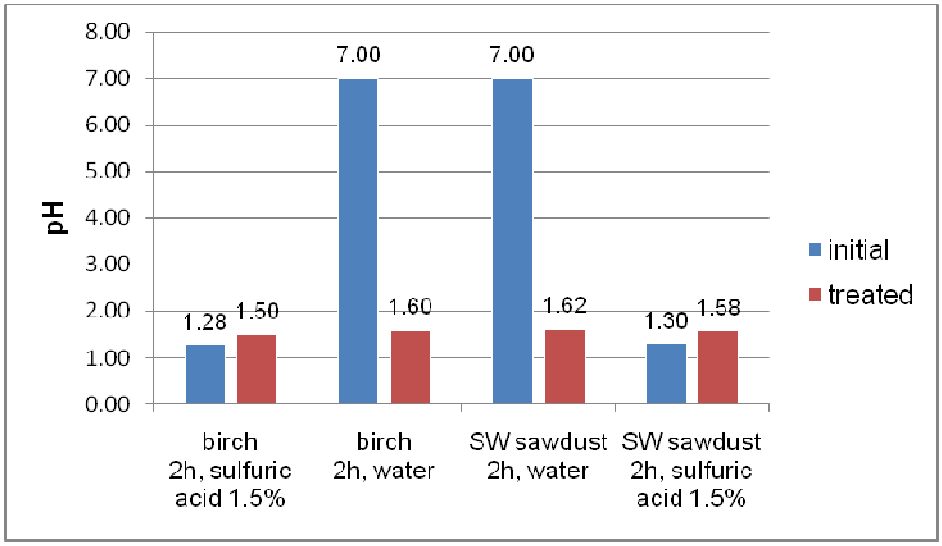

Figure 5.1 The pH values of solvents prior to extraction and pH values of hydrolyzates from autoclave.

As can be seen from the figure 5.1, pH of autohydrolysis liquors significantly drops after extraction, from 7 to 1.6. Decrease in pH is affected by released acetic acid contained in wood. However, pH slightly increases after extraction in case of sulfuric acid-catalyzed hydrolysis. This increase occurs due to the fact that released acetic acid is much weaker than sulfuric acid. Also part of the sulfuric acid can be consumed in the neutralization reactions with various wood compounds. The acidity of initial solution does not impact directly the final acidity of hydrolyzates, but acidity developed during hydrolysis within the lignocellulosic biomass does. (Testova 2006, p. 16).

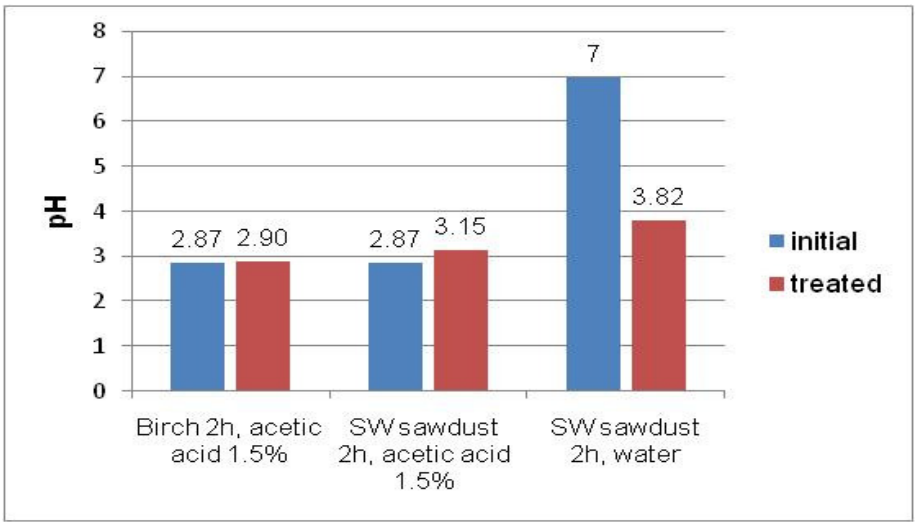

Figure 5.2 The pH values of solvents prior to extraction and pH values of hydrolyzates. The liquors were received by treatment in the glycol bath.

As can be seen from the figure 5.2, there is the same trend of pH changes as in previous graph. The pH of hydrolyzate drops from 7 to 3.82, due to liberation of acetic acid from wood. However, the pH of hydrolyzates, to which acetic acid was added initially, did not change considerably. The pH of hydrolyzate from birch chips treatment increased from 2.87 to 2.90, whereas the pH of SW sawdust hydrolyzate changed from 2.87 to 3.15. The higher value of pH is observed in the second case, which might be due to dissolution of bigger amount of neutral compounds of the wood in case of SW sawdust.

5.2 Dry Solids Content

The samples of hydrolyzates from each treatment were placed in oven at 40 ^{o}C in order to evaporate liquid and measure dry solids content. The results can be found in the figure 5.3.

