CHALMERS
UNIVERSITY OF TECHNOLOGY

Comparative study of steam explosion pretreatment of birch and spruce

Master's thesis within the Innovative and Sustainable Chemical Engineering programme

VANJA UZELAC

Department of Chemical and Biological Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2014

MASTER'S THESIS

Comparative study of steam explosion pretreatment of birch and spruce

Master's thesis within the Innovative and Sustainable Chemical Engineering programme

VANJA UZELAC

Comparative study of steam explosion pretreatment of birch and spruce
Vanja Uzelac

Department of Chemical Engineering
Chalmers University of Technology

SE-412 96 Göteborg
Sweden
Telephone: +46 (0)31-772 1000

Abstract

The environmental concerns related to CO_2 and the rising price of petroleum, have increased the interest in alternative renewable resources. The use of biomass as raw material is considered to be the most suitable and renewable primary energy resource for production of alternative fuels. The biorefinery is an emerging field that has a goal to compete with petroleum-based industries. However, a lot of development is required and one of the main challenges to the biorefinery is the complex chemical composition and physical structure of the biomass. That is why pretreatment is necessary to open up the biomass structure and break down the lignocellulosic bonding in order to promote enzymatic accessibility to cellulose and hemicellulose for hydrolysis. A promising pretreatment is steam explosion, which has several advantages compared to other alternatives such as lower environmental impact, reduced capital investment, greater energy efficiency and less hazardous process chemicals

The aim of this thesis is compare the effectiveness of steam explosion of the hardwood birch and the softwood spruce. This is done by an extensive literature review, which compares the chemical and structural analysis, and steam explosion experiments. The experiments are analyzed by mercury porosimetry and high-pressure liquid chromatography for the sugar analysis. The mercury porosimetry gives information about the change in the physical structure and the sugar analysis will compare the degradation of hemicellulose and cellulose between the wood species. A comparison of lignin content is also made. The results of mercury porosimetry showed that pore size and intrusion volume have increased more for spruce than birch which indicates that steam explosion pretreatment is potentially more effective on spruce. The literature review revealed no significant difference and the results of sugar and lignin analysis showed that considerable degradation occurred. However, slightly favorable trends were obtained for spruce regarding the effectiveness of steam explosion.

Acknowledgments

This master's thesis has been performed at Chalmers University of Technology in Göteborg, Sweden. The work has been supervised by Muhammad Muzamal at Chalmers University of Technology. I would like thank my supervisor Muhammad Muzamal for his help and guidance throughout this thesis project. I would also like to thank Anders Rasmuson for the opportunity to let me conduct my master's thesis at the division of Chemical Engineering.

Table of contents

1 Introduction

1.1 Background

The environmental concerns related to CO_2 have increased over the past years and that have strengthened the interest in alternative, nonpetroleum-based resources for use as raw materials. The use of biomass as raw material is considered to be the most suitable and renewable primary energy resource for production of alternative fuels such as bioethanol, where the demand is increasing (Sun and Cheng 2002). The transformation from crude oil-based refinery to biomass-based refinery has attracted strong scientific attention. Also the increased petroleum prices have raised the interest of alternative transportation fuel. Currently, starch and sugar crops are mostly used for the production of bioethanol. However, there is little possibility of process improvements and the sustainability of these raw materials is questioned (Alvira et al. 2010). There is less concern of lignocellulosc biomass compared to conversion of starch and sugar crops since there is no competition between lignocellulosic biomass and food crop. Therefore lignocellulosic biomass is an interesting alternative. Also lignocellulosic biomass has a good potential for the production of affordable bioethanol because it is less expensive compared to starch and sugar crops (Zheng et al. 2009).

Lignocellulosic biomass such as forestry residue, agriculture residue and wood products are example of renewable resources that stores energy from sunlight in their chemical bonds (Zheng et al. 2009). Lignocellulose is the most abundant renewable biomass in Earth with an estimated annual production of 10-50 billion ton worldwide (Sanchez and Cardona 2008). Lignocellulosic biomass can produce a range of different products such as of liquid transport fuels, highly purified cellulose, power and chemicals. The production of chemicals requires far lower volumes of biomass compared to the energy and fuel production. The biological production of chemicals is central to the sustained development of biorefining technologies, because there is a demand of high value and relatively low material utilization in this industry (FitzPatrick et al. 2010). The bio-based chemicals have been used in the automotive industry for a long time. Henry Ford had plant materials for car tires in his original manufacturing plan of car production. Today, there are many automotive parts manufactured that use biomass as a raw material, e.g. sunshades, seat cushions and armrests (FitzPatrick et al. 2010).

The biological conversion of lignocellulosic raw material for ethanol production provides a lot of benefits but the development is still hindered by economic and technical difficulties. In order to reduce the cost of ethanol production and make the conversion more feasible, there is a need of efficient utilization of the raw material to obtain high ethanol yields, high productivity and process integration in order to reduce energy demand (Galbe and Zacchi 2007; Tomás-Pejó et al. 2008)). The conversion of lignocellulosic biomass to bioethanol requires the following steps: hydrolysis of cellulose and hemicellulose, sugar fermentation, lignin residue separation and finally recovery and purification of ethanol in order to fulfil fuel specifications (Alvira et al. 2010). The most viable strategy to provide benefits over other chemical conversion routes is the employment of enzymes for the lignocellulose hydrolysis (Zheng et al 2009).

The hydrolysis of cellulose and hemicellulose is technically difficult because the digestibility of the two wood components is hindered by physico-chemical complexities in biomass. These structural complications are the reason why a pretreatment step is necessary for obtaining various chemicals and fermentable sugars during the hydrolysis step. The reason for the implementation of

pretreatment is to open up the biomass structure and break down the lignocellulosic bonding in order to promote enzymatic accessibility to cellulose and hemicellulose for hydrolysis (Mosier et al. 2005). The current research for pretreatment is focusing on different approaches to optimize the process of effective utilization of lingocellullose for ethanol production. The aim of the research is to identify, evaluate, develop and demonstrate promising techniques to primary enhance lower dosage of enzyme for the enzymatic hydrolysis of biomass and reduce the bioconversion time (Alvira et al. 2010). The pretreatment is an important step in the conversion of biomass to fermentable sugars, but it has been viewed as one of the most expensive processing steps. The benefits with pretreatment are the great potential for improvement of efficiency and with the help of research and development; it can reduce the economic costs as well as facilitate the pretreatment process (Mosier et al. 2005).

1.2 Aim

The aim of this thesis is to compare and analyze the effects of steam explosion pretreatment on the hardwood birch and the softwood spruce. This is done by an extensive literature review, experiments consisting of steam explosion pretreatment and the subsequently analysis tools which are mercury porosimetry and high-pressure liquid chromatography for the sugar analysis. The literature review will, among other things, compare the chemical and structural analysis on different steam exploded wood species. The sugar analysis will compare the degradation of hemicellulose and cellulose between birch and spruce. A comparison of lignin content is made as well. The mercury porosimetry will study the change in the physical structure of birch and spruce, i.e. analyzing the change in pore size distribution and intrusion volume due to steam explosion.

The purpose of the thesis is to better understand the steam explosion process by comparing the effectiveness of steam explosion on softwood and hardwood. The effects of steam explosion on the softwood spruce have been analyzed and therefore it is interesting to compare it with the effects on the hardwood birch. The potential difference of the efficiency of steam explosion between the wood species and the explanation behind it will also help to increase the understanding of the process

1.3 Limitations

The initial plan for this thesis was to solely perform experiments on the hardwood birch and analyze the physical structure for the three main process steps which are the treatment step, the explosion step and the impact step. However, there was a problem with the steam generator. Because of that reason the experiments were performed in lab scale equipment instead of the initial larger steam explosion equipment. The lab scale equipment is not able to perform the impact step (the impact of wood chips mixed with other chips and vessel walls) and the steam explosion is performed at milder conditions, i.e. lower temperature and pressure.

An experimental comparison of chemical analysis regarding the optimal sugar yields after enzymatic hydrolysis (saccharification) is not performed. However, a literature comparison of optimal sugar yields between different hardwood and softwood species is done. The subsequently enzymatic hydrolysis and fermentations steps will not be experimentally investigated and analyzed in this thesis. These processes will solely be mentioned and explained in the report in order to get a holistic perspective and better understating of the biorefinery process.

2 Theory

2.1 Wood

Wood is made from seed-bearing plants and belongs to the group *Spermatophytae*. Wood has a complex hierarchic structure that determines the mechanical and physical properties of all the timber products including pulp and sawn wood. The properties of wood are governed by the structure itself which are mainly anatomical organization and cell wall ultrastructure (Daniel 2004). Wood has a highly complex physical structure and chemical composition which can be used in many applications such as building construction, fuel, chemicals and furniture. Wood can be divided into to two types of groups which are softwood produced from gymnosperm trees and hardwood produced from angiosperm trees (Daniel 2004). There are a lot of wood species around the world with an estimated 30 000 angiosperm and 500 gymnosperms species. Spruce, pine and fir are examples of softwoods and birch, aspen and poplar are examples of hardwoods. The most common wood species used in Swedish pulp and paper industries are birch, pine and spruce, where the most used raw material in many biorefineries is spruce (Daniel 2004; Muzamal 2014).

2.1.1 Structure of wood

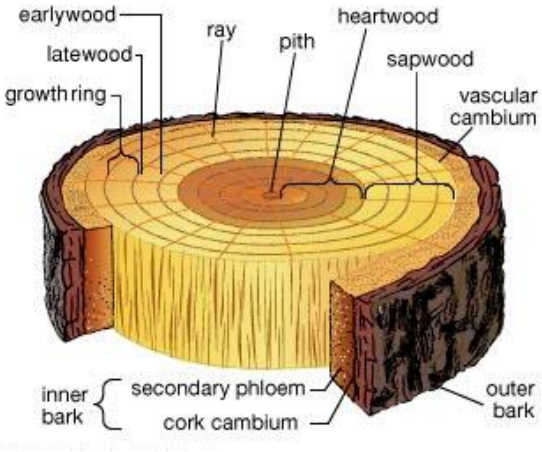

Figure 1: The structure of a wood stem (Merriam-Webster 2006).

A tree can be separated into three primarily parts referred to as the crown, trunk (stem) and root system. These parts are composed of different tissues which in turn are comprised of specific wood cells. The wood stem can be divvied into several complex layers in which each has an own specific function as shown in Figure 1. The first outer dead layer of the stem is called bark and its function is to protect the wood from physical, mechanical and biological degradation (Daniel 2004). The innermost layer of the bark is referred as phloem and is a living tissue that allows transportation of nutrients and the storage of products. The next layer after phloem is vascular cambium and is thin layer of cells that provides production of phloem cells to the outside and xylem cells to the inside. The xylem constitutes the major part of the wood of a tree and is divided into sapwood and heartwood (Daniel 2004).

Sapwood is composed of both living and dead cells, and its main function is transportation of water from the roots to the leaves. Heartwood constitutes of only dead cells and primarily serves as a support tissue. Finally, at the center of the steam is the pith which represents the development of

tissue during the first years of tree growth. The xylem is organized into separate concentrically orientated rings called annual growth rings and each ring is representing one years' tree growth (Daniel 2004). The growth ring is composed of earlywood and latewood. During the spring large earlyood cells are formed when the water supply is large and latewood cells are produced during the summer when the cells grow slower and become thicker. The major physical difference between the two is that earlywood cells have a larger cross-section and thinner walls compare to latewood (Muzamal 2014).

Wood consists of axial and radial cell systems and is produced in the vascular cambium. Cells in the trunk are arranged in longitudinal and radial directions. There are two types of cells referred as tracheids and parenchyma. Tracheids are extended cells in the xylem of vascular plants and their function is water and mineral salts transportation. Parenchyma cells consist of relatively large, thin-walled cells and can primarily be found within the ray canals. The main function of ray is to store and redistribute storage materials. It contributes 5-11% of the total softwood and up to 30% of the total hardwood (Daniel 2004).

Hardwoods are considered to have much more complex structure than softwoods and have cell types with much greater cell morphology. Hardwoods have also a larger number of different cell types than softwoods. That includes vessels (pores), libriform fibers, fiber tracheids, longitudinal parenchyma and ray parenchyma, which all constitute the major part of the tissue of hardwood and are present in all known hardwoods (Daniel 2004). In softwoods, there are lower amounts of parenchyma cells present compare to hardwoods. The presence of longitudinal parenchyma in softwoods are even more limited and only a number of species have it. When softwood species do have it, they only occur in reduced amounts. The width and height of ray cells in hardwoods can vary a lot (Daniel 2004). In the softwoods, the early and latewood tracheid are responsible for the fluid conduction and support. In the hardwoods, they have developed specialized cells to take care of these functions. The support and strength are taken care by libriform fibers and the fluid conduction is provided by specialized cells called vessels or pores (Daniel 2004).

