

Rabins Gaudel

# Pressurised Hot Water Extraction (PHWE) and Alkaline Extraction of Spruce

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| Instructor(s)  | Hannu Ilvesniemi, Professor at The Finnish Forest Research Institute<br>Teemu Tikkanen, Former Researcher at The Finnish Forest Research Institute<br>Esa Toukoniitty, Senior Lecturer at Metropolia UAS |
| <p>Lignocellulosic biomass is among the potential renewable feedstock for the production of energy and chemicals. The pathway to biofuel from biomass involves four core steps: pretreatment, hydrolysis, fermentation, and separation process to obtain highly concentrated bioethanol. The study is mainly focused on the pretreatment of the Norway spruce sawdust. Two pretreatment methods, namely Pressurised hot water extraction (PHWE) and alkaline extraction were applied separately and in combination, in order to fractionate lignocelluloses of the Norway spruce sawdust. NaOH (0.55 M) solution was used during alkaline extractions. The consequences of cooling inbetween the combined extractions in the composition of the residual wood were analysed in the analysis part. Cellulose and hemicellulose in the wood residual were analysed by acid hydrolysis and acid metanalysis, respectively. Both the Klason lignin and acid soluble lignin, in the wood residual were determined to find the total amount of lignin content in the sample. The extracts were analysed for brix and pH.</p> <p>Separate PHWEs (180 °C and 210 °C) and alkaline extractions (150 °C and 170 °C) were effective in removing hemicellulose and lignin, respectively. Cellulose recovery from both the PHWEs and the alkaline extractions was above 90%. Combined extractions (PHWE/alkaline) 180 °C /150 °C and 180 °C /170 °C were found more effective than combined extraction 210 °C /150 °C and 210 °C /170 °C in terms of cellulose yield. However, lignin contents in all of the combined extractions were higher than the expected. More than 60% of the cellulose was lost during combined extractions 210 °C /150 °C and 210 °C /170 °C. Surprisingly, lignin content in the wood residual from the combined extractions 210 °C /150 °C and 210 °C /170 °C was higher than the lignin content in the wood residual from combined extractions 180 °C /150 °C and 180 °C /170 °C. Cooling resulted in condensation of lignin into the wood fibre, resulting in high lignin content in the wood residual. In combined extraction, the temperature of PHWE affected the cellulose recovery and the delignification process. Higher temperature PHWE resulted in low cellulose yield and high lignin content in the extracted spruce sawdust.</p> |  |
| Keywords   | pressurised hot water extraction, alkaline extraction, pretreatment of spruce, combined extraction of spruce, bioethanol for spruce  |

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## Abbreviations

|             |  |
|-------------|--|
| 4-O-Me-GlcA | glucuronic acid                                |
| Ara         | arabinose                                      |
| DP          | degree of polymerisation                       |
| FID         | flame ionise detector                          |
| Gal         | galactose                                      |
| GalA        | galucuronicacids                               |
| GC          | gas chromatography                             |
| Glc         | glucose  |
| GlcA        | glucuronicacid                                 |
| HMDS        | hexamethyldisilazane                           |
| HMF         | hydromethyl furfural                           |
| Man         | mannose  |
| MeoH        | methanol                                       |
| mL          | mililiter                                      |
| PHW         | pressurised hot water                          |
| PHWE        | pressurised hot water extraction               |
| Rha         | rhamose  |
| SHF         | separate hydrolysis and fermentation           |
| SSF         | simultaneous saccharification and fermentation |
| TDS         | total dissolved solid                          |
| Tmcs        | trimethylchlorosilane                          |
| UV          | ultraviolet                                    |
| Xyl         | xylose   |

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## 1 Introduction

Lignocellulose biomass has been considered as a potential source of biofuel and high value chemicals [1]. With abundant source of lignocellulose biomass and a need to address global climatic and energy agendas, the interest on biofuel is ever growing. Today bioethanol is mainly produced from the sugar- or starch-based feedstock, like sugarcane and corn. The cost of such raw materials is almost 40-70% of the total production cost (Claassen et al., 1999 cited in [2]). In addition, the cultivation of such sugar-or starch-based feedstock as an energy crop is limited by the availability of agricultural land. In order to reduce the raw material cost of bioethanol production, the use of cheaper raw material is inevitable. Using lignocelluloses biomass would ensure, low raw material cost with abundant supply, throughout the world. However, using complex matrix of lignocelluloses-based raw material is not easy, but requires different physical, chemical and biological procedures to fractionate and ferment the plant biomass sugars. The challenge becomes to make these procedures cost-effective, efficient and sustainable. Basically, biofuel production from lignocelluloses biomass has three principle steps, pretreatment, hydrolysis-fermentation and a separation process [1]. Pretreatment step is a very important stage in bioethanol production as it plays an important role for the efficiency of hydrolysis and fermentation. Pretreatment is the bottleneck into the development of bioprocess from lignocelluloses biomass (Cheng et al., 2008; Alvarado et al., 2009; Zhang et al., 2009 are cited in [1]). In lignocellulose-based biorefineries, it is indispensable to break down the feedstock's structure to increase the availability of sugars from cellulose and hemicellulose for hydrolysis-fermentation process. [1]