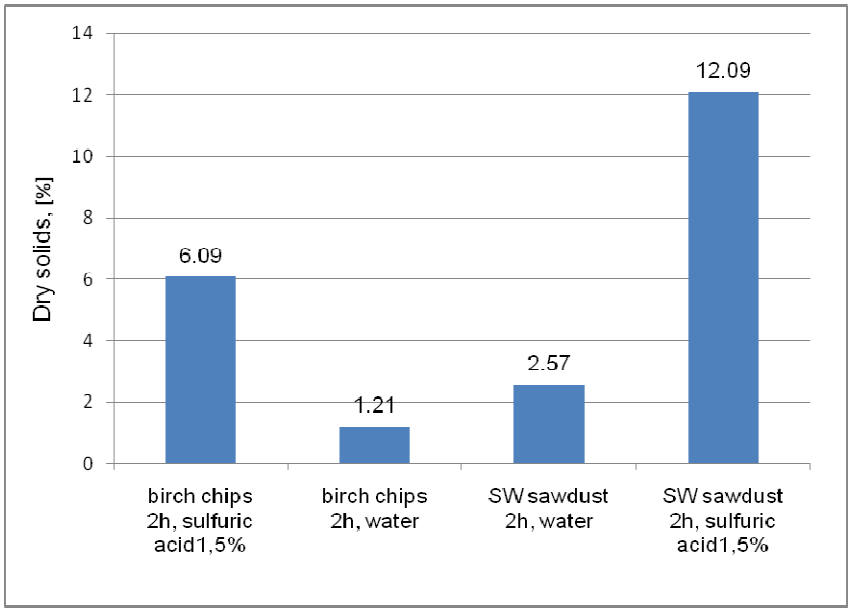

Figure 5.3 The dry solids content of the samples

As can be seen from the figure 5.3, dry solids content in hydrolyzates obtained by treatment with acid catalyst is higher than that in case of hot water extraction. For example, dry solids content of birch liquors are 6.09% and 1.21% for acid-catalyzed and hot water extraction respectively. Furthermore, it was observed that the concentration of solids is higher in SW sawdust hydrolyzates than the amount of solids dissolved in liquors from birch chips. The

values are 1.21% and 2.57% for hydrolyzates of birch chips and SW sawdust respectively. Furthermore, the same tendency can be seen in samples received after acid catalyzed hydrolysis. The amount of solids dissolved in hydrolyzate received from birch chips treatment is 6.09%, whereas it is 12.09% in SW sawdust sample.

The observations support theoretical supposition that fine particles provide higher yield of dissolution products (Pettersen 1984). Moreover, acid catalyzed hydrolysis is more effective in a comparison with Autohydrolysis, so that more solids were transferred into solution (Azarov et al. 1999, Gellerstedt et. al. 2009).

5.3 Comparison of Extracts after Treatment

Visual comparison of hydrolyzate samples have shown that all liquids have high content of dissolved hemicelluloses due to their rich color (Testova 2006, p. 33). The extraction liquors are shown in the figure 5.4. Also it was observed that after some time a solid-phase precipitation was formed on the bottom of each bottle. The solid phase might contain small pieces of fibers and brownish powder-like particles that may resemble lignin. During water hydrolysis, less lignin was dissolved in a comparison with acid catalyzed hydrolysis, thus hot water hydrolyzates have less precipitate (Springer et al. 1982). Also dissolved solids and other components of lignocellulosic material might contribute to the density of the liquors (Appendix 1: Figure 1).

Figure 5.4 The extraction liquors.

Moreover, the solutions received from acid catalyzed hydrolysis have dark color, due to the fact that catalyst might cause formation of furfural and hydroxyl-methylfurfural (Qian et al. 2005; Maarten et. al. 2009). The scheme of reaction steps occurring to polysaccharides can be seen in the figure 5.5. Also, degradation products of furfural consist of humic substances, which are dark colored high molecular compounds (Nova Scotia Environment 2008). Humic substances are formed as the result of condensation reaction under high temperature and mineral acid acting as catalyst.

The figure bellow represents the stages of monosaccharides formation and their degradation into other products. The furfural and HMF are products of sugars decomposition, while in the presence of temperature and acid catalyst furfural and HMF continue splitting into levulinic and formic acids.