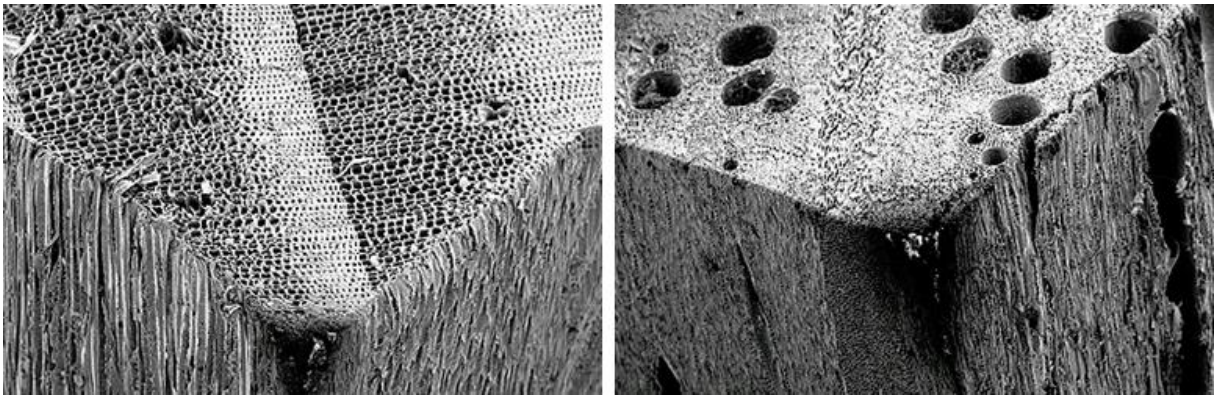

Figure 2: The image on the left shows the absence of vessels (pores) in the softwood pine and the image on the right shows the presence of vessels in the hardwood oak (Mckdandy 2006).

Vessels are a characteristic and dominant feature that separates hardwoods from softwoods (see Figure 2). They may show considerable variation in size and shape and can be recognized by the naked eye in sawn wood (Daniel 2004). The vessels are only present in hardwoods and are comprised of single cells which are joined end to end for the formation of longitudinal tubes. The vessels are the main conducting part in hardwoods and the ends of the cells can vary between entirely open or

perforated (Daniel 2004). There is also a difference in vessels size and cellular morphology between various hardwood species. The softwoods are known as “non-porous woods” and the hardwoods as “porous woods” due to the appearance of vessels in transverse wood sections as holes. The cell walls of vessels are relatively thin compared to fibers and softwood tracheids (Daniel 2004).

One of the most typical microstructures that transpire in cell walls of softwoods and hardwoods are pits. They can be describes as canals that regulate the liquid flow directions through the cell walls, i.e. both laterally and vertically. There is a variation of shapes and sizes of wood pits and they can be divided into three types which are simple pits, borderer pits and cross-field pits (Daniel 2004). The different types of pits connect different types of cells in softwood and hardwood. In the latter case, simple pits are responsible for connecting parenchyma cells and vessels with parenchyma cells. The connections between vessel elements as well as between fiber tracheids are regulated by bordered pits. Lastly, the cross-field pits connect fiber tracheids and parenchyma cells (Daniel 2004).

2.1.2 Components of wood

The walls of wood cells are composed of three main chemical components i.e. cellulose, lignin and hemicelluloses. The structure can be described as a skeletal matrix formed by cellulose and is surrounded and covered by the hemicelluloses and lignin (Daniel 2004). The most abundant organic compound in nature is cellulose. In biomass there is an annual production and break down of about 150 billion tonnes cellulose. Its composition is approximately 38-50% in wood (Lennholm and Blomqvist 2004). There are a lot of applications for cellulose such as paper products, textiles, composite plastics and building material. It is a linear polysaccharide in which the monomers, B-D-glucose units are linked together by (1-4) glucosidic bonds (Muzamal 2014). It has a high molecular weight with an average degree of polymerization of about 8000 for wood cellulose. The chains of cellulose are self-arranging in order to make fibrils. It has crystalline and amorphous regions. The stability of the crystalline region is better for chemical and thermal conditions compared to the amorphous region (Muzamal 2014; Lennholm and Blomqvist 2004).

Hemicelluloses generally occur as heterogeneous polysaccharides and have a degree of polymerization of 100-200 monomers. Hemicellulose constitutes about 25-30% in softwood and 30-35% in hardwood. It can be found in the matrix between cellulose fibrils in the cell wall. The building units that are mainly present in hemicellulose are hexoses and/or pentoses but there are also small amounts of deoxyhexoses and certain uronic acids (Teleman 2004). It has been shown that hemicelluloses have a lower chemical and thermal stability than cellulose. The cell walls of hemicelluloses have a low stiffness and a high moisture absorption capacity due to the low degree of polymerization and crystallinity (Muzamal 2014). The most common hemicelluloses are xylans and glucomannans. The more abundant of the two are xylans which are the main components of secondary cell walls (Gírio et al. 2010).

Lignin is the most abundant aromatic polymer in the nature and is a random polymer composed of phenyl propane units (Suhas et al. 2006). It makes up 15-35% of wood and is present in the middle lamella as well as in the cell wall. The structure of the lignin is immensely complex and forms a three dimensional network. The network is formed mainly by three types of monolignols that are linked together by different ether and carbon-carbon bonds (Norberg 2012). Lignin can be divided into three main groups called softwood lignin, hardwood lignin and grass lignin. The function of lignin is to give the cell wall a stiffness which gives mechanical strength to the wood. It can also act as a barrier

to protect the tree against microbial degradation and making the cell wall hydrophobic to give an efficient transportation system of water and nutrition (Suhas et al. 2006; Norberg 2012).

2.2 Biorefinery

A biorefinery can be described as a facility that integrates processes of biomass conversion and equipment to produce fuels, power and value-added chemicals from biomass. The concept of biorefinery is similar to the current crude oil refineries, which provide production of several fuels and petroleum-based products. It is analogous to many conversion technologies from oil refinery such as fluid catalytic cracking, thermal cracking and hydrocracking technology (Demirbas 2010). Another similarity is that most of the oil refinery and biorefinery are not using all the available conversion technologies. They utilize the technologies that are most cost effective regarding the conversion of one type of biomass into specific desired end products (Demirbas 2010). The biorefinery process converts the biomass raw material into a multitude of valuable chemicals and energy with minimal waste and emissions (Demirbas 2010). In a theoretical point of view, a biorefinery is anything that utilizes biomass and produces more than one product. Some examples of existing biorefineries are corn processors and pulp and paper mills. The latter is considered to be the first generation of biorefineries. The concept of biorefinery is not a new idea. It has been around for a long time, e.g. sugarcane has been used in bioethanol production since 6000 BC (Demirbas 2010).

There are problems with the implementation and commercialization of biorefineries which are both technical and non-technical. The major technical complications are the production costs of crops and difficulties in harvesting and storing the grown material. Also the costs of transportation are a significant problem. The main non-technical obstacles are restrictions on land use and environmental and ecological impacts of large areas of monoculture (Demirbas 2010). However, the future development of biorefineries would involve mimicking the energy efficiency of present oil refining, with the help of large heat integration and co-product progress. It will likely have an integration of both bioconversion and chemical cracking technologies. There are four main technologies for the chemical production from biomass, which are pretreatment, thermochemical conversion, fermentation and bioconversion and finally product separation and upgrading (Demirbas 2010). The pretreatment process is one of the main economic costs in the biorefinery process. There has been a lot of research and development to improve the pretreatment techniques that enhance the following enzymatic hydrolysis of the treated biomass (Muzamal 2014).

2.3 Factors limiting enzymatic hydrolysis

Main factors that affect the enzymatic hydrolysis can be divided into enzyme-related and substrate-related. There is also an interrelation between the two groups. It is necessary to have a pretreatment step to modify some structural characteristics of lignocellulose and increase the accessibility of glucan and xylan to the enzymatic attack (Alvira et al. 2010). The factor that reduces the efficiency of hydrolysis is the linkage of lignin with hemicellulose and cellulose. It inhibits enzyme accessibility and in a result of that makes the biomass hard to digest (Mansfield et al. 1999). The pretreatment can break down the lignin structure and make enzyme accessibility to the cellulose. The accessibility of enzymes is one of the key factors limiting the enzymatic hydrolysis and that is why one of the main aims of the pretreatment is to increase the available surface area for the enzymatic attack.

The mean pore size of the substrate can be increased by the removal of hemicellulose and the consequence of this is an increased accessibility and probability of the cellulose to be hydrolyzed

(Chandra et al. 2007). It has been shown that the relation between the pore size of the substrate and the size of the enzyme is the main limiting factor in enzymatic hydrolysis of lignocellulosic biomass (Chandra et al. 2007). Some important parameters in determining the hydrolysis rates of comparatively refined cellulose substrates are the degree of polymerization and cellulose crystallinity. However, other sources have suggested that these factors alone do not explain the recalcitrance of lignocellulosic substrates (Puri 1984).

2.4 Pretreatment process

The overall purpose of the pretreatment is to break down the shield formed by lignin (see Figure 3), increase the available surface area, disrupt the crystalline structure and reduce the degree of polymerization of cellulose in order to enhance enzyme accessibility to the cellulose during hydrolysis (Zheng et al. 2009; Mosier et al. 2005). There are studies of various pretreatment technologies for the utilization of biomass for bioethanol production. There are advantages and disadvantages for all the different pretreatments and it is necessary to adapt suitable pretreatments based on the properties of the raw material. An effective pretreatment should have qualities such as avoiding size reduction, preservation of hemicellulose fractions and be cost-effective (Zheng et al. 2009). Some of the most common pretreatment processes are discussed below.

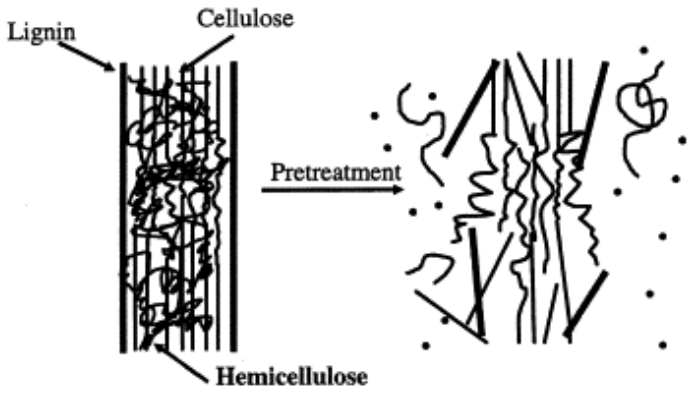

Figure 3: The pretreatment effect on lignocellulosic biomass (Mosier 2005).

2.4.1 Alkali pretreatment

Alkali pretreatment is a method that increases the digestibility of cellulose by affecting the lignin in the biomass. The effectiveness of the alkali pretreatment depends on the lignin content. It can be performed at room temperature and the time ranges from a few minutes to days (Carvalheiro et al 2008). Some examples of suitable alkaline pretreatments are sodium, potassium and ammonium hydroxides. The advantages with this pretreatment are that it causes less degradation of hemicelluloses and cellulose compared to acid and hydrothermal pretreatments. Also there is less sugar degradation than acid pretreatment and it is more effective on agriculture residue than on wood materials (Kumar et al 2009). The drawbacks with alkali pretreatment are the loss of fermentable sugars and production of inhibitory compounds. These problems must be handled in order to optimize the pretreatment conditions (Alvira et al 2010).

2.4.2 Acid pretreatment

In acid pretreatment, hemicelluloses are solubilized to increase the cellulose accessibility to enzymes. This pretreatment can either be performed by concentrated acid or diluted acid. The drawbacks with concentrated acid are that it is less suitable for ethanol production because of the hemicellulose and

cellulose degradation and formation of inhibiting compounds (Wyman 1996). Other problems include equipment corrosion and acid recovery. Furthermore, there are high operation and maintenance costs which limit the commercialization development (Wyman 1996). The dilute acid pretreatment is a better method for industrial applications and can pretreat a wide range of lignocellulosic biomass. It can be performed at high temperatures for a short time period or at low temperatures for a longer time period. It also presents the advantage of solubilizing hemicellulose and converting it to fermentable sugars (Saha et al. 2005).