Of the entire renewable energy sources, biomass is one of a kind raw material in the production of liquid fuels. Biofuel can be fed directly, or as a blend with other fuels, into combustion engine to generate power. The technology is not new as Ford's model T was designed to run either with gasoline or alcohol, back in 1908 (DiPardo, 2000 cited in [2]). Today there are many flexifuel automobiles which can run by a blend up to 85%, and in many countries, the ordinary gasoline contains some percentage of ethanol. In EU countries, the share of renewable resources in all gasoline and diesel transportation fuel rose to 5.75% by 2010 compared to 1.2% in 2005 [2].

Enormous attentiveness into biofuel sector is due to the fact it can subsidise the use of fossil fuels and ultimately reduce the greenhouse gas emissions. Considerable amount of greenhouse gas emissions come from the transportation sector. The combustion of the ethanol does not contribute to the net increase in carbon dioxide, as it becomes the part on natural closed carbon cycle [2]. As shown in Figure 1, plants capture atmospheric carbon dioxide during photosynthesis and later release it during combustion of bioethanol. In this way there is always natural carbon cycle, and there is no net change in carbon dioxide in the atmosphere in the long term. Another driving factor for bioethanol development is to create energy security and independence of petroleum products [2]. Fossil fuels are limited and could be finished any time in the future. Without prioritising renewable energy sectors, there is no way to eliminate energy crisis in the future. The demand of petroleum product is supposed to increase by 60% between the year 2000 and 2030 [2]. In this case, the right way would be to introduce considerable amount of renewable energy into the global energy market. Today biomass can be directly fed as fuel into the co-fired power plant, or valuable chemical can be extracted by fractionation methods to produce fuel or chemicals.

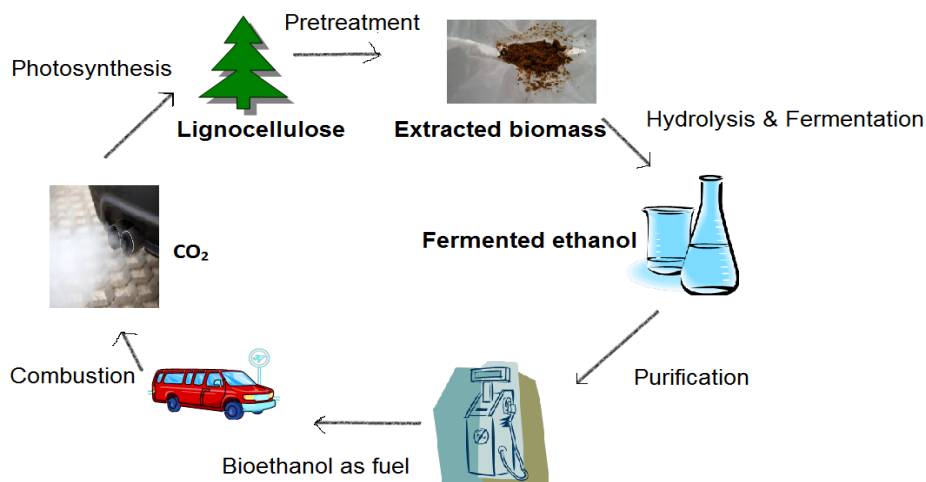


Figure 1. Carbon Cycle of biofuels

Sustainable production of biofuel has less impact in the environment, but assessment of impact with respect to wildlife and different field of business such as agriculture, forestry and food industry is necessary. There has always been a debate about the impact of 'biomass to energy' into food and agriculture industry. The debate can continue, but the solution is only one and that is the sustainable planning and implementation.

## 2 Aim of study

In biorefinery, pretreatment of the biomass is considered as a crucial stage in terms of energy and water consumption. In general, the goal of the pretreatment is to provide the best physical and chemical conditions, for example pH, temperature, moisture and pressure, which results in fractionation of complex lignocellulose matrix. This thesis project focuses on the high temperature PHWE and low temperature alkaline extraction of Norway spruce. During the experimental part of this thesis project, both the extraction methods were carried out separately and in combination. In the combined pretreatment methods, PHWE was followed by alkaline extraction. Furthermore, to understand the effects of cooling on fractionation of lignocellulose, combined extraction was performed with and without cooling between the PHWE and alkaline extraction. After extraction, all the samples were analysed for cellulose content, hemicellulose content, lignin content and ash content. Apart from the chemical analysis, ethanol projection based on the complete hydrolysis and heating power required for each extraction were calculated.