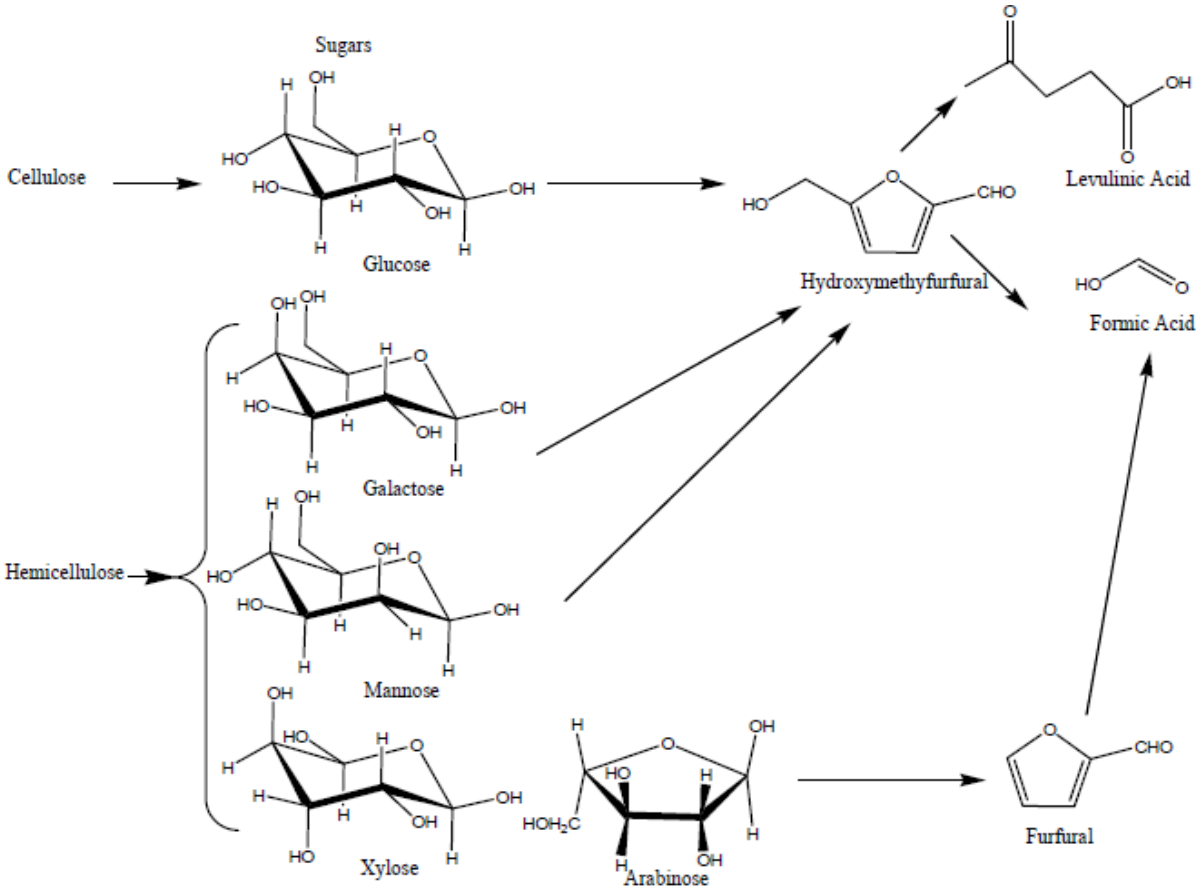

Figure 5.5 Reactions occurring to carbohydrates during hydrolysis of lignocellulosic materials. (Gellerstedt et al. 2001)

5.4 Comparison of Electropherograms

The identification of monosaccharides pictured in electropherograms was processed according to a certain order. The migration order of monosaccharides was as follows: xylitol, cellobiose, glucose, fructose, rhamnose and mannose. The respective order is applicable for all electropherograms, because standard solution and experimental conditions were identical for all samples. Despite the fact that migration times of sugars in standard solution and in sample may differ, the order remains as described. The quantity of each monosaccharide can be calculated via its peak area and corresponding calibration line. The examination of electropherograms, which were obtained from different hydrolyzates, revealed approximate composition of monosaccharides in each sample and unknown compounds were pointed out.

5.4.1 Analysis of Birch Hydrolyzates

In the obtained descriptions of reference sugars, there was a migration time of xylitol. However, the xylitol is not a product of reactions in the hydrolyzate and it might be mistaken with xylose. Due to the fact that hydroxyl group in xylitol is substituted for aldehyde group in xylose, both compounds might have the same migration time. Furthermore, xylose is a main monosaccharide by its quantity in hardwood, whereas it is the second in softwood as can be seen from the figure 2.3. Also the cellobiose, which is a disaccharide and the product of incomplete hydrolysis of glucose, can be found in big quantities in the hydrolyzates.

The electropherograms of birch hydrolyzate are shown in the Appendix 2: Figure 1. On the second electropherogram can be seen that xylose is at 10.037 minute, cellobiose forms a peak at 10.632 minute, glucose is at 13.1 minute, and poorly separated mannose is at 15 minute.

The electropherograms of liquor sample from hydrolysis catalyzed by sulfuric acid are illustrated in the Appendix 2: Figure 3. The sugars are identified according to the first electropherogram, where xylose and cellobiose appears at 7.33 and 7.79 minutes respectively. Then glucose might be at 10.975 minute with corresponding area of 1670. Other two peaks at 12.018 and 12.39 did not separate well, thus it can be said that one of them is mannose and another is monosaccharide (arabinose, rhamnose, galactose).

The electropherograms of hydrolyzate sample of birch treated by hydrolysis with acetic acid as catalyst are presented in the Appendix 2: Figure 4. Due to the poor resolution it is difficult to identify compounds, but xylose might be at 10.196 minute, cellobiose followed by glucose at 10.59 and 10.852 respectively.

On the basis of CE analysis it can be said that principle monosaccharides of birch hydrolyzates are xylose and glucose. However, other sugars (arabinose, rhamnose, galactose) are present in insignificant quantities or cannot be defined. The same conclusion was made by Rovio et al. in his work. Moreover, the common unidentified compounds were found in all electropherograms of birch samples. The two peaks are formed clearly between 20^{th} and 22^{nd}

minutes, because sugars migrate early it is possible to suggest that these compounds are not sugars.

5.4.2 Analysis of Sawdust Hydrolyzates

The hydrolyzate sample of SW sawdust after Autohydrolysis is shown in the Appendix 2: Figure 2, where the migration order of sugars is as follows: xylitol, mannitol, cellobiose, glucose, fructose, rhamnose and mannose. The xylose is at 7.32 minute, cellobiose at 8.067 minute, galactose is at 9.80 minute and mannose is at 10.614 minute. As can be seen lots of monosaccharides occurred in the hydrolyzate, despite the size of the peaks area the quantity of the sugars cannot be defined directly from the figure.

The hydrolyzate sample of SW sawdust after treatment by 1.5% acetic acid is illustrated in the Appendix 2: Figure 5. By following the same procedure as described above, it can be said that hydrolyzate is composed of lots of monosaccharides. However, it can be seen that xylose might migrate at 7.47 minute, glucose is at 10.069 minute and mannose can be at 11.604 minute.

In a comparison with birch hydrolyzates, there are various monosaccharides clearly visible and they might have approximately even quantities. The approximate monosaccharide composition of liquors can be seen in the table 5.1.