2.4.3 Liquid hot water pretreatment

This pretreatment with liquid hot water does not require employment of catalysts or chemicals. The temperature of the water is at 160-240^{o}C and high pressure is needed to maintain the water in the liquid state. The aim of the liquid hot water pretreatment is to make cellulose more accessible and to prevent the formation of inhibitors (Alvira et al. 2010). This is achieved by solubilizing the hemicellulose and lignin degradation during the processing of liquid hot water. The benefits with this pretreatment are the economical savings. There is no use of catalyst and there is a low-cost reactor construction due to low corrosion potential. The drawbacks are that there is no development on the commercial scale because of the high demand for water and high energy requirement which make this process hugely expensive (Alvira et al. 2010).

2.4.4 Steam explosion

The process of steam explosion was first developed and introduced by William H. Mason in 1925 for the production of a type of hardboard called Masonite and was patented the following year (Mason 1926). The description of the patent was a steam explosion process which was used for the pretreatment of wood. In this process wood chips are fed in a Masonite gun and then steam heated at certain temperature, pressure and time. Thereafter the pressure is increased and the chips are discharged and then exploded at atmospheric pressure to produce pulp (Mason 1926). Today the steam explosion process has become one of the most common and widely employed physico-chemical pretreatments for lignocellulosic biomass (Alvira et al. 2010). It is a hydrothermal pretreatment that consists of three main steps which are the treatment step, the explosion step and the impact step. The first experiment step can be described as treatment of lignocellulosic biomass with pressurized steam for a specific amount of time. The explosion of wood chips due to the rapid release of pressure is corresponding to the explosion step and the impact of wood chips mixed with other chips and vessel walls is representing the impact step (Muzamal 2014).

There is a combination of mechanical forces and chemical effects in the steam explosion pretreatment. A chemical process called autohydrolysis is occurring during the steam explosion pretreatment. Autohydrolysis takes place when acetic acid from acetyl groups is formed because of the high temperature (Alvira et al. 2010). Water can also act as an acid at elevated temperatures which facilitates the hydrolysis of hemicelluloses. The cause for the mechanical effects is sudden pressure reduction which results in separation of fibers due to the explosive decompression (Alvira et al. 2010). The lignin is reorganized and to some extent removed from the material owing to the combined effect of partial hemicellulose hydrolysis and solubilization (Pan et al. 2005). The surface of the cellulose is going to be exposed because of the hemicellulose removal and that will increase the accessibility of the enzyme to the cellulose microfibrils (Alvira et al. 2010). However, degradation of hemicelluloses is not beneficial for some processes as hemicelluloses can also be used to produce fermentable sugars.

The major parameters which affect the effectiveness of steam explosion are particle size, temperature (or pressure), residence time and the combined effect of both temperature and time. Overend and Chornet (1987) have made observations that it is possible to trade time of treatment and the temperature of treatment such that equivalent final effects such as pulp quality or enzyme accessibility are obtained. A severity factor has been developed that is commonly used for the optimization of steam explosion processes of biomass. The severity factor characterizes the steam explosion process and is a function of the reaction time and temperature and can be described by the following equation (Overend and Chornet 1987):

$$R_0 = t * e^{\frac{(T-100)}{14,45}} \quad (1)$$

The limitation of this model is that it does not consider the moisture content in the raw material and particle size which both have strong effect on the kinetics of steam explosion pretreatment, i.e. both physical and chemical changes of the biomass. It has been shown that the kinetics are slow due to the high moisture contents in the raw material. This is caused when biomass voids are filled with condensate before the temperature of the steam is reached (Overend and Chornet 1987).

There are several advantages that are obtained from the steam explosion process compared to other pretreatment technologies. That includes the potential for significantly lower environmental impact, reduced capital investment, greater energy efficiency, less hazardous process chemicals and complete sugar recovery of wood biopolymers (Avellar and Glasser 1998). Other favorable features are the option to use large chip size, unnecessary addition of acid catalyst in hardwood, good hydrolysis yields in enzymatic hydrolysis and the possibility to be developed on a commercial scale (Alvira et al. 2010). The main disadvantages of the steam explosion process are the partial degradation of hemicellulose and that toxic compounds are generated, which could affect the subsequent hydrolysis and fermentations steps (Olivia et al. 2003). Which toxic compounds are produced and what amount are decided by the feedstock and the severity of the pretreatment. That is the reason why it is necessary to use a robust thermotolerant yeast strain, which is capable of ethanol fermentation of glucose from cellulose (Oliva et al. 2003).

2.5 Effect of steam explosion on chemical changes

2.5.1 Optimal sugar yield condition regarding pressure and time

There are two related studies, performed by Asada et al. (2011; 2012) and at the same location, which compare the utilization of hardwoods and softwoods in the bioethanol production by using steam explosion and enzymatic saccharification. The term saccharification stands for when soluble polysaccharides are broken into its component sugar molecules by hydrolysis. Asada et al. (2012) considered the softwood Japanese cedar and Asada et al. (2011) considered the hardwood aspen chopsticks. The steam explosion pretreatment for both woods was carried out in a batch pilot unit equipped with a 2 l of reactor for Japanese cedar and with a 1 l of reactor for aspen chopsticks at the Japan Chemical Engineering and Machinery Co., Ltd. in Osaka, Japan (Asada et al. 2011; Asada et al. 2012).

The samples of Japanese cedar and aspen chopsticks where chopped into wood chips with a length of 4-5 cm. Regarding the Japanese cedar, an amount of 200 g of the cedar wood chips was put into the reactor and exposed to the saturated steam. After the saturated steam exposure for a steaming

time of 1-10 min, a ball valve at the bottom of the reactor was abruptly opened to bring the reactor rapidly to atmospheric pressure. Thereafter the steam exploded product containing liquid-solid materials was obtained in the receiver (Asada et al. 2012). The same procedure was done for the aspen chopsticks with an exception of introducing 100 g of chopsticks into the reactor and exposed to the saturated steam for a steaming time of 5 min (Asada et al. 2011).

The enzymatic saccharification was carried out on the water extracted Japanese cedar and Aspen chopsticks by using cellulolytic enzyme. The result showed that the amount of reducing sugars and glucose produced from both Japanase cedar and aspen chopsticks were rapidly increased with the increase of time and reached their maximum values at 48 h. However, the optimal sugar yield values between Japanese cedar and aspen chopsticks were different. The sugar yield is the ratio of amount of sugar production to the initial amount of sugar contained (Asada et al. 2012). For the Japanese cedar the condition at 45 atm and 3 min provided the highest sugar yield. For the aspen chopsticks the highest value of sugar yield and amount of reducing sugar and glucose production were reached at a treatment of 25 atm and 5 min (Asada et al. 2011).

The results show that the softwood required higher steam conditions than the hardwood. That means the steam explosion pretreatment is probably more effective for the hardwood compared to the softwood according to these studies. The steam explosion condition of hardwood provides a more positive effect on the enzymatic saccharification due to the breakdown of the wood chip and enhancing the enzyme accessibility as well as the cellulose digestibility (Asada et al. 2011). This thesis will perform a similar experimental comparison regarding pressure and time, but it will be a physical structure analysis instead. The results from the theoretical chemical analysis together with the experimental structure comparison and analysis of sugar and lignin, will provide better information on which of the wood species are more suitable for the steam explosion pretreatment.

2.5.2 Optimal sugar yield condition regarding temperature and time

There are also studies analyzing the optimal sugar yields by comparing temperature and time instead of pressure and time. The raw materials that are evaluated are from several different studies. The ones that are considered for this case are the hardwoods salix, poplar and birch and the softwoods considered are pine and spruce forest residue. It must be emphasized that no study is available in which experiments are performed on hardwood and softwood under similar conditions. Instead there is going to be a comparison between different studies. The poplar, salix and birch were chopped into wood chips length size of 20 mm, 2-10 mm and 10 mm respectively. The steam pretreatments for poplar and salix wood were both performed in a 10 l reactor and for birch it was in a 20 l reactor. The results showed the optimal sugar yield condition after enzymatic hydrolysis for poplar were at 210^{o}C and 15 min, while for the salix the highest total yield were at 200^{o}C, 14 min and for the birch it was at 220^{o}C and 10min (Schutt et al. 2011; Sassner et al 2005;Vivekanad et al 2013).

Regarding the softwood pine, the steam explosion pretreatment was carried out in a stainless-steel autoclave reactor and the optimal condition for sugar yield was at 220^{o}C and at short reaction times (San Martin et al 1995). While the pretreatment for spruce was carried out in a 10 l reactor with wood chips length at 10-60 mm and the highest product yield at 220^{o}C (the time had minor influence) (Janzon et al. 2014). The results shows that the majority of the hardwood species need a milder steam condition compare to softwood, with the exception of birch which had its highest sugar yield at the same temperature as the softwood (220^{o}C).

2.5.3 Possible reasons for different effectiveness results

There has not been made any clear reasons for why steam explosion pretreatment is potentially less effective on softwood compared to hardwood (Clark and Mackie 1987). However, one of the reasons may be that softwood contains a relatively large amount of condensed-type lignin which can make it harder for the pretreatment to reorganize and remove the lignin (Asada et al. 2012). It is generally considered that softwoods are one of the most difficult lignocellulosic raw materials to hydrolyze to sugars for fermentation. It is mainly because of the nature and the amount of lignin, which consists of 25-30% in softwood compare to 20-25% in hardwood. The steam explosion pretreatment has to consider critical process steps such as lignin separation and utilization when softwood is chosen as a substrate. In the modern steam explosion pretreatment, a delignification step is a requirement in order to achieve a feasible process. However, it must be emphasized that there are structural differences between the hardwood species (i.e. birch had the same optimal conditions as spruce) and that is why it is difficult to draw accurate general conclusions for all the hardwood species.

3 Methodology

3.1 Material and sample preparation

Wood is a complex material and has an internal structure that varies a lot between different kinds of woods. In order to study and make a comparison between the different steam explosion steps, the wood materials were cut into appropriate samples. The raw material used for the experiments was Norwegian dry birch. A birch stem was cut into a smaller stem with a machine saw. The small stem was then again cut with a machine saw into wood pieces with dimensions of 100 mm x 20 mm x 4 mm. These large pieces were divided into three and four parts with dimensions of 20 mm x 20 mm x 4 mm, as shown in figure 4. One part was used for reference and the rest (2 or 3 parts) were used for the experiments. These wood pieces were analyzed by mercury porosimetry. For the sugar and lignin analysis, the original large pieces were divided into two parts with dimensions of 30 mm x 20 mm x 4 mm. One part is reference and the other is for experiments (see figure 4). The samples for mercury porosimetry are made smaller because the mercury is not able to penetrate into larger wood pieces. Before the steam explosion experiments, the wood samples were water impregnated. The reason for that is if there is already water inside the samples, it will become easier to create explosion. The wood samples were fully impregnated with de-ionized water for 24 hours at room temperature in concealed vessels.

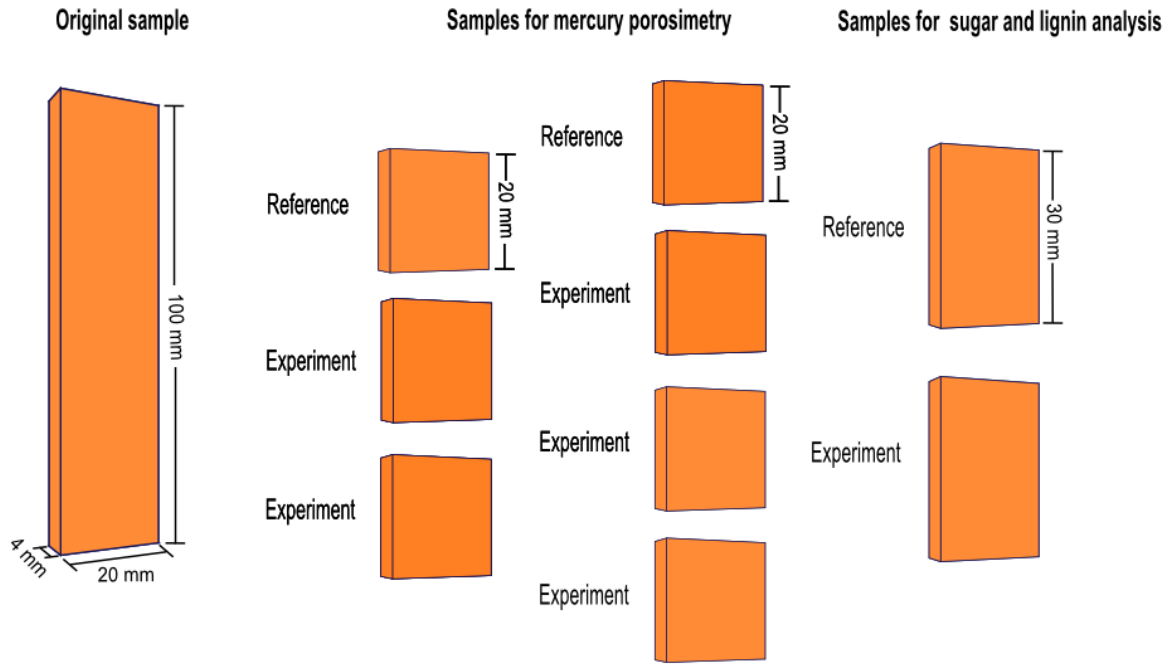

Figure 4: Dimensions of the wood samples for the mercury porosimetry and sugar and lignin analysis

3.2 Steam explosion equipment

The steam explosion experiments were performed in lab scale equipment consisting of a modified steel autoclave with a volume of 1.2 liters. The lid of the autoclave has an inlet for the steam and a device for temperature measurement which can be attached to the lid. The release of the pressure can be done by opening the vent, which is located in the middle of the lid. The autoclave is then placed in the insulation. The purpose of the insulation is to better maintain the desired temperature.