## 3 Literature Review

### 3.1 Lignocellulose

Lignocellulosic biomass is referred to higher plants, softwood or hardwood [3]. Lignocellulose material mainly consists of cellulose, hemicelluloses and lignin. These three component cover almost 90% of the total dry mass and the remaining parts is ash and extractives [4]. Lignin and hemicelluloses are connected by lignin-carbohydrate bond and as a result, they form hydrolysis-resistant protecting sheet around cellulose. The proportion of cellulose, hemicellulose and lignin differs from types of species, but in general, two-third of the total lignocellulosic dry biomass consists of cellulose and hemicellulose [4]. Cellulose and some of the hemicelluloses are the polymers which can be hydrolysed and fermented in order to produce biofuel from lignocellulos biomass (Chandal and Singh, 2010 cited in [4]). Lignocellulose can be separated into hard wood, soft wood and agriculture residue [4]. Composition of different lignocellulosic biomass is given in Table 1 below.

Table 1. Composition of different lignocellulosic materials [3]

| Lignocellulosic materials      | Cellulose % | Hemicellulose % | Lignin % |
|--------------------------------|-------------|-----------------|----------|
| Hardwood Stems                 | 40-50       | 24-40           | 18-25    |
| Softwood stems                 | 45-50       | 25-35           | 25-35    |
| Nut shells                     | 25-30       | 25-30           | 30-40    |
| Corn cobs                      | 45          | 35              | 15       |
| Grasses                        | 25-40       | 35-50           | 10-30    |
| Paper                          | 85-99       | 0               | 0-15     |
| Wheat Straw                    | 30          | 50              | 15       |
| Sorted refuse                  | 60          | 20              | 20       |
| Leaves                         | 15-20       | 80-85           | 0        |
| Cotton seed hairs              | 80-95       | 5-20            | 0        |
| Newspaper                      | 40-55       | 25-40           | 18-30    |
| Waste paper from chemical pulp | 60-70       | 10-20           | 5-10     |
| primary waste water solids     | 8-15        | NA              | 24-29    |
| Swine Waste                    | 6           | 28              | NA       |
| Solid cattle manure            | 1.6-4.7     | 1.4-3.3         | 2.7-5.7  |
| Coastal Bermuda grass          | 25          | 35.7            | 6,4      |
| Switchgrass                    | 45          | 31.4            | 12       |

According to the above table, softwood consists of 45-50% cellulose, 25-35% hemicellulose, and 25-35% lignin. Similarly, softwood contains 0-10% extractives and less than 1% inorganic content, ash. Softwood generally has high lignin content. [2]

### 3.1.1 Cellulose

Cellulose is the major constituent of the plant cell wall and consists of linear linkage between glucose units, as shown in the figure 2. The number of repeated units or the degree of freedom varies between 8000-15000 glucose units (Brown, 2004 is cited in [4]). In cellulose, D-glucopyranose units are linearly connected by  $\beta$ -1, 4-glucosidic bonds, and this bond allows the polymer to arrange in long straight chains [3]. This linkage forms cellobiose components, which forms polymer. Each chain is linked together by hydrogen bonds and van der Waals forces, which form rigid and solid polymer with high tensile strength [8]. Hemicelluloses and lignin cover microfibrils, forming a complex matrix around the cellulose polymer. Because of this complex association of cellulose with hemicelluloses and lignin, intense pretreatment is required in order to isolate cellulose from other polymers (Palonen, 2004 cited in [4]). Cellulose can be found in crystalline or amorphous form. Crystalline structure, more common structure, is non-susceptible to the enzyme reaction while the amorphous structure is easily degradable as compared to the crystalline structure [8, 4]. Figure 2 illustrates the structure of a cellulose molecule.