5.5 Concentration of Hydrolyzates

The capillary electrophoresis analysis revealed large concentration of various monosaccharides in the liquid samples. However, the significant amounts of glucose, mannose, xylose and cellobiose were detected in a comparison to other sugars. The concentrations of major monosaccharides are given in table 5.1.

Table 5.1 Concentrations of monosaccharides in hydrolyzate samples

Compounds [g/l]	Birch, acid	SW sawdust, acid	Birch, water	SW sawdust, water
Glucose	18.27	50.80	1.60	1.35
Cellobiose	9.13	23.85	0.40	0.34
Mannose	5.48	15.39	0.33	4.63
Xylose	21.15	12.97	6.87	3.72
Total Sugars	54.20	103.01	9.20	10.04
Other	6.70	17.89	2.90	2.56

As can be seen from the table 5.1, there is a great difference between acid catalyzed hydrolysis and hot water hydrolysis. The distinction is also observed between hydrolyzate samples of hardwood and softwood, which were compared to results obtained by Rovio et al. The concentration of total sugars is very important for further conversion, generally, the higher concentration of sugars, the more efficient conversion is. It was found out that the highest concentration of sugars was obtained after acid-catalyzed hydrolysis of SW sawdust (around 103 g/l), which may occur due to better diffusion of reagents and larger reaction area of fine sawdust particles. However, acid-catalyzed hydrolysis of birch chips yielded only a half amount of sugars in a comparison with results of SW sawdust, around 54.20 g/l. Hot water hydrolysis provided very low concentration of sugars, from 9 g/l to 10 g/l, and hence that cannot be regarded as prospective in given conditions.

Concentration of certain sugars shows a clear difference between hardwood and softwood such as non-cellulosic sugars, xylose and mannose. One reason is difference in raw materials, which are birch chips and SW sawdust. Birch hydrolyzate is rich in xylose, because a principle hemicellulose unit is *O*-acetyl-4-*O*-methylglucuruno-β-D-xylan which counts almost to half of whole amount of released sugars (Azarov, 1999). On the other hand, birch hydrolyzate contains very small amount of mannose (5,48 g/l), while SW sawdust hydrolyzate contains three times more mannose (around 15,5 g/l), because glucomannans contribute to 15-20% of SW composition (Azarov, 1999). Relatively low amount of glucose is in the birch hydrolyzate (18,27 g/l), which can be explained by fact that hydrolysis in this case occurred only in easy-accessible part of cellulose while crystalline part remained untouched. Amount of glucose in case of SW

sawdust is much higher and because of that, it is possible to say that hydrolysis occurred in crystalline part of cellulose as well.

5.6 Discussion

The hydrolysis of softwood chips should be performed and compared with results of hardwood chips. Moreover, sawdust of hardwood and softwood should be treated at the same conditions and compared. The results may provide complete information of what monosaccharides can be obtained at the same conditions from different wood species. Therefore the hydrolysis efficiency can be identified.

The various hydrolysis catalysts of different concentrations can be studied in order to find the most suitable one. For example, more experiments with acetic acid are worth to perform and to try higher concentrations with small addition of strong acid acting as catalyst. The example of the work can be seen in the article of Abad et al.

The capillary electrophoresis was found to be an effective method of compounds separation for identification. However, different experimental conditions should be studied in order to get a clear characterization of each sample. Moreover, not only sugar analysis has to be performed, but also it is interesting to know the uronic acids composition. Also it can be important to know whether any furfural or HMF is in the sample, because these chemicals act as inhibitors during biofuel production.

6 SUMMARY

The aim of this study was to investigate the effect of different hydrolysis techniques on the two types of raw material, birch chips and sawdust mixture of softwood. The obtained hydrolyzates were analyzed, e.g. pH measurement, measurement of dry solids content, and monosaccharides identification by the

capillary electrophoresis method. The results revealed that sulfuric acid catalyzed hydrolysis was more effective in a comparison with hot water treatment and hydrolysis with acetic acid catalyst. The hydrolysis with sulfuric acid catalyst is the most prospective approach for sugars´ extraction among tested methods according to the results. Moreover, hydrolyzates received from SW sawdust treatment contained more products than others, because of larger surface area and better diffusion. Moreover, it is also possible that hydrolysis occurred in crystalline part of cellulose as well in softwood.

The obtained experimental results e.g. change of pH values of solution, different concentrations of monosaccharides and the total product yield support both the study of the wood chemistry and the study of acid-catalyzed hydrolysis of hemicelluloses from wood by other researchers. On the other hand, the study results show that in order to obtain the certain type of monosaccharides for further conversion, the hydrolysis process could be carefully selected. Xylose-rich liquors of acid hydrolysis of birch chips can be used in xylitol production after required cleaning procedures, while glucose-rich hydrolyzates of SW sawdust treatment can be utilized in biofuel production.