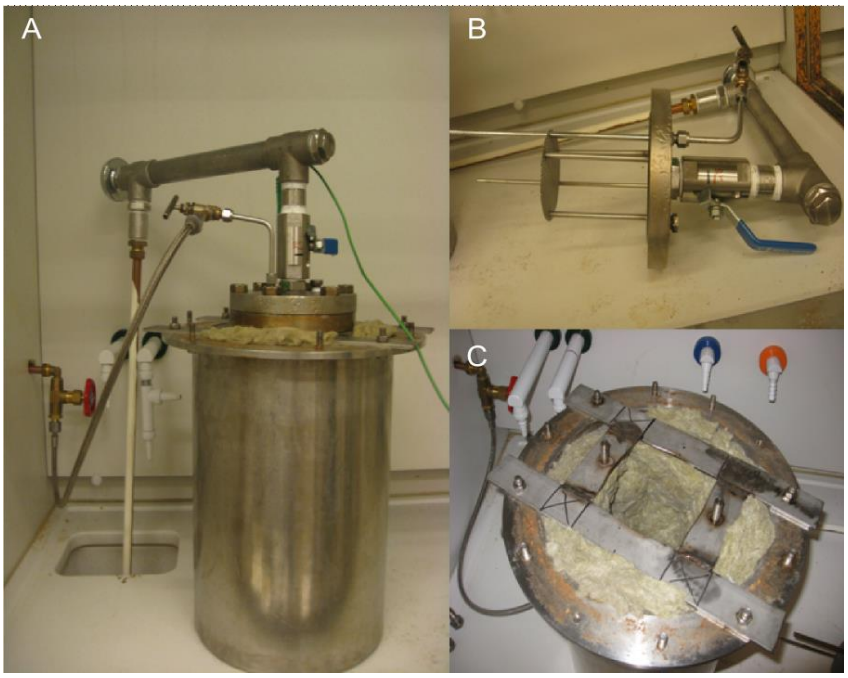

Figure 5: The lab scale steam explosion equipment. A): The autoclave with lid and the connected steam. B): The lid with the steam inlet and the large vent used for the pressure release. C): The autoclave put in an insulation to better preserve the desired temperature (Jedvert 2012).

3.3 Experimental conditions

A variation of experimental conditions on the wood chips was performed in order to study in detail the different mechanisms of the steam explosion pretreatment. For the mercury porosimetry a total of 14 samples were studied, in which there were 10 experimental and four reference (untreated) samples. Each of the samples has a duplicate so there were five unique experimental conditions performed (see Table 1). This was done due to the risk of experimental errors and for obtaining more accurate results. All experiments were performed with saturated steam at 7 bar which corresponds to around 165^{o}C in the steam explosion equipment. The treatment time of the experiments varied between 10 and 20 min for both birch and spruce samples. An additional experiment was done for the birch sample, which was treating the sample with only heat for 10 min. That means slow release of pressure which will prevent the explosion, in contrast to rapid release of pressure for obtaining explosion.

Table 1: Steam explosion conditions of different samples for the mercury porosimetry.

Experiment	Pressure (bar)	Type	Time (min)	Heat	Explosion
SE-1	7	Birch	10	Yes	Yes
SE-2	7	Birch	20	Yes	Yes
SE-3	7	Birch	10	Yes	No
SE-4	7	Spruce	10	Yes	Yes
SE-5	7	Spruce	20	Yes	Yes

For the sugar and lignin analysis a total of eight samples were studied, in which there were four experimental and four reference samples. In the sugar and lignin analysis case as well, each of the samples has a duplicate so there were two unique experimental conditions performed as shown in Table 2. The samples for sugar and lignin analysis are, like mentioned before, a little larger than the samples for mercury porosimetry.

Table 2: Steam explosion conditions of different samples for the sugar and lignin analysis.

Experiment	Pressure (bar)	Type	Time (min)	Heat	Explosion
SE-6	7	Birch	10	Yes	Yes
SE-7	7	Spruce	10	Yes	Yes

3.4 Sugar and lignin analysis

3.4.1 Procedure

Before the samples can be used in the sugar analysis equipment called high-pressure liquid chromatography (HPLC), they must undergo a certain procedure. The samples used for the sugar and lignin analysis are first oven dried for two hours before they are grind down in a grinder machine. 200 mg of the dried wood chips are grind down and put in 150 ml beakers. Thereafter 3 ml of H_2SO_4 are added and mixed carefully with a glass rod. After they are vacuumed in 15 min, the beakers are put in a 30^{o}C water bath for 60 min. The beakers are then diluted with 84 g de-ionized water and autoclaved at 125^{o}C during 60 min. After the glass filters are weighed, the lignin is filtrated into 100 ml laboratory flasks. Then 5 ml of the hydrolysate are put in 50 ml laboratory flasks. Thereafter 2 ml of 200 mg/l standard fucose solution are added and diluted to 50 ml. This solution are going to be used for the UV spectroscopy, where the absorbance values are obtained for the lignin calculations, and for the subsequently HPLC technique.

3.4.2 Equipment

The equipment that was used for the sugar analysis of the wood chips is called Dionex ICS-5000 HPLC system, equipped with CarboPac PA1 columns (see figure 6). Regarding the detection, an Electrochemical Detector was used. The considered software was Chromeleon 7, Chromatography Data System, version 7.1.0.898, with a fucose concentration of 8 mg/l.

Figure 6: Dionex ICS-5000 HPLC system, equipped with CarboPac PA1 columns.

3.5 Mercury porosimetry

3.5.1 Theory

Mercury porosimetry is a useful technique for characterization of structural changes in wood. The technique provides a wide range of information such as pore volume, porosity, pore size distribution and density (Moura et al. 2002). The pores that can be examined have a size between about 500 µm and 3.4 nm. The analysis time of a complete mercury porosimetry procedure may take as little as half an hour (Giesche 2006) but the mercury porosimetry equipment that are used in this thesis have an total analysis time of around 4 hours. The most relevant limitation with this technique is that the largest entrance towards the pore is measured instead of the actual inner size of a pore. The network of pores such as cross-linking structure can be achieved through different software methods and interpretation. However, a lot of assumptions are made in that process which will make the final results rather arbitrary (Giesche 2006). Some structures like closed pores are impossible to measure by mercury porosimetry because the mercury cannot enter the pore (Giesche 2006).

The principle of this technique is based on that the surface tension of mercury is very high for most materials and therefore there is no pore penetration through capillary action (Moura et al. 2002). The penetration can only be obtained if force is applied. The main assumption in mercury porosimetry is the pore shape, which is assumed to be perfect cylinders (Giesche 2006). According to these assumptions the smaller pores will be filled with increased pressure. The diameter of a pore is inversely proportional to the applied pressure and can be calculated by the Washburn equation (Washburn 1921):

$$D = -\frac{4\gamma \cos\theta}{P} \tag{2}$$

where D is the pore diameter, γ is the surface tension, θ is the contact angle and P is the pressure. For the present calculations a contact angle of 130^{o} and a surface tension of 0.485 N m^{-1} were used since mercury has similar contact angle values for many different substances (Pfriem et al. 2009; Ritter and Drake 1945). The average pore diameter can be calculated as (Micromeritics 2008):

$$D_{avg} = \frac{4V}{A} \tag{3}$$

where V is the total intrusion volume and A is the total pore area calculated using the assumption of cylindrical pores. The difference between the real shape of the pores and the assumed cylindrical pores must be taken into consideration when interpreting the obtained results. Figure 6 illustrates the structural difference and the pressure dependence of mercury in pores that are assumed cylindrical and in pores of real shape. When the pressure is zero there is no mercury that penetrates the wood material but with increased pressure there will be increased penetration of the wood cells (Muzamal 2014).

The mercury penetration will even occur at low pressure but the mercury can only access the wood cells at the core through the pores in the cell walls. When the pressure is increased sufficiently the mercury will penetrate through the pores into the inner cells and eventually fill the volume. Therefore, the size of the pore diameters will give large intrusion volume peaks which originate in the lumen volumes (Muzamal 2014).

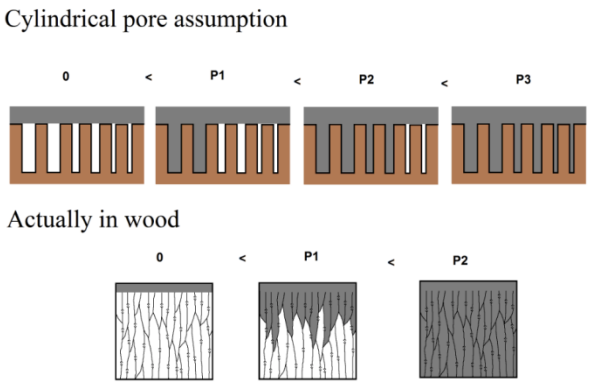

Figure 7: Mercury porosimetry analysis of assumed cylindrical pores and real shape pores (Muzamal 2014).

3.5.2 Equipment

The mercury porosimetry equipment used for this thesis is called AutoPore IV 9500. It is a 228 MPa mercury porosimetry that covers the pore diameter range from 360 to 0.003µm. It has two low-pressure ports and one high-pressure chamber. All aspects of low-pressure and high-pressure analysis, data collection, reduction and display are processed by the computer (Micromeritics 2008).

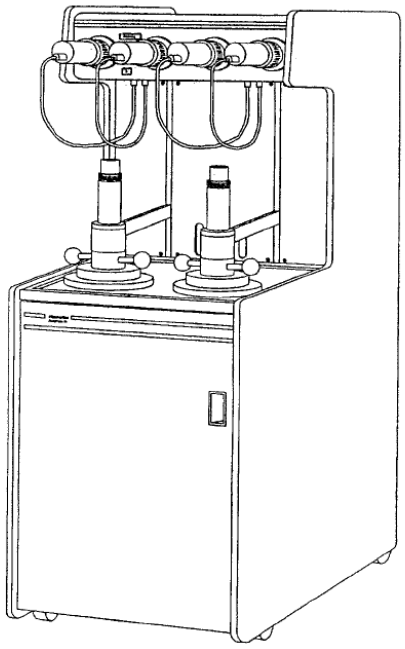

Figure 8: The mercury porosimetry equipment (Micromeritics 2008).

3.5.3 Procedure

The samples that are used for the mercury porosimetry are first dried in a freeze dryer for a week. The drying process prior to analysis is important especially for porous material such as wood, which are almost impossible to evacuate without fluidization unless they are dried first (Micromeritics 2008). Dry samples will provide better productivity and reduction in instrument maintenance, as well as improved data quality. After the samples are dried, they are weighed and put in a mercury penetrometer, which is an electrical capacitance dilatometer (see figure 6). The penetrometer is sealed by applying a light coating of vacuum grease to the lip of the bulb. The grease is used in order to fill the unavoidable roughness of the ground glass lip and polished surface of the cap

(Micromeritics 2008). Thereafter the seal is applied on the bulb opening and is tightened up. Before inserting it into the low pressure port, silicone high vacuum grease is applied on the outside of the stem as well as a spacer. The low pressure analysis takes around two hours before the penetrometer is inserted in the high pressure chamber. After the penetrometer in the high pressure chamber is locked and ready, the analysis will run for one and a half hour. It is finished when a window of the summary report comes up on the computer screen.

Figure 9: A penetrometer constructed of an insulated glass and a metal stem (Micromeritics 2008).