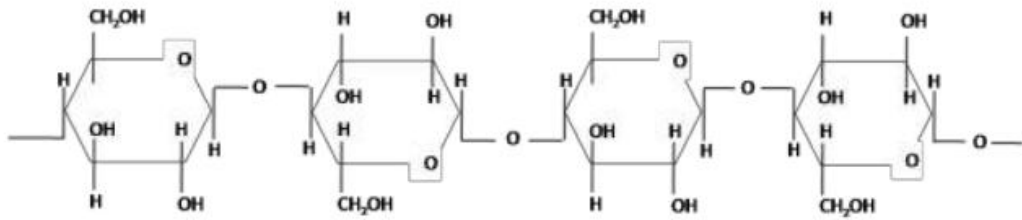


Figure 2. Structure of cellulose molecule [4]

Cellulose is relatively hygroscopic in nature and absorbs about 8-14% of the water under normal atmospheric conditions (20 °C, 60% relative humidity). The solubility of the cellulose increases with the increase in temperature. High temperature provides energy to break the hydrogen bonds which hold the crystalline structure of the cellulose molecule (Figure 3). Cellulose is generally soluble in acid solution. On the other hand, alkali solution causes the swelling of the cellulose as well as dissolution of the low molecular weight fraction of the polymer. Decomposition of cellulose starts at 180 °C (thermo-wood handbook, 2003 cited in [3]). [3]

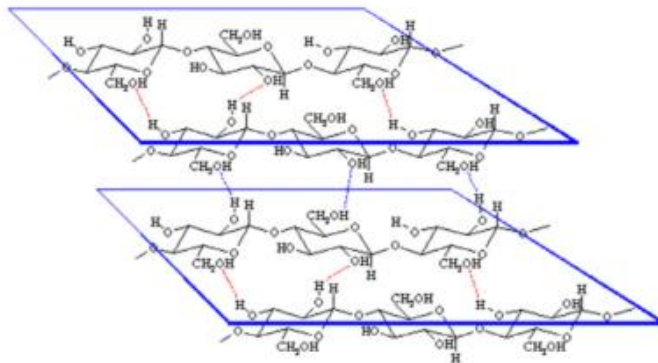


Figure 3. Hydrogen bond between the cellulose polymer chains[3]

### 3.1.2 Hemicellulose

Hemicellulose is a complex carbohydrate structure consisting of pentoses ( $\beta$ -D-xylose,  $\alpha$ -L-arabinose), hexose ( $\beta$ -D-mannose,  $\beta$ -D-glucose and  $\alpha$ -D-galactose) and uronic acids ( $\alpha$ -D-glucuronic,  $\alpha$ -D-4-O-methylgalacturonic and  $\alpha$ -D-galacturonic acids). Similarly, small amount of  $\alpha$ -L-rhamnose and  $\alpha$ -L-fucose can be present in hemicellulose. Sugar are connected by either  $\beta$ -1,4-glycosidic bonds, or  $\beta$ -1,3 linkages [7]. Figure 4 shows a schematic representation of hemicellulose.

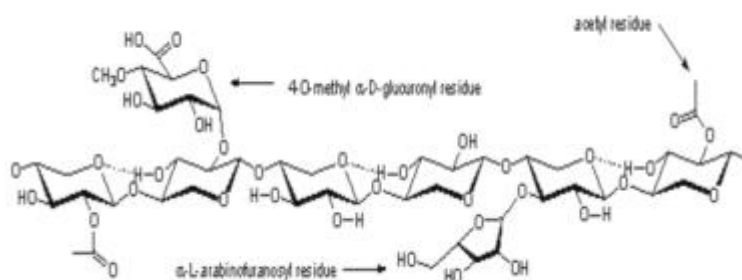


Figure 4. A schematic representation of the hemicellulose

In comparison with cellulose, hemicellulose is short (100-200 units) and has several lateral branches of hexoses and pentoses[3]. The branched structure of hemicellulose makes it amorphous in nature and more sensitive to enzymes [3]. Unlike cellulose, hemicellulose has short lateral branches of sugar, lower molecular weight and is easily hydrolysed [8]. Hemicellulose acts as a connector between lignin and the cellulose fibre and makes the whole cellulose-hemicellulose-lignin network more rigid. The solubility of hemicellulose increases with the increase in temperature. Hemicellulose starts to solubilize in water around 150 °C under neutral condition. However, the solubility of hemicellulose also depends on the moisture content and pH. In lignocellulose material, hemicellulose is the most thermo-chemical sensitive polymer and reacts first during thermal treatment. [5]

Xylans are the most abundant hemicellulose which can consist of about 20-30% of dry weight of biomass such as hardwood and herbaceous plants [7]. Galactoglucomannan-type hemicelluloses are the second abundant hemicellulose and are found in relatively large amount in soft wood. Spruce, the raw material used in this study, contains a higher amount of mannan than xylan [4]. The high amount of mannan is an advantage for the bioethanol production as mannan can be hydrolyzed and fermented to ethanol.