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APPENDIX 1

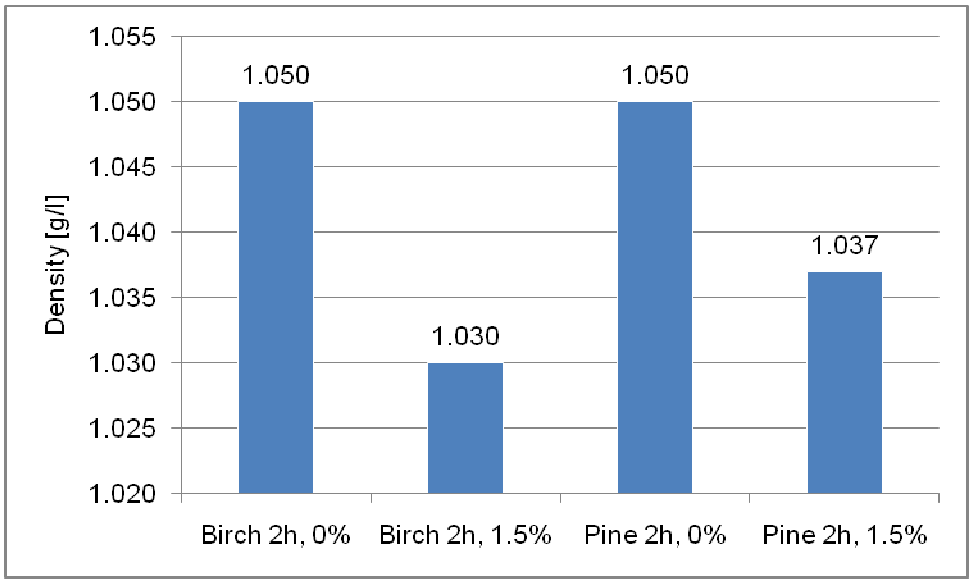

Figure 1 Density of liquors received during hot water and sulfuric acid catalyzed hydrolysis in the revolving digester.

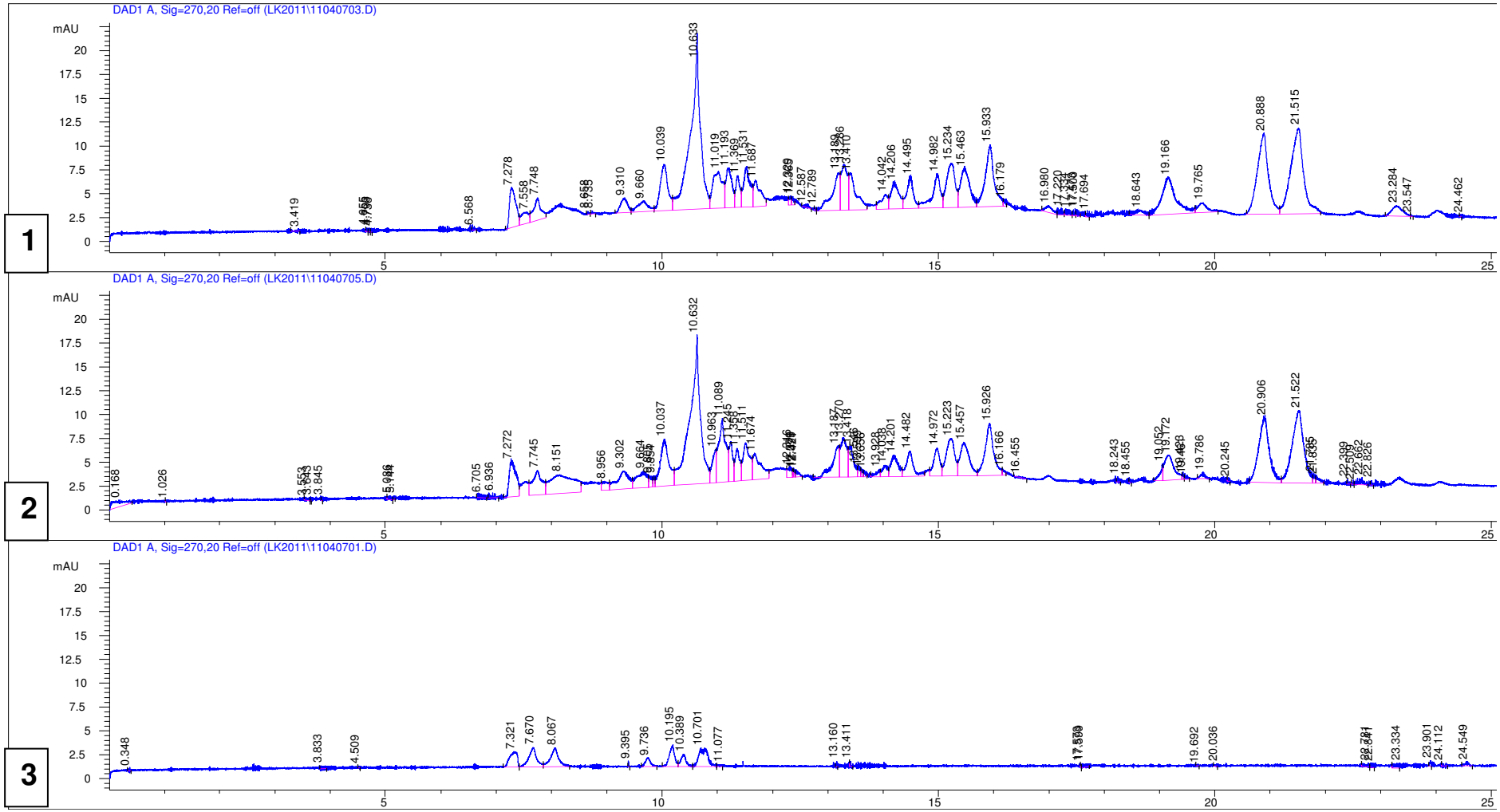

Figure 1Electropherogram of hydrolyzate sample from birch chips autohydrolysis during 2 hours, where 1 –hydrolyzate sample, 2 – sample and standard solution of fructose (25 ppm), 3 – standard solution of fructose (25 ppm)