4 Results

4.1 Mercury porosimetry results

4.1.1 Summary

The results from the mercury porosimetry on the different wood chips are summarized in Table 3. The table shows the obtained average pore diameters and total intrusion volumes for the steam exploded wood chips as well as the reference samples. The result values are the average of two samples except for one of the birch references, which was lost due to absence of nitrogen supply in the mercury porosimetry equipment. The conditions of the different experiments are given in Table 1.

Table 3: Average pore diameter and total intrusion volume for different steam explosion experiments.

Experiment	Average pore diameter (nm)	Total intrusion volume (mL/g)
Reference birch	266.09	1.0988
SE-1	302.25	1.1102
SE-2	343.55	1.2353
SE-3	317.80	1.1080
Reference spruce	278.25	1.5727
SE-4	547.60	1.5917
SE-5	436.15	1.6404

The values shows that the average pore diameter for the birch samples (SE-1 – SE-3) are affected by the steam explosion since all the pore diameters have increased compared to the birch reference. The largest average pore diameter was obtained for SE-2 which has a treatment time of 20 min. The results for the spruce samples indicates that the effect of steam explosion is more on spruce as compared to birch, since the average pore diameters for the spruce samples have increased significantly more than for birch. It also shows that the difference between the steam exploded spruce and the reference is larger compare to the difference of steam exploded birch and the reference. The conditions for the highest pore diameter for birch and spruce respectively differed since the highest pore diameter for birch was obtained at a higher treatment time (SE-2: 20 min) compared to the spruce sample (SE-4: 10 min). Regarding the experiment with only heat (SE-3), it shows less increase in pore diameter than experiment SE-2 but has increased more compared to experiment SE-1.

The total intrusion volumes show similar trends as the average pore diameters. Both spruce and birch were affected by steam explosion and showed increased intrusion volumes. However, there is a higher intrusion volume obtained for steam exploded spruce samples (SE-4; SE-5) compare to steam exploded birch samples (S.E-1; SE-2). The difference of intrusion volumes between treated samples and the reference showed different trends compare to the obtained difference of pore diameters. Both birch and spruce (SE-2 has the highest increase compare to reference) had similar values. Also, the highest intrusion volumes for birch (SE-2) and spruce (SE-5) were obtained at higher treatment time, which was not the case regarding the pore diameter for spruce (SE-4).

4.1.2 Experimental results of birch

The different experimental results for birch samples are presented here. Comparisons are made for different birch sample experiments and presented in logarithmic scale plots. The pore diameter in µm is plotted against the incremental intrusion volume in mL/g.

4.1.2.1 Results comparing untreated and treated samples

It is important to understand if the explosion step actually has any effect on the treated wood samples and that is why a comparison between untreated and treated samples is made.

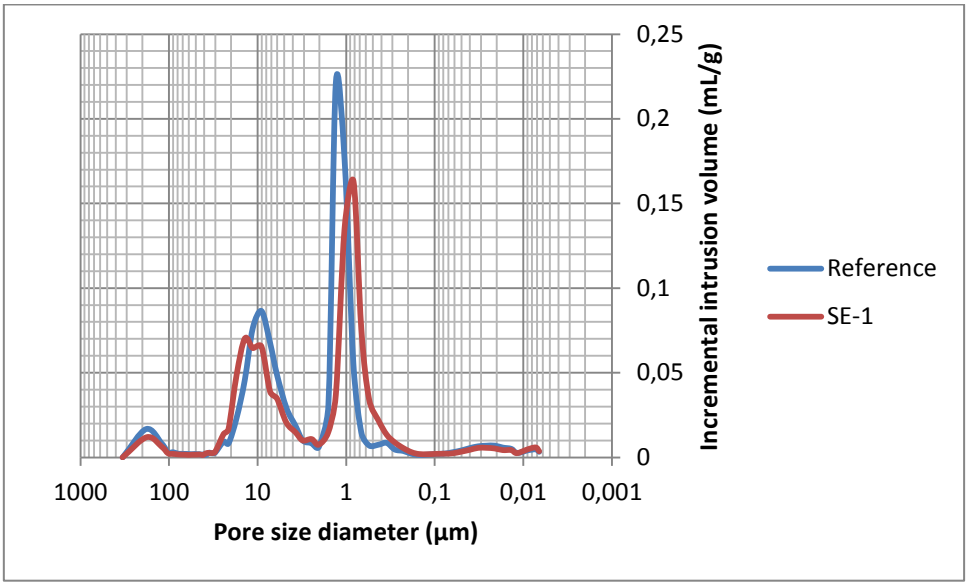

Figure 10: Incremental intrusion volume for reference and SE-1.

Figure 10 shows the incremental volume for different pore sizes in wood. It compares the reference (untreated) birch sample with experiment SE-1 (treatment time of 10 min) and it displays structural changes in wood after the steam explosion. There are two large peaks in Figure 10 for both samples. The peak of the reference is in the range of 1-2 µm which corresponds to cross-field (half bordered) pits. The peak of SE-1 is in the range of 0.8-1 µm and corresponds also closer to cross-field pits. The two lower peaks in Figure 10 are in the range of 10 µm and correspond potentially to ray cells. The diameter for ray cells in softwood is in the range of 2-50 µm and the ray cells of hardwood have similar size. The lowest peak in the plot is in the range of 110 µm which corresponds to vessels (vessels in hardwood have a diameter in the range of 30-130 µm).

4.1.2.2 Results comparing the effect of time

The following plot compares the treatment time between SE-1 and SE-2 which are 10 and 20 min respectively. In order to get more accurate results, the duplicates SE-1.2 and SE-2.2 (with the same experimental conditions as SE-1 and SE.2) are presented and compared as well. That is because specific samples that are cut from a wood stem can differ in physical structure depending on where on the wood stem the sample is cut.

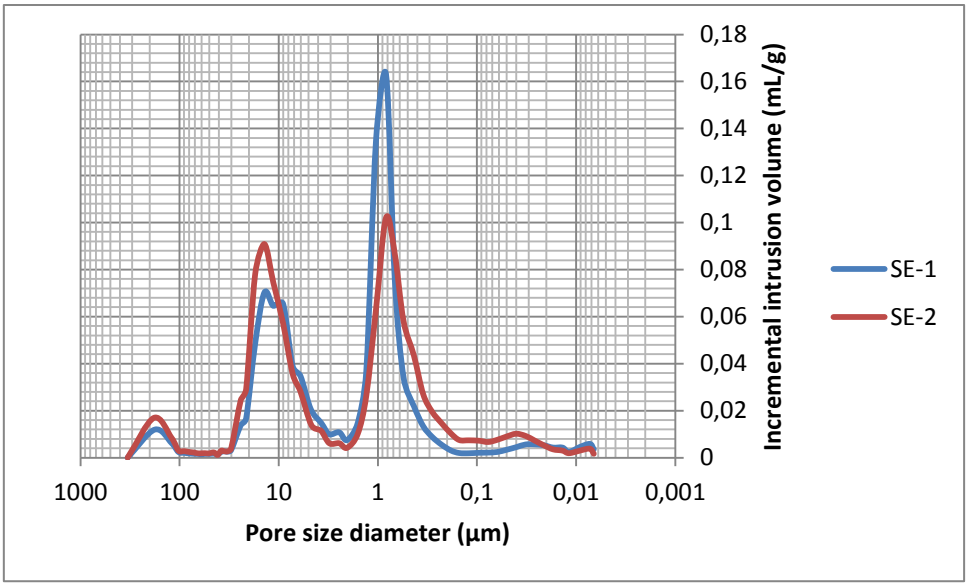

Figure 11: Incremental intrusion volume for experiment SE-1 and SE-2.

Figure 11 shows that most of the penetration of experiment SE-1 occurs through cross-field pits but for experiment SE-2 (treatment time of 20 min) is more even with slightly more penetration through cross-field pits. Another takeaway from the plot is that the penetration through ray cells is larger for SE-2 than SE-1 but the opposite is taking place for the penetration through cross-field pits.

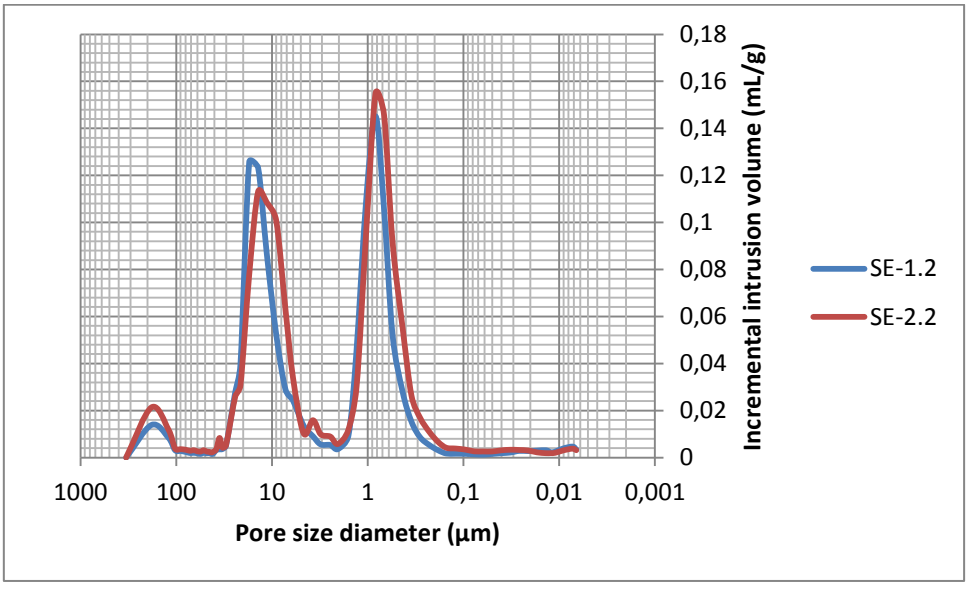

Figure 12: Incremental intrusion volume for experiment SE-1.2 and SE-2.2, which are the duplicates of SE-1 and SE-2.

Figure 12 shows little different trends compare to Figure 11. In this case both experiments have more similar trends where SE-2.2 has slightly more penetration through cross-field pits than SE-1.2, which is opposite in Figure 11. There is opposite trends for the ray cell penetration, in which there is little more penetration occurring for SE-1.2 than SE-2.2. Also, it appears that there is overall more penetration through ray cells compared to corresponding curves in Figure 11.

The common trends between Figure 11 and Figure 12 are that the most of the penetration for both experiments occurs through cross-field pits and that the influence of time is not affecting the

different birch samples significantly. Both diagrams show potential vessels in the range of 110 µm and the other curves show similar trends to each other except for SE-1, which has a lot more penetration taking place through cross-field pits than SE-2.

4.1.2.3 Results comparing the effect of steam treatment and explosion step

In this section, the samples treated with only heat (slow release of pressure to prevent explosion) are compared with the steam exploded samples. Both duplicates of the experiments are presented in the two diagrams.

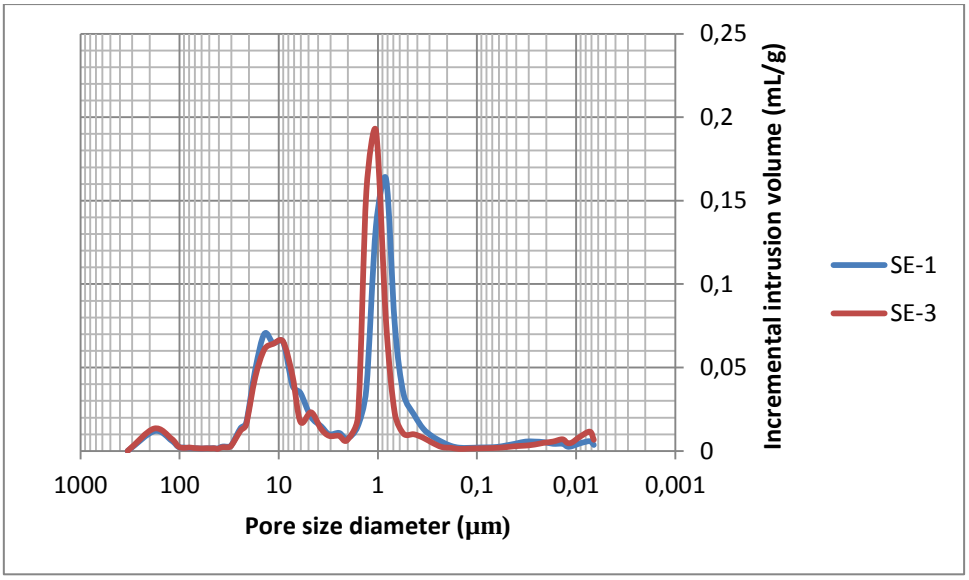

Figure 13: Incremental intrusion volume for experiment SE-1 and SE-3.