### 3.1.3 Lignin

Lignin is the third abundant polymer after cellulose and hemicellulose, which is present in the plant cellular wall. It consists of three phenylpropane units, *p*-coumaryl, coniferyl and sinapyl alcohol, held together by different kinds of linkage (Figure 5). In plant structure, lignin provides the structural support, impermeability and resistance against microbial attack and oxidative stress. Similarly, it plays a role in transportation of water, nutrient and other metabolites in the plant cell. Lignin is heteropolymer which is non-water soluble and this characteristic of lignin makes difficult to degrade. Lignin starts to dissolve into water at 180 °C under neutral conditions. Solvent such as alcohol, dioxane, acetone, pyridine, and dimethyl sulphoxide significantly dissolve lignin. [3, 5]

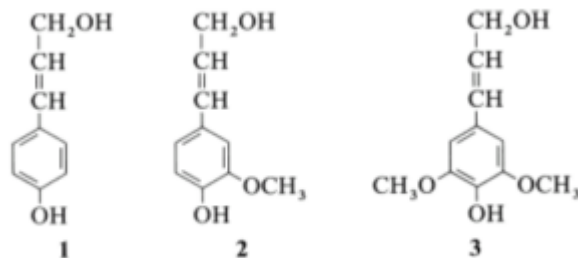


Figure 5. Building block of three-dimensional polymer lignin, *p*-coumaryl (1), coniferyl (2) and sinapyl alcohol (3) [3]

### 3.1.4 Extractives and others

In addition to cellulose, hemicelluloses and lignin, lignocellulose materials contain small amounts of extractives and ash. Within the plant cell, extractives play a role in biological and anti-microbial protection. Some examples of extractives are phenols, tannins, fats and sterols. On the other hand, ash contains inorganic compounds such as silica, alkali and salts. Norway spruce contains about 2% extractives and 1% residual constituents (Sjöström, 1993 is cited in [2]). [4]

### 3.2 Norway spruce

The raw material used in the experimental part of this thesis is Norway spruce (*Picea abies*). Composition of spruce, according to different sources, is shown in Table 2.

Table 2. Composition of spruce according to different sources [4]

| S.N | Glucan(%) | Galactan(%) | Mannan(%) | Xylan(%) | Arabinan(%) | Lignin(%) | References               |
|-----|-----------|-------------|-----------|----------|-------------|-----------|--------------------------|
| 1   | 44.0      | 2.3         | 13.0      | 6.0      | 2.0         | 27.5      | (Sassner et al, 2008)    |
| 2   | 43.4-45.2 | 1.8-2       | 12-12.6   | 4.9-5.4  | 0.7-1.1     | 27.9-28.1 | (Tengborg, 2000)         |
| 3   | 49.9      | 2.3         | 12.3      | 5.3      | 1.7         | 28.7      | (Söderström et al, 2003) |
| 4   | 44.8-45.0 | 2.2         | 12.0      | 5.2      | 2.0         | 29.9-32.3 | (Hoyer et al, 2010)      |

Norway spruce, which is native in Europe, is highly potential resource for paper products, sawn goods and wood-base panels. Due to its hardness and high adaptability characteristic, it is grown in different parts of the world. In Finland, forest industry depends mainly on the soft wood, as 80% of the consumption relied on softwood in 2000s [9]. Therefore Norway spruce is considered as very important biomass related with the country's economy. In Finland, since 2000, the annual growth rate of Norway spruce is about 27 million m<sup>3</sup>, while the average annual removal of commercial roundwood was 25 million m<sup>3</sup> [9]. Norway spruce is considered as potential biomass for bioethanol production. Characteristics of Norway spruce such as fast and easy growth, tallness and a reasonable amount of cellulose and galactoglucomannan (hemicellulose) can be beneficial for the production of bioethanol. In Norway spruce, cellulose and galactoglucomannan sum up to roughly 55% of the dry wood weight, and they can be hydrolysed and fermented to produce ethanol.

### 3.3 Pretreatment of Lignocellulose Biomass

The basic procedure for the production of ethanol from lignocellulose is given in Figure 6 below.