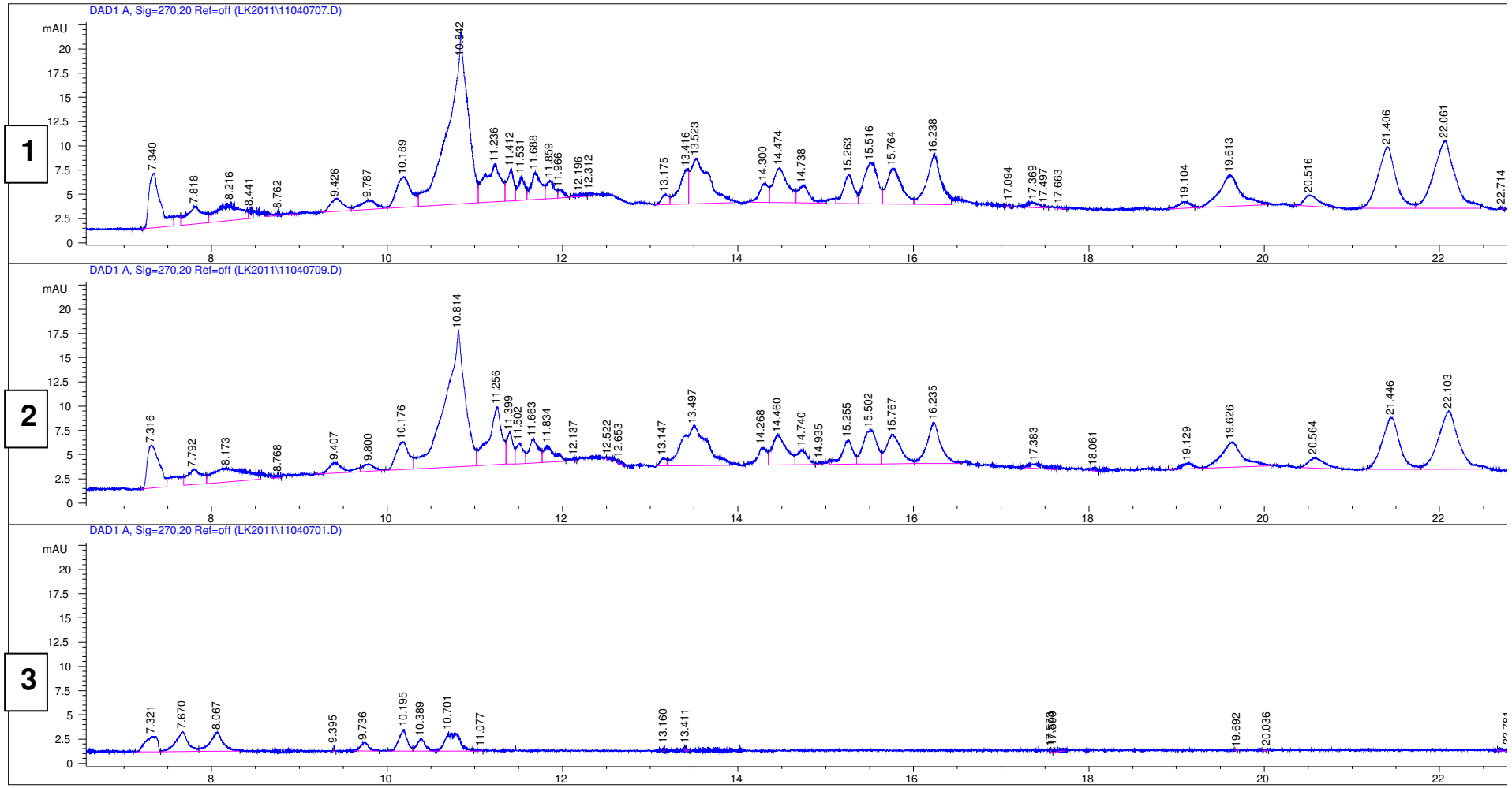

Figure 2 Electropherogram of hydrolyzate sample from SW sawdust autohydrolysis during 2 hours, where 1 – hydrolyzate sample, 2 – sample and standard solution of fructose (25 ppm), 3 – standard solution of fructose (25 ppm)