Figure 13 shows that there is more penetration through cross-field pits for the only heat (unexploded) experiment than the explosion experiment. Table 2 shows also that there are structural changes occurring in unexploded wood samples. There is an increase of total intrusion volume from 1.0988 mL/g to 1.1080 mL/g. The structural changes in unexploded wood samples might be due to degradation and removal of hemicellulose and lignin during steam (only heat) treatment (Donaldson et al. 1988). The highest peak for the only heat sample has larger pore size diameter than the explosion sample. Regarding the penetration through ray cells, both samples showed almost identical trends with a highest peak in a range of 10-11 µm.

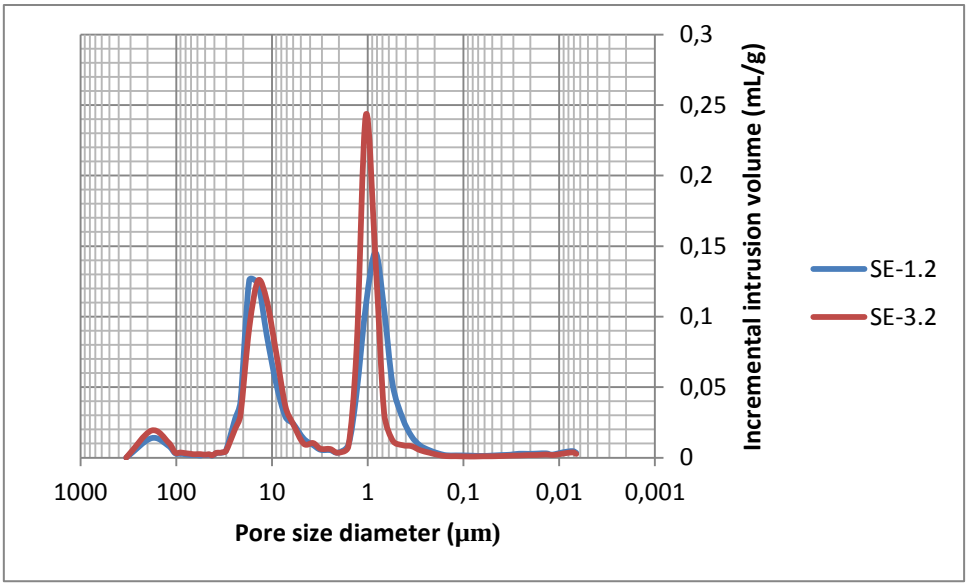

Figure 14: Incremental intrusion volume for experiment SE-1.2 and SE-3.2, which are the duplicates of SE-1 and SE-3.

Figure 14 shows very similar trends as Figure 13. The difference between the penetrations through pits for SE-1.2 and SE-3.2 is larger compared to the corresponding curves in Figure 13. Another difference is that all the peaks (except penetration through pits for SE-1.2) in Figure 14, which are in the range of 0,25 and 0,13 mL/g, are larger than the peaks in Figure 13, which are in the range of 0,2 and 0,07 mL/g. There are a lot more penetration through ray cells in Figure 14 compared to Figure 13.

Both of the diagrams (Fig 13 and Fig 14) show that there is more penetration through cross-field pits for the unexploded wood samples. However, it must be emphasized that there are structural differences between the wood pieces that are cut from the same original sample. Regarding the penetration through ray cells, it shows no difference between steam-treated and exploded samples since the curves were almost identical. Also there are curves that correspond to vessels for both of the diagrams.

4.1.3 Experimental results of spruce

In this section all the spruce samples (including the duplicate) are compared and presented. The difference from the birch experiments is that the steam (only heat) treatment was not performed for the spruce samples. The two diagrams show the reference sample and the two samples with 10 and 20 min treatment time.

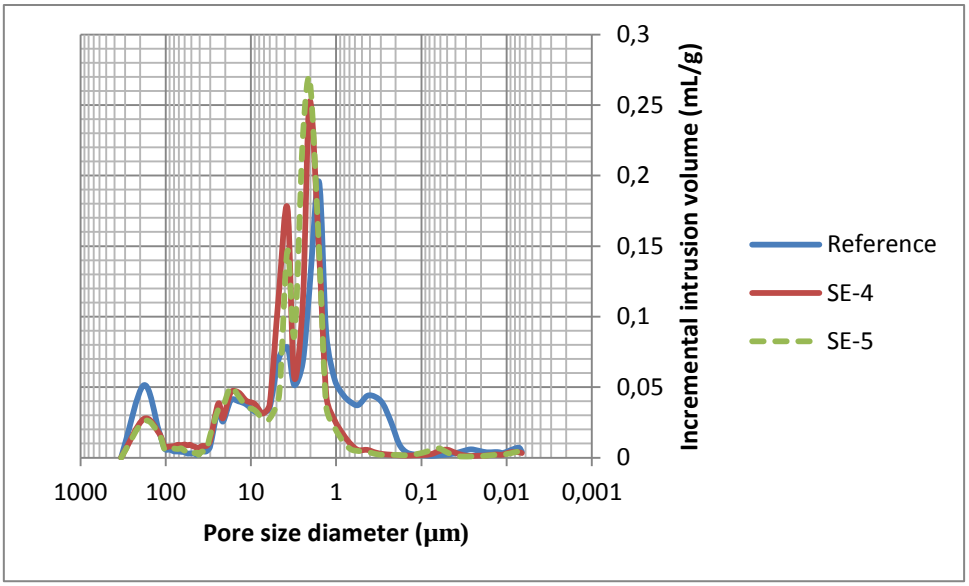

Figure 16: Incremental intrusion volume for reference, SE-4 and SE-5.

Figure 16 shows that most of the penetration occurs through pits. The highest peak for SE-4 and SE-5 is in the range of 1-2 µm, which corresponds to cross-field pits. The second highest peak for SE-4 and SE-5 is in the range of 3-4 µm, which corresponds to bordered pits. There has been clear structural changes after steam explosion treatment since the plots of treated samples show significant variation from the untreated sample.

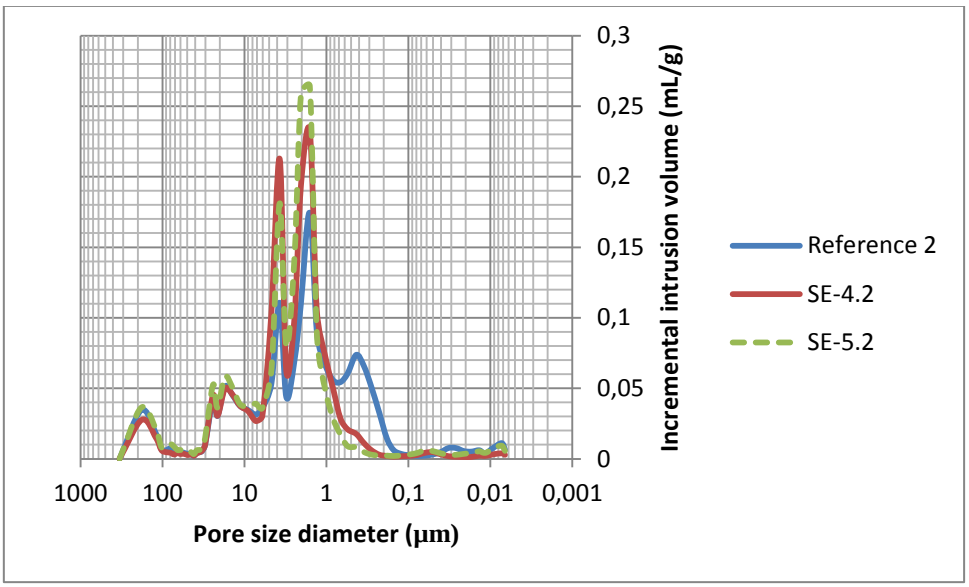

Figure 17: Incremental intrusion volume for reference 2, SE-4.2 and SE-5.2, which are the duplicates of reference SE-4 and SE-5.

Figure 17 shows almost identical trends of the curves as Figure 18. There are some differences in the second highest peaks for SE-4.2 and SE-5.2. They are a little bit higher than the corresponding peaks in Figure 16. However, the main takeaway from these two spruce graphs is that the most of the penetration into wood is taking place through pits in the cell walls. The spruce plots show a more uniform penetration pattern compared to the birch plots which have a more divided penetration pattern.

4.2 Results of sugar and lignin analysis

4.2.1 Summary

The results from the sugar and lignin analysis are summarized in Table 4. The Klason lignin results are defined as the residual material after the samples have been subjected to hydrolysis treatment with 72% H_2SO_4 (the formula to obtain the Klason lignin can be found in the appendix). The acid soluble lignin (ASL) is calculated by the following expression of Beer's law (Dence 1992):

$$\text{ASL (g/l)} = \frac{\text{Absorbance (A)}}{\text{b (light path in cm) x a (absorptivity in 1 } g^{-1}cm^{-1})} \quad (4)$$

, where the absorbance values are obtained from the UC spectroscopy and b = 1 and a = 110.

The cellulose, glucomannan and xylan values are calculated by converting the values obtained from the HPLC (the conversion formulas can be found in the appendix). All the result values in Table 4 are average of two samples except for cellulose. One of the samples showed increased amount of cellulose after explosion which is not probable and that is why it is not included. The conditions of the two experiments are given in Table 2 (SE-6: birch & 10 min; SE-7: spruce & 10 min). Other components that could be present are extractives such as terpenes, terpenoids, fats and fatty acids which are present in parenchyma cells.

Table 4: Klason lignin results, acid soluble lignin (ASL) results from the UV spectroscopy and converted results from the HPLC.

Experiment	Yield [100%]	Klason lignin	ASL	Cellulose	Glucomannan	Xylan	Other	Detection
Ref. birch	100	22.10	4.22	35.97	2.86	20.34	14.50	0.85
SE-6	100	22.53	3.98	33.97	2.71	19.30	17.51	0.82
Ref. spruce	100	27.10	0.75	37.06	15.64	5.24	14.21	0.86
SE-7	100	27.99	0.64	36.76	15.38	4.66	14.57	0.85

4.2.2 Degradation results of xylan

Figure 18 shows the different degradation values of xylan between the birch and spruce samples. Regarding birch, the percentage amount of xylan that degraded between the reference and treated wood sample was 5.11% as shown in Figure 18. That value is obtained by taking the difference between the reference (20.34%) and the birch sample (19.30%) and divides it by the reference (20.34%). For the spruce, a degradation of 11.07% occurred between the reference and the treated wood sample ((5.24% - 4.66%)/5.24%*100). A higher degradation of xylan occurred for the spruce compared to birch.

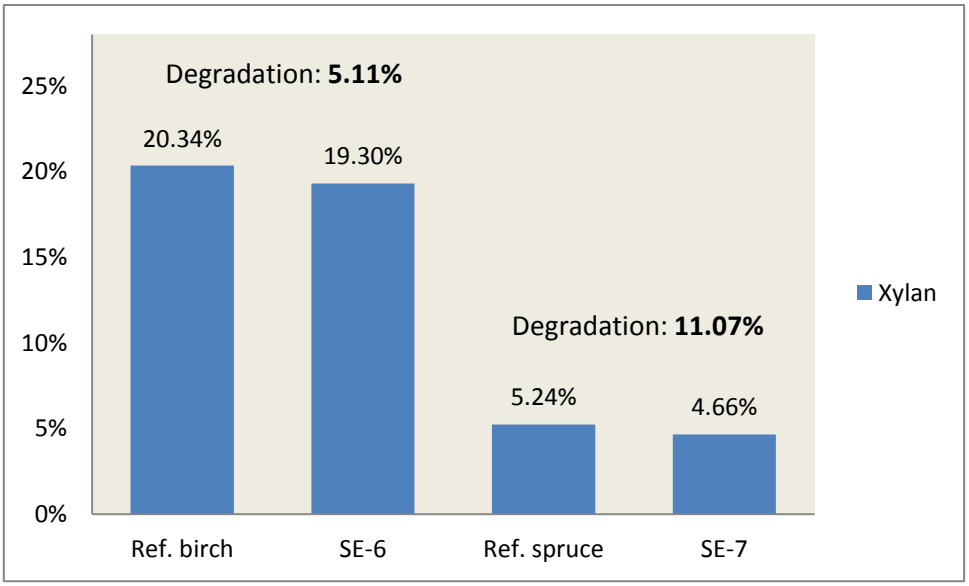

Figure 18: Degradation values of xylan for birch and spruce samples.

4.2.3 Degradation results of glucomannan

Figure 19 shows that results of glucomannan degradation between birch and spruce samples. The amount of decreased glucomannan between the reference of birch and the treated birch sample was 5.24%. For the spruce, the decreased amount of glucomannan between the reference and the treated spruce samples was 1.66%. The degradation of glucomannan for birch was higher than for spruce.