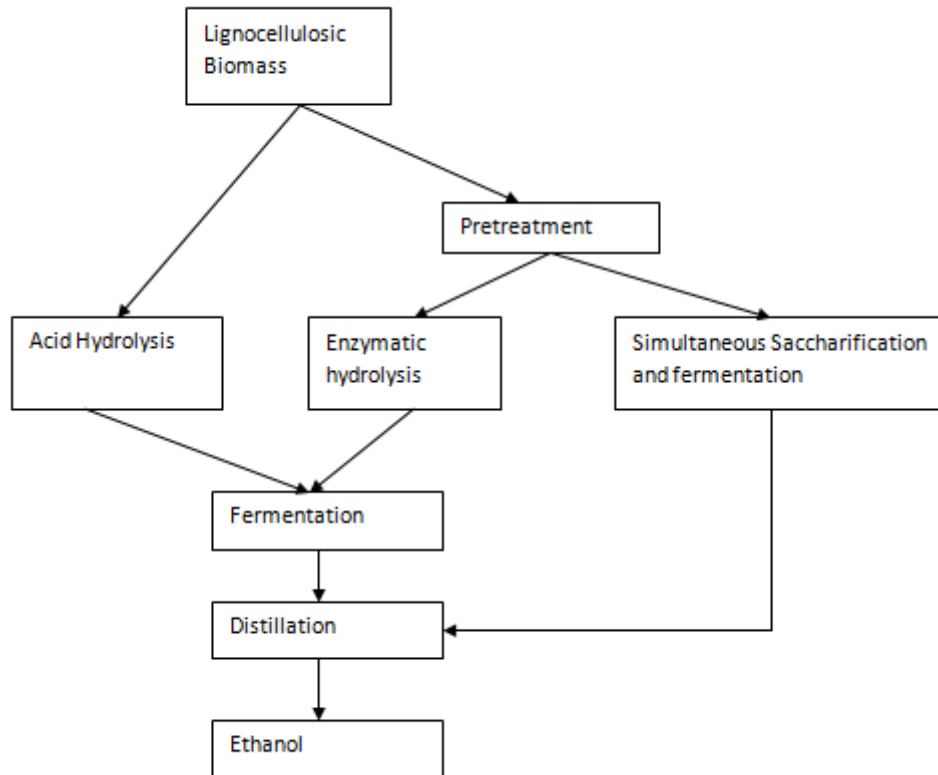


Figure 6. Biomass to bioethanol [14]

The objective of the pretreatment is to break down the structure of lignocellulose matrix, in order to get cellulose and hemicellulose in a form, from which they could be easily hydrolysed to sugars [1]. The effects of pretreatment on lignocellulose are increase in the surface area and porosity, reduction in the crystallinity of cellulose, modification and removal of lignin, depolymerisation of hemicelluloses and removal of hemicelluloses (Figure 7) [3]. Similarly, limiting the production of inhibitory compounds, avoiding the degradation of carbohydrate and minimising the cost are the challenges of pretreatment process [11]. Economically, pretreatment step is one of the expensive phases in biofuel production process as it requires considerable amount of energy. [6]

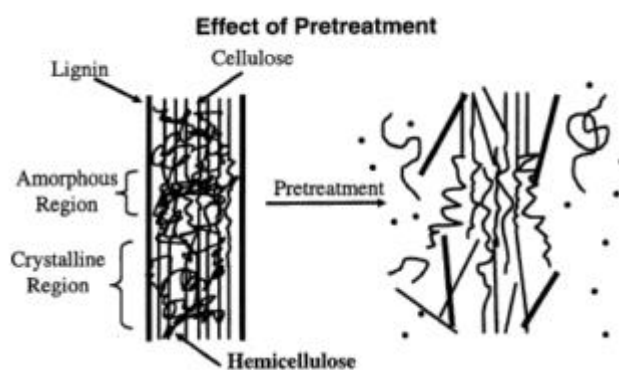


Figure 7. Effect of pretreatment on lignocellulosic biomass [6] (Hsu et al, 1980 is cited in [3])

There are many available pretreatment methods such as physical, chemical, biological, electrical, physiochemical and their combination [11]. However, this thesis mainly focuses on mechanical, thermal and chemical pretreatment methods.

### 3.3.1 Mechanical pretreatment

Mechanical pretreatment of biomass is done by chipping, cutting, grinding, milling, and many other methods [3]. The goal of mechanical pretreatment is to reduce the particle size and increase surface area. This is also a phase which requires significant amount of energy. The power requirement for the mechanical size reduction of biomass depends on the final particle size and the biomass characteristic (Cadoche and López., 1989 in [6]). Other physical pretreatment methods are pyrolysis where cellulose rapidly decomposes into gaseous products and char when treated at high temperature with limited oxygen [11].

### 3.3.2 Thermal pretreatment

Thermal pretreatment is the process of fractionation of biomass under the application of temperature. Pretreatment methods such as pressurised hot water extraction, steam explosion, ammonia fibre explosion and CO<sub>2</sub> explosion use elevated temperature to cause the disruption of the lignocellulose. The use of the solvents depends on the goal of the fractionation. For example, PHWE is effective for hemicellulose removal, whereas alkaline extraction is effective for lignin removal.