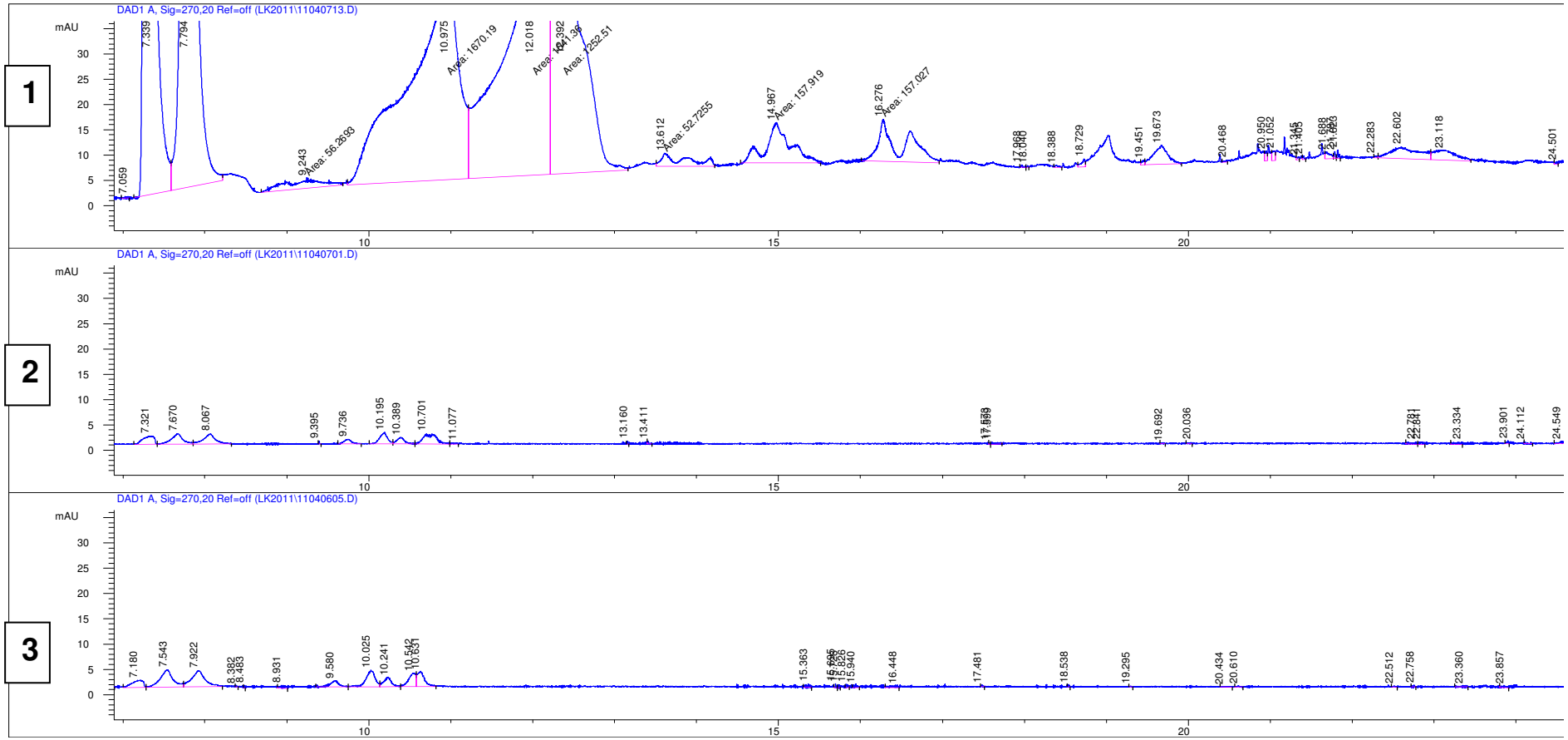

Figure 3 Electropherogram of hydrolyzate sample from birch chips treatment during 2 hours with sulfuric acid as catalyst (1.5%), where 1 –hydrolyzate sample, 2 – sample and standard solution of fructose (25 ppm), 3 – standard solution of fructose (25 ppm)