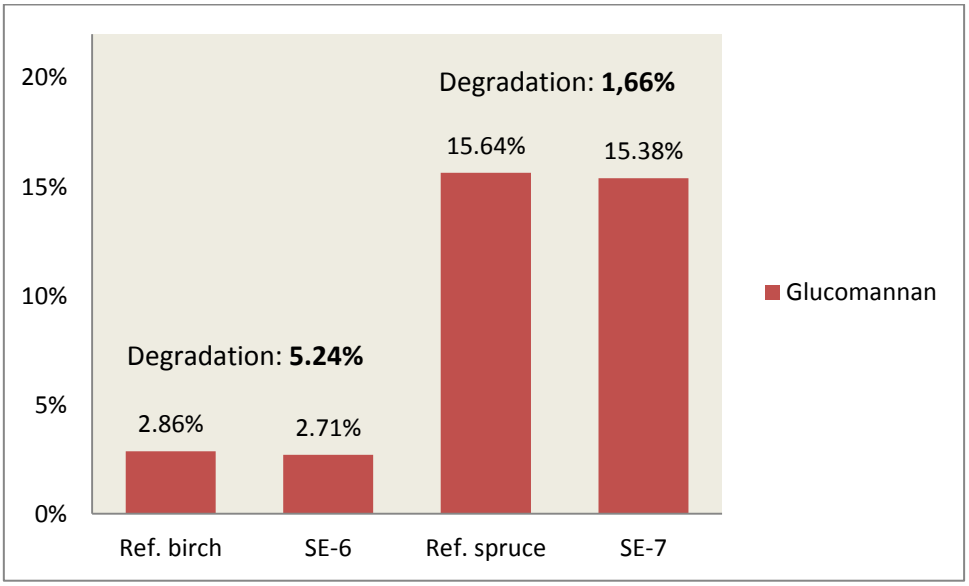

Figure 19: Degradation values of glucomannan for birch and spruce samples.

4.2.4 Degradation results of cellulose

The degradation of cellulose between birch and spruce samples are shown in Figure 20. The cellulose degradation of birch was 5.56%. For the spruce sample, the cellulose decreased with 0.81% between the reference and treated sample. A relatively higher degradation value occurred for the birch experiments compared to the spruce experiments.

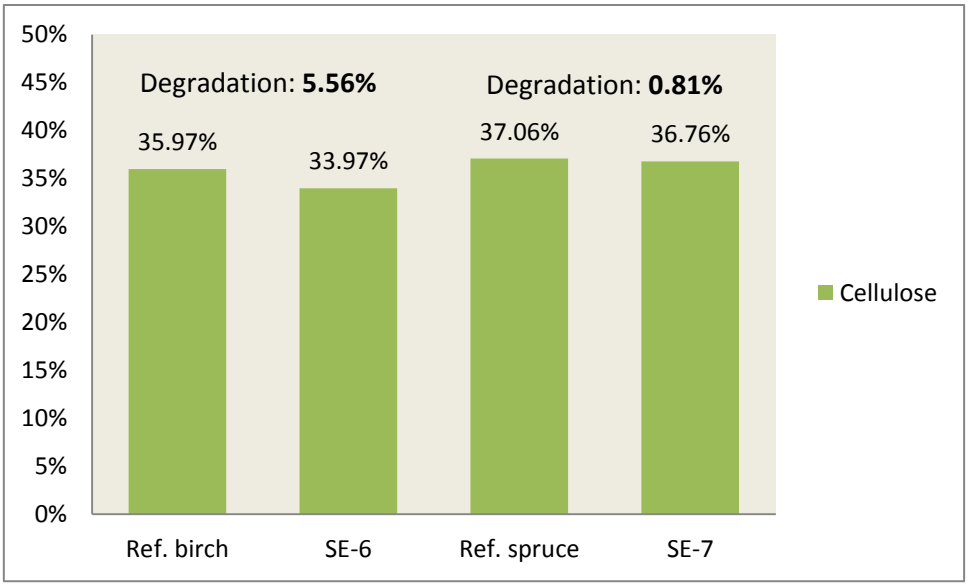

Figure 20: Degradation values of cellulose for birch and spruce samples.

4.2.5 Comparison of total lignin content

The total lignin content can be calculated as following: Total lignin content = Klason lignin + acid soluble lignin. The results of the total lignin content for the different experiments are presented and compared in Figure 21.

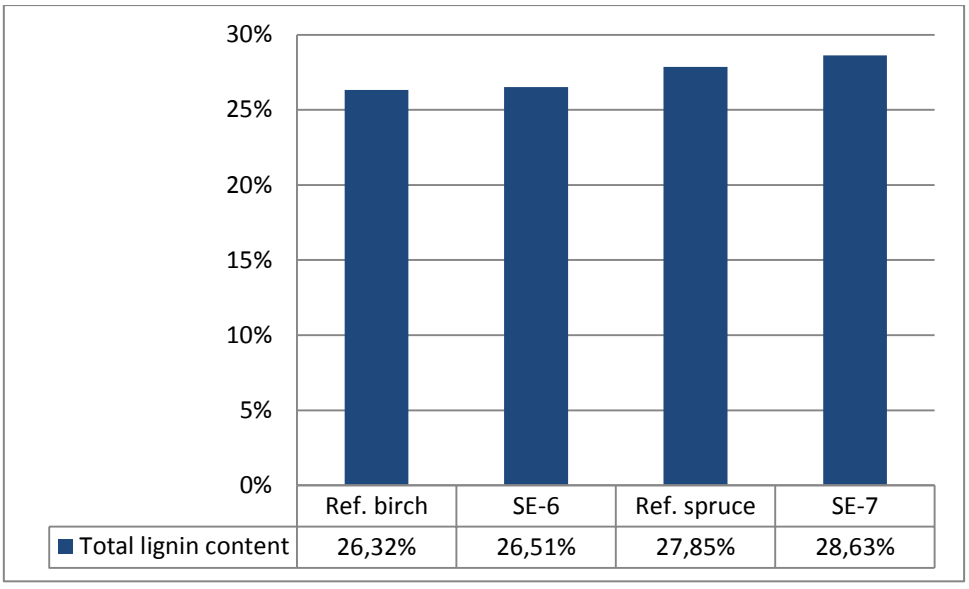

Figure 21: Comparison of the total lignin content between birch and spruce samples.

Figure 21 shows that there is an increase of total lignin content for both the birch and spruce samples. The birch experiments have a lignin increase of 0.72% ((26.51% - 26.32%)/26.32%*100), while the spruce experiments have a cellulose increase of 2.80%. The increase of total lignin content is slightly higher for the spruce experiments.

5 Discussion

5.1 Mercury porosimetry results

According to the results in Table 3, both the average pore diameters and total intrusion volumes showed an indication that the steam explosion is more effective on spruce. An increase in pore size and intrusion volume will increase the accessibility of chemical reagents and enzymes. However, it must be taken into consideration that the total intrusion volume of spruce reference was much larger than the total intrusion volume of all the birch samples. The intrusion volume of spruce reference was high without steam explosion treatment which tells more about the material rather than the steam explosion process itself. The results of spruce show that most of the penetration occurs through bordered and cross-field pits in the cell wall. While for the birch, the penetration distribution was more complex with high peaks in the range of 10 µm which potentially corresponds to ray cells.

Plant cells (lumina) can also have a width (diameter) in the range of 10 µm but the length is much longer than ray cells. That is because ray cells in hardwood only consist of ray parenchyma which is horizontal and radially arranged. That is why the lower peaks in the birch diagrams (Fig 10- Fig 14) probably correspond to ray cells. If the penetration is taking place through plant cells the peaks would be a lot higher, i.e. the intrusion volume would be a lot higher. A potential reason for the higher total intrusion volumes obtained in spruce is because there are more penetration through bordered pits and cross-field pits. That is because bordered pits and cross-fields pits are more common in softwoods, while simple pits are more common in hardwoods (Nelson 2001). Another explanation for the higher intrusion volumes obtained in spruce is the structure of the cell walls. Hardwood species have normally higher densities and thicker walls than softwood species (Asif 2009). This will make penetration harder and make it more difficult for the steam explosion treatment to disrupt the thicker walls in the hardwood birch compare to the softwood spruce.

Another potential reason why lower intrusion volumes are obtained in birch is because of the structure of hardwoods. The internal structure and liquid permeability of hardwoods is more complex and more variable compared to the softwoods, which can somewhat complicate the steam explosion process (Nelson 2001). It must be taken into consideration that ray parenchyma in hardwoods has a form and structure that tends to be short with isodiametric cells (Daniel 2004). A significant amount of penetration is taking place through ray parenchyma in birch which results in low intrusion volumes because of their short structural size. The reason that penetration through ray cells takes place in birch but not spruce could be due to the presence of ray tracheids in the softwood. Softwood rays are composed of a combination of ray parenchyma and ray tracheids which are dead cells.

Regarding the explosion experiments, all of the penetration through cross-field pits in birch has reached lower peaks than spruce. A potential reason for that is the structure of the pit membrane in which the penetration is taking place. The openings in the pit membrane are about 10 times smaller in hardwoods than in softwoods (Nelson 2001). If the openings are larger there will be more penetration occurring and it will become easier for the steam explosion treatment to open up the larger openings than the smaller ones. For the samples with treatment time of 10 min (SE-1 and SE-4), the highest increase in total intrusion volume regarding the reference was for spruce but for a

treatment time of 20 min (SE-2 and SE-5) the highest was for birch. That indicates that a higher treatment time may affect the birch experiments more than the spruce experiments.

5.2 Results of sugar and lignin analysis

Regarding the degradation of the hemicelluloses i.e. xylan and glucomannan, there were considerable degradation for both of the birch and spruce samples. There were more degradation of xylan for spruce (Figure 18) and more degradation of glucomannan for birch (Figure 19). Since there are opposite trends for birch and spruce it is difficult to draw any accurate conclusions other than that hemicelluloses degraded considerable for both samples. However, the degradation of glucomannan for spruce showed relatively low values (1.66% in Figure 19). The results from the cellulose degradation showed that there is significantly higher cellulose degradation for birch (difference of 4.75% in figure 20, i.e. 5.56%-0.81%) than for spruce. A possible explanation for that could be the structure of the cellulose fibers. The cellulose fibers in hardwood are shorter than the fibers in softwood (Wahren 1983). This could make the degradation process of cellulose easier for hardwood with shorter fibers.

Regarding the total lignin content, there has been an increase of lignin for both of the birch and spruce as shown in Figure 21. The acid soluble lignin has decreased for both samples but it is the Klason lignin that has increased. One of the reasons for the lignin increase according to Miranda et al. (1978) is due to the hemicellulose degradation product, furfural and polymerization of lignin. During autohydrolysis, reactive hemicellulose degradation products, such as furfural and its predecessors are able to react with the lignin and thereby be accountable for increase in lignin content. Miranda et al. (1978) suggest that two types of reactions occur during autohydrolysis, which are depolymerization and repolymerization. The depolymerization is the faster reaction between the two and is responsible for the solubility of the lignin in the solvent. When the heating is increasing the repolymerization (condensation) reaction is taking over, which results in increasing amounts of insoluble residual lignin (Miranda et al. 1978).

The literature review instigated the optimal sugar yield for three softwood species (Japanese cedar, pine and spruce) and four hardwood species (aspen chopsticks, salix, poplar and birch). Most of the results showed that the hardwoods needed lower optimal steam conditions compared to softwoods, except for birch species. Birch had an optimal steam condition at the same temperature as the spruce. It must be taken into consideration that the structures can vary significantly between the hardwood species. Also, the optimal conditions of sugar yields are compared after enzymatic hydrolysis (saccharaifcatoin) is carried out, which is not the case for the sugar analysis experiments. That is why it can be difficult to fully compare the literature review and the experimental results since the conditions are significantly different.

6 Conclusions

In this thesis it was shown that the obtained average pore size diameter and the total intrusion volume for spruce were larger compared to birch. This indicates that steam explosion is more effective on spruce due to the increased accessibility of chemical reagents and enzymes. A possible explanation is that the cell walls in birch are thicker than the cell walls in spruce and that there is more penetration taking place through bordered and cross-field pits in spruce compared to birch. The openings in the pit membranes are about 10 times bigger for softwood than for hardwood, which may provide more effective steam explosion treatment for spruce. Also the internal structure of hardwood is more complex which can somewhat reduce the effectiveness of steam explosion.