### 3.3.3 Chemical pretreatment

Pretreatment methods which are initiated by chemicals are categorised as chemical pretreatment methods. Different kind of chemicals such as dilute acid, strong acid, alkaline, ammonia, hydrogen peroxide and organic solvent are used as solvent to initiate the disruption of biomass. [3]

## 3.4 PHWE and alkaline extraction

### 3.4.1 PHWE

The PHWE method utilises the pressurised water at elevated temperature in order to dissolve the hemicellulose and lignin from the lignocellulose matrix. PHWE is also called as hydrothermolysis, hydrothermal pretreatment, aqueous fractionation, solvolysis or aquasolv (Mosier et al., 2005 cited in [3]). PHWE is an environmentally friendly method compared to extraction methods which use organic solvent. In addition, water is easily available and can be recycled.

PHWE can be performed either in batch or flow-through mode. Compared to static or batch extraction, dynamic extraction requires more volume of water. However, extraction efficiency is higher in the dynamic extraction [12]. According to (Yang and Wyman., 2004 cited in [5]) flow through extraction removes more hemicelluloses and lignin than the batch extraction. Continuous flow of water during extraction lowers the concentration of hemicelluloses and lignin in the extraction capsule and this minimise the risk of formation of degradation products like furfural and condensation and precipitation of lignin [5]. The efficiency of the batch extraction depends on the partition-equilibrium constant and the solubility of the compounds at applied temperature and pressure. Batch extraction of a highly concentrated sample or a low solubility sample might lead to incomplete extraction. [12]

At room temperature and atmospheric pressure, water is highly polar solvent due to the presence of hydrogen bond in water. Thus, water is not considered as a suitable solvent for the extraction for non-polar or organic compounds at room temperature. However, when the temperature is raised, permittivity, viscosity and surface tension of water decrease, but diffusivity characteristic increases. With applied pressure (50 bar), the dielectric constant ( $\epsilon$ ) decreases from 80 at 25 °C to 27 at 250 °C. This dielectric constant of water at 250 °C and 50 bar falls between methanol ( $\epsilon=33$ ) and ethanol ( $\epsilon=24$ ).

Under such conditions, water behaves like an organic solvent and also solubilises non-polar compounds. PHWE involves basic four mechanisms: (1) desorption of solute under applied pressure and temperature, (2) diffusion of the extraction fluid into the matrix, (3) partition of solute into the extraction fluid and (4) chromatographic elution out of the extraction capsule into a collection vial [12].

At higher temperature and pressure, lignocellulose of the biomass starts to solubilise into water. During the pretreatment process, hemicelluloses and some lignin fractions solubilise first (Garrote et al., 1999 cited in [5]). The pressure is applied to keep water in condensed form. At 200 °C, the minimum pressure required to keep water in condensed form is 15 bar and 85 bar at 300 °C. If the pressure goes below the boiling point, superheated steam is formed and it makes hard to control the extraction. [12]

The main factors effecting PHWE are temperature, dynamics of extraction, pressure, retention time and flow rate. Temperature affects the physicochemical properties of water and increase vapour pressure and thermal desorption of the biomass. In PHWE, the applied temperature is usually above the boiling point and that changes water properties into low viscous, low surface tension and high diffusion characteristic. However, selection of temperature is crucial and depends on the goals of extraction. During high temperature extractions, possibility of formation of inhibitors is high. Besides temperature, also pH affects to inhibitor formation. Few inhibitors are typically formed at neutral pH. Similarly, pressure plays a great role in distributing water throughout the area of biomass, which would not be possible without it. [12]

#### 3.4.2 Alkaline extraction

Alkaline pretreatment methods use alkaline earth metal such as sodium, potassium or calcium water solution, in order to remove lignin from the biomass. Alkaline pretreatment methods are very effective for the solubilisation of lignin along with some fraction of cellulose and hemicelluloses. Compared to other pretreatment methods, alkaline extraction can be carried out at low temperature and pressure. However, the extraction time is usually long. The chemistry of delignification during alkaline pretreatment is that the hydroxyl ion in alkaline solution cleaves the bond in lignin molecules [13]. During the pretreatment, salivation and saponification of the biomass occurs. Swollen state increases the surface area of the biomass and makes it more accessible for enzymes or bacteria during hydrolysis. High concentration of alkaline cause alkaline hydrolysis

and degradation and decomposition of dissolved polysaccharides. Alkaline pretreatment also causes peeling of biomass, which might be advantage for later conversion process, but there is a risk of degradation and loss of carbon, in the form of carbon dioxide. [5]

Alkaline pretreatment of lignocellulose biomass cause swelling of biomass and the consequences are increased surface area, decreased DP and crystallinity of cellulose, breakage of linkage between lignin and carbohydrates and disruption of lignin (Fan et al., 1987 cited in [6]). Delignification has three steps; (1) initial delignification where 20-25% of lignin is removed, (2) bulk delignification where most of the lignin is removed through the cleavage of  $\beta$ -aryl linkage in non-phenolic lignin units, (3) residual delignification where remaining lignin is removed and C-C bond in propane chain and condensate product are cleaved (Gierer, J., 1985.Cited in [13]).