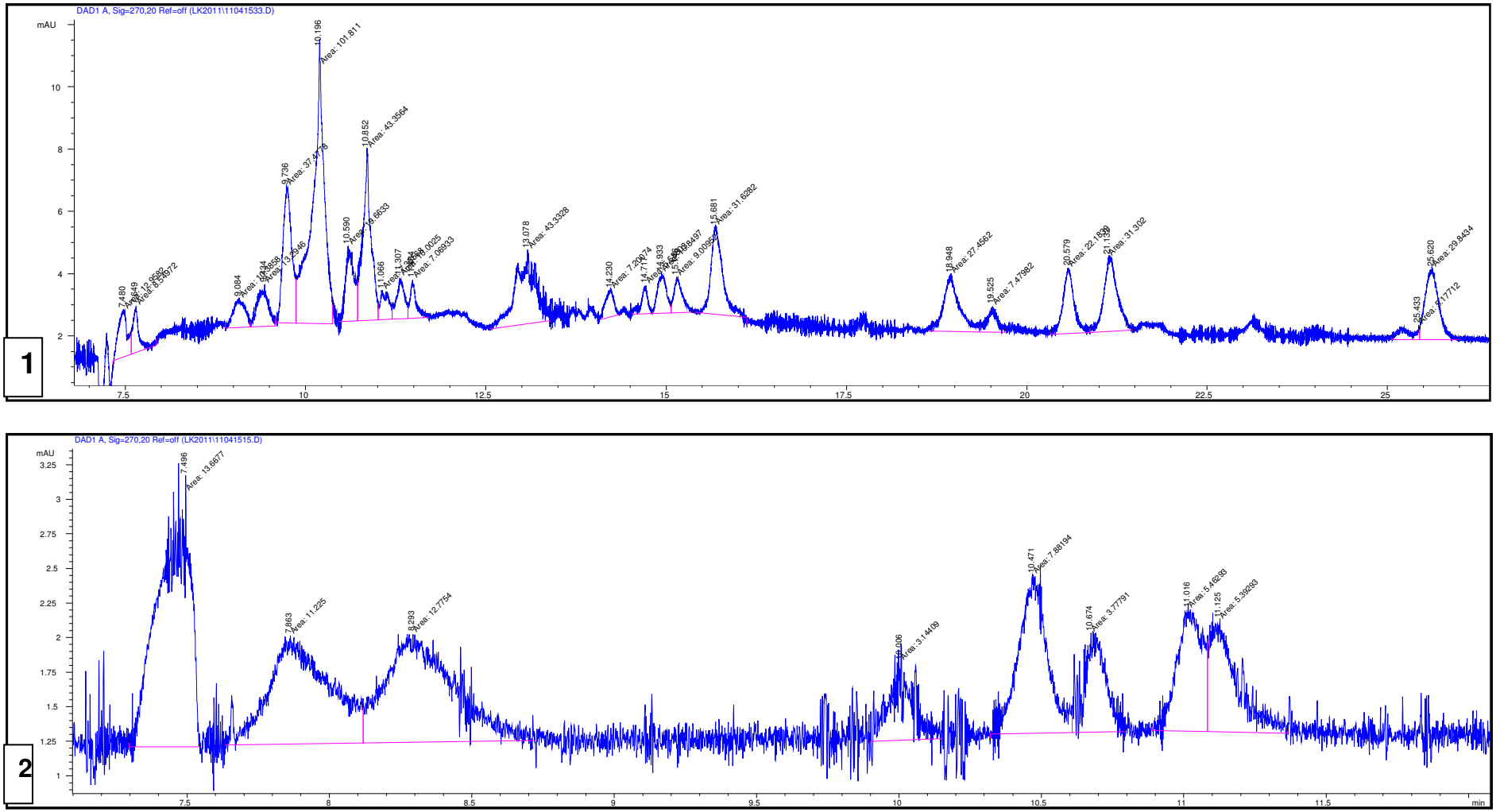

Figure 4 Electropherogram of hydrolyzate sample of birch chips treatment during 2 hours with acetic acid as catalyst (1.5%), where 1 – hydrolyzate sample, 2 – standard solution of fructose (25 ppm)

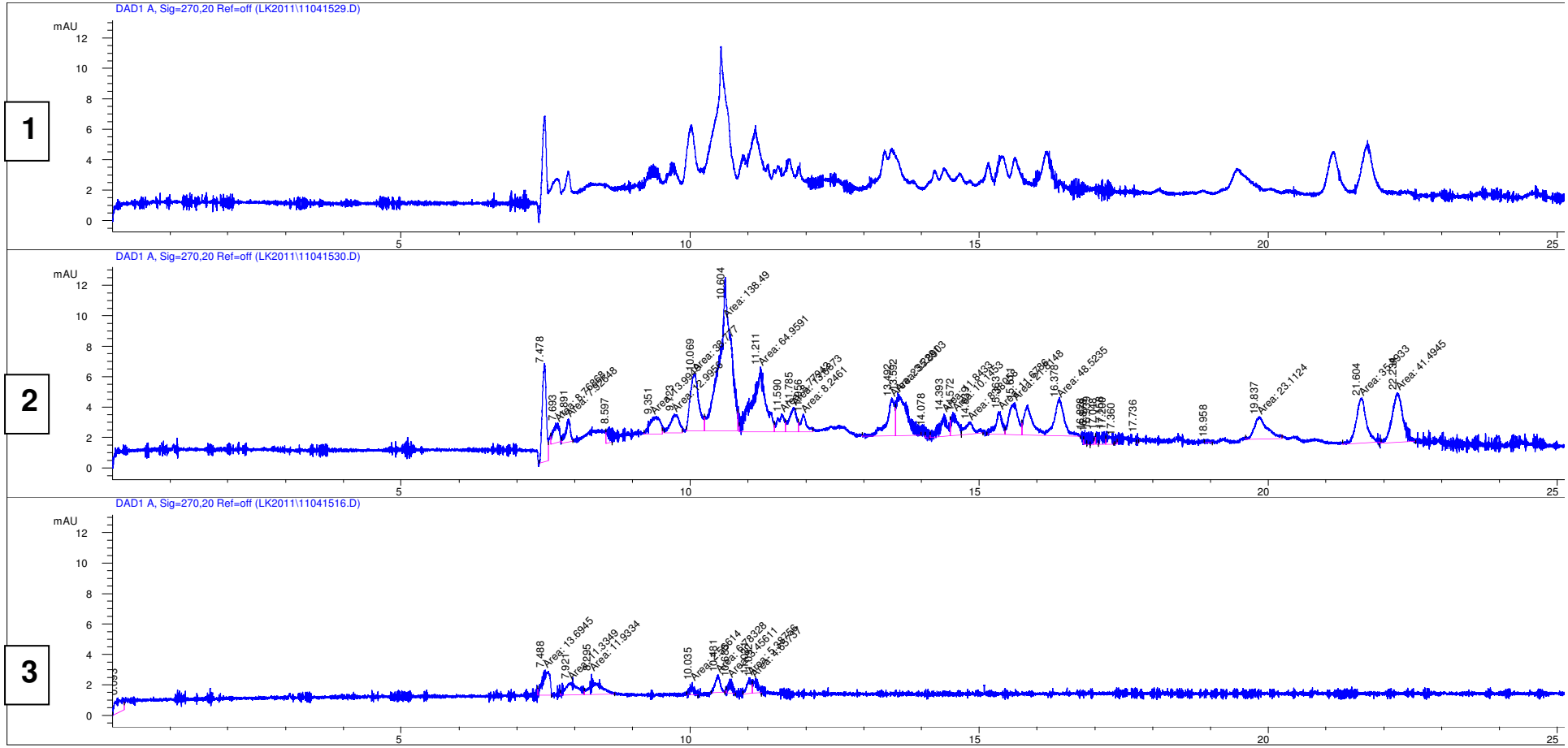

Figure 5 Electropherogram of hydrolyzate sample from SW sawdust treatment during 2 hours with acetic acid as catalyst (1.5%), where 1 - hydrolyzate sample, 2 – sample and standard solution of fructose (25 ppm), 3 – standard solution of fructose (25 ppm)