The sugar analysis results for the hemicelluloses xylan and glucomannan showed that considerable degradation occurred. For cellulose there was significantly more degradation for birch compare to spruce. The total lignin content increased relatively for both wood species which was potentially due to lignin polymerization and furfural, which can react with lignin during autohydrolysis. The overall conclusion from the sugar analysis and lignin results is that both birch and spruce showed rather similar results with slightly more favorable outcomes for spruce regarding steam explosion effectiveness. The literature review showed that the optimal sugar yield for birch and spruce occurred at the same steam conditions.

7 Future work

For the future work it is recommended to perform the steam explosion pretreatment on the hardwood birch at higher steam conditions. The experiments in this thesis were only performed in mild steam explosion at 7 bar which corresponds to around 165^{o}C. If the experiments were performed in larger steam explosion equipment with steam conditions at 14 bar which corresponds to 195^{o}C, the results would probably be more distinctive. The wood samples would potentially be more affected by higher steam conditions and it would be easier to draw conclusion whether if birch or spruce is more effective for steam explosion treatment.

Another relevant aspect to investigate is the effect of the impact step in steam explosion treatment (the impact of wood chips mixed with other chips and vessel walls), which was initially planned to be performed for this thesis. According to a previous study of steam explosion by Muzamal (2014), the impact step did the most damage to the wood material compare to the explosion step. The wood samples were disintegrated into small pieces because of the impact of highly softened wood chips mixed with other chips and the equipment walls. The explosion step did not have the same effect on the wood material, neither for the experiments performed in this thesis or the study performed by Muzamal (2014).

An analysis tools that can be used for investigating the effectiveness of steam explosion treatment on woods is the scanning electron microscope (SEM). The SEM is capable of obtaining 3D-like images of the surfaces of a large variety of materials. It is one of the most adaptable instruments for the examination and analysis of the microstructural characteristic of solid objects. One of the main reasons for the usefulness of SEM is the high resolution which can be obtained after examination of bulk objects (Goldstein et al. 2003).

8 References

Alvira, P., Tomás-Pejó, E. and Ballesteros, M. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour. Technol.* 101 (13): 4851-4661.

Asada, C., Kita, A., Sasaki, C. and Nakamura, Y. 2011. Ethanol production from disposable aspen chopsticks using delignification pretreatments. *Carbohyd. Polym*. 85 (1): 196-200.

Asada, C., Sasaki, C., Yoshihiro, U., Sakafuji, J. and Nakamura, Y. 2012. Effect of steam explosion pretreatment with ultra-high temperature and pressure on effective utilization of softwood biomass. *Biochem. Eng. J.* 60: 25-29.

Asif, M. 2009. Sustainably of timber, wood and bamboo in construction. *Sust. Cons. Ma*. 2 (1): 31-54.

Avellar, B.K. and Glasser, W.G. 1998. Steam-assisted biomass fractionation. I. Process considerations and economic evaluation. *Biomass Bioeng*. 12 (3): 205-218.

Carvalheiro, F., Duarte, L.C. and Gírio, F.M. 2008. Hemicellulose biorefineries: a review on biomass pretreatments. *J. Sci. Ind. Res*. 67 (11): 849-864

Chandra, R.P., Bura, R., Mabee, W.E., Berlin, A., Pan, X. ans Saddler, J.N. 2007. Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulisocs? *Adv. Bioche. Eng/Biotechnol*. 108: 67-93.

Clark, T.A. and Mackie, K.L. 1987. Steam Explosion of the Softwood *Pinus Radiata* with Sulphur Dioxide Addition. I. Process Optimisation. *J. W. Chem. Technol*. 7 (3): 373-403.

Daniel, G. 2004. Wood and fibre morphology. In The Ljungberg Textbook (eds.) Wood Chemistry. 1-24. Stockholm: KTH.

Demirbas, A. 2010. *Biorefineries - For Biomass Upgrading Facilities*. London. Springer-Verlag London Ltd.

Dence, C.W. 1992. The Determination of Lignin. Springer-Verlag Berlin Heidelberg.

Donaldson, L. A., Wong, K. K. Y. and Mackie, K. L. 1988. Ultrastructure of steam-exploded wood. *Wood Sci. Technol*. 22 (2): 103-114.

FitzPatrick, M., Champage, P., Cunningham, M.F. and Whitney, R.A. 2010. A biorefinery processing perspective: Treatments of lignocellulosic materials for the production of value-added products. *Bioresour. Technol.* 101 (23): 8915-8922.

Galbe, M. and Zacchi, G. 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Adv. Biochem, Eng/Biotechnol*. 108: 41-65.

Giesche, H. 2006. Mercury Porosimetry: a General (Practucal) Overview. *Part. Part. Syst. Charact.* 23 (1): 1-11

Gírio, F.M., Fonesca, C., Carvalheiro, F., Duarte, L.C., Marques, S. and Bogel-Lukasik, R. 2010. Hemicelluloses for fuel ethanol: A review. *Bioresour. Techno.* 101 (13): 4775-4800.

Goldstein, J.I., Newbury, D.E., Echlin, P., Joy, D.C., Lyman, C.E., Lifshin, E., Sawyer, L and Michael, J.R. 2003. *Scanning Electron Microscopy and X-ray Microanalysis*. 3rd ed. Springer Science + Business Media New York.

Janzon, R., Schutt, F., Oldenburg, S., Elmar, F., Körner, I. and Saake, B. 2014. Steam pretreatment of spruce forest residues: Optimal conditions for biogas production and enzymatic hydrolysis. *Carbohyd. Polym.* 100: 200.210.

Jedvert, K. 2012. *Mild Stem Explosion of Spruce Wood*. Photograph. Viewed 5 May 2014.

Kumar, P., Barett, D.M., Delwiche, M. and Stroeve, P. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res*. 48 (8): 3713-3729.

Lennholm, H. and Blomqvist, K. 2004. Cellulose. In The Ljungberg Textbook (eds.) Wood Chemistry. 1-31. Stockholm: KTH.

Lens, F., Gasson, P., Smets, E. and Jansen, S. 2003. Comparative Wood Anatomy of Epacrids (Styphelioideae, Ericaceae s.l. *An. Bot*. 91 (7): 835-856.

Mansfield, S.D., Mooney, C. and Saddler, J.N. 1999. Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnol. Prog.* 15 (5): 804-816.

Mason, W.H. 1926. Process and apparatus for disintegration of wood and the like. US Patent: 1578609.

Mckdandy. 2006. *Hardwood.* Photograph. Viewed 28 March 2014. <http://en.wikipedia.org/wiki/File:Hard_Soft_Wood.jpg>

Merriam-Webster. 2006. *Wood*. Photograph. Viewed 23 March 2014. <http://www.merriam-webster.com/concise-images/72251.htm>

Micromeritics Intrsumental Corporation. 2008. *AutoPore IV 9500 – Operator's Manual V1.09*. Norcross, USA.

Miranda, G. S. C. and Wayman, M. 1978. Characterization of autohydrolysis aspen (*P. tremuloides*) lignins. Part 1. Composition and molecular wright distribution of extracted autohydrolysis lignin. *Can. Jour. Chem*. 57 (10): 1141-1149.

Mosier, N., Wyman, C.E., Dale, B.D., Elander, R.T., Lee, Y.Y., Holtzapple, M. and Ladisch C.M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* 96 (6): 673-686.

Mosier, N. 2005. *Features of promising technologies for pretreatment of lignocellulosic biomass.* Photograph. Viewed 5 April 2014. <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0960852404002536-gr1.gif>

Moura, M.J., Ferreira, P.J. and Figueiredo, M.M. 2002. The use of mercury intrusion porosimetry to the charaxterization of eucalyptus wood, pulp and paper. Iberoamerican Cingress in Pulp Pap. Res.

Muzamal, M. 2014. Steam Explosion of Wood. Chalmers University of Technology. Licentiate thesis. 2013:3. ISSN: 1652-943X.

Nelson, R. M Jr. 2001. Water Relations of Forest Fuels. *Forest Fires: Behavior and ecological effects*. 79-149.

Norberg, I. 2012. Carbon fibres from Kraft lignin. KTH Royal Institute of Technology. Doctoral thesis. 2012:13. ISSN: 1654-1081.

Olivia, J.M., Sáez, F., Ballesteros, I., Gónzalez, A., Negro, M.J., Manzanares, P. and Ballesteros, M. 2003. Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast *Kluyverinyces narxianus*. *Appl. Microbiol. Biotechnol*. 105 (1-3): 141-153.

Overend, R.P. and Chornet, E. 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. *Phil. Trans. R. Soc. Lond. A*. 321 (1521): 523-536.

Pan, X., Xie, D., Gilkes, N., Gregg, D.J. and Saddler, J.N. 2005. Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content. *Appl. Biochem. Biotechnol*. 124 (1): 1069-1079.

Pfriem, A., Zauer, M. and Wagenfuhr, A. 2009. Alteration of the pore structure of spruce (Picea abies (L.) Karst.) and maple (Acer pseudoplatanus L.) due to thermal treatment as determined by helium pycnometry and mercury intrusion porosimetry. *Holzforsch*. 63 (1): 94-96.

Puri, V.P. 1984. Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification. *Biotechnol. Bioeng*. 26 (10): 1219-1222.

Ritter, H. L. and Drake, C. 1945. Pore-size distribution in porous materials. *Ind. Eng. Chem*. 17(12): 782-786.

Saha, B.C., Iten, L.B., Cotta, M.A. and Wu, Y.V. 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Process Biochem.* 40 (12): 3693-3700.

Sánchez, Ó.J. and Cardona, C.A. 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour. Technol.* 99 (13): 5270-5295.

San Martín, R., Perez, C. and Briones, R. 1995. Simultaneous production of ethanol and kraft pulp from pine (*pinus radiate*) using steam explosion. *Bioresour. Technol*. 53 (3): 217-223.

Sassner, P., Galbe, M. and Zacchi, G. 2005. Steam Pretreatment of Salix with and without SO_2 Impregnation for Production of Bioethanol. *Appl. Biochem. Biotechnol*. 121-123: 1101-1117

Schutt, F., Puls, J. and Saake, B. 2011. Optimization of steam pretreatment conditions for enzymatic hydrolysis of poplar wood. *Holzforschung*. 65: 453-459.

Suhas, Carrott, P.J.M. and Carrott M.M.L. Ribeiro. 2006. Lignin-from natural adsorbent to activated carbon: A review. *Bioresour. Techn.* 98 (12): 2301-2312.

Sun, Y. and Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour. Technol.* 83 (1): 1-11.

Teleman, A. Hemicellulose and pectins. 2004. In The Ljungberg Textbook (eds.) Wood Chemistry. 1-20. Stockholm: KTH.

Tomás-Pejó, E., Olivia, J.M. and Ballesteros, M. Realistic approach for full-scale bioethanol production from lignocellulose: a review. *J. Sci. Ind. Res*. 67 (11): 874-884.

Vivekanand, V., Olsen, E.F., Eijsink, G.H. and Horn, S.J. 2013. Effect of different steam explosion conditions on methane potential and enzymatic saccharification of birch. *Bioresour. Technol*. 127: 343-349.

Wahren, D. 1983. Refining of hardwoods and softwoods. The institute of paper chemistry, Appleton Wisconsin.

Wyman, C.E. 1996. Handbook on Bioethanol: Production and Utilization. Taylor Francis, Washington. 417.

Zheng, Y., Pan, Z. and Zhang, R. 2009. Overview of biomass pretreatment for cellulosic ethanol production. *Int. J. Agric. Biol. Eng.*2 (3): 51-68.

9 Appendix

The results of xylan, glucomannan and cellulose were calculated after sugar analysis by using following assumptions and conversion formulas (Jedvert 2012):

- Anhydro sugars were calculated from sugar monomers by the removal of water, i.e. multiplication by 0,88 in the case of pentosans and by 0,9 in the of hexosans. Glucomannan was calculated as the sum of galactan, mannan and part of the glucan (see equations below).
- All galactan measured is included in galactoglucomannan except for the acetyl groups.
- Xylan was calculated as the sum of xylan and arabinan. All arabinan measured is included in the xylan.
- Cellulose was calculated as the content of glucan after removal of the glucan connected to the galactoglucomannans.

Galactoglucomannan = Galactose + (1+(1/3,5)) * Mannose

Xylan = Xylose + Arabinose

Cellulose = Glucose – (1/3,5)* Mannose

The results of the analysis were summarized into a mass balance with the assumptions that the sugars (carbohydrates) were divided into (galacto)glucomannan, xylan and cellulose.

The results of Klason lignin are obtained by calculating the difference between the weights of the filter (the lignin are stuck on the filter) before and after drying in an oven drier and thereafter divide that value by the weight of the initial grinded sample.