### 3.5 Formation of inhibitors

Although pretreatment process is so vital in the biofuel production process, it has tendency to produce chemicals which may prohibit the hydrolysis and fermentation process. Pretreatment can produce different chemical during degradation process and which will limit the ethanol yield and productivity. Degradation of (hemi) cellulose intends to produce furfural and hydroxymethyl furfural (HMF). These chemicals affect the growth and respiration of the micro-organism used in the hydrolysis and fermentation process. Temperature over 160 °C and residence time of 4 h for acid pretreatment, help to produce significant amount of furfural and HMF( Mckillip and Collin, 2002 is cited in [3]). Similarly degradation of lignin can produce several aromatic, polyaromatic, phenolic and aldehydic compounds, which can have more inhibitory effect than furfural and HMF. Other potential inhibitory chemicals are extractives and heavy metal ions from equipment. [3]

In order to minimise the formation of inhibitory compound, lignin degradation should be controlled, and the temperature and residence time, should be kept as low and short as possible [3].

### 3.6 Hydrolysis

Hydrolysis is the process of converting carbohydrate polymers into simple fermentable sugars. After the pretreatment process, cellulose-rich biomass is broken down into glucose and hemicellulose into pentoses and hexoses. Hydrolysis and fermentation can be done together (SSF, simultaneous saccharification and fermentation) or separately (SHF, separate hydrolysis and fermentation). SSF has several advantages, such as reduced number of steps, lower enzyme requirements, reduced end product inhibition because of continuous, shorter process time, rapid conversion of glucose into ethanol and reduce contamination due to the presence of ethanol [6]. However, in SSF, temperature for the hydrolysis cannot be optimised independently from the fermentation and produced ethanol may inhibit to cellulose activity [6].

In general, there are three types of hydrolysis methods, and they are (1) concentrated acid hydrolysis (2) dilute acid hydrolysis (3) enzymatic hydrolysis [2].

#### 3.6.1 Strong acid hydrolysis

In strong acid hydrolysis, strong acid such as sulphuric acid, sulphurous acid, hydrofluoric acid, hydrochloric acid, phosphoric acid and formic acid are applied to the biomass under moderate temperature [14]. The acid breaks down hydrogen bonds between cellulose chains. Strong acid hydrolysis requires low temperature and has high yield (i.e. 90% of theoretical glucose yield) (Lenihan et al., 2010 cited in [14]). Strong acid can penetrate lignin without any pretreatment of biomass. However, use of strong acid could lead to equipment corrosion and need an acid recovery system in order to make the process economical (Galbe & Zacchi., 2002 cited in [2]).

#### 3.6.2 Weak acid hydrolysis

Dilute acid is the common hydrolysis method, where the biomass is treated with dilute acid (around 0.5%). The temperature for the weak acid hydrolysis is higher than the strong acid hydrolysis. The main advantages of this method are low acid consumption and fast reaction. On the other hand, low sugar yield, high temperature requirement, hemicellulose sugar degradation and formation of inhibitors are the negative aspects of dilute acid hydrolysis. [2]

### 3.6.3 Enzymatic hydrolysis

Enzymatic hydrolysis uses cellulase for the hydrolysis of cellulose. Both bacteria and fungi can produce cellulase. Bacteria belonging to *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora*, and *Streptomyces* are capable of producing cellulases (Bisaria, 1991 is cited in [6]). Out of these, *Cellulomonas fimi* and *Thermomonospora fusca* have been studied for the cellulase production. Similarly, fungi such as *Sclerotium rolfsii*, *P. chrysosporium* and species of *Trichoderma*, *Aspergillus*, *Schizophyllum* and *Penicillium* produce cellulases (Sternberg, 1976; Fan et al., 1987; Duff and Murray, 1996 are cited in [6]). Among these fungi, *Trichoderma* are widely used in the enzyme industry for producing wide range of enzymes [2].

Cellulases are a mixture of many enzymes. The three major groups are (1) endoglucanase, which attacks cellulose from the region of low crystallinity; (2) exoglucanase or cellobiohydrolase, which help for further desentrification of cellulose by removing cellobiose units from the free-chain ends; (3)  $\beta$ -glucosidase, which hydrolyse cellobiose into glucose [6]. In addition, there are ancillary enzymes such as glucuronidase, acetyltransferase, xylanase,  $\beta$ -xylosidase, galactomannanase and glucomannanase, which hydrolyse hemicellulose (Duff and Murray, 1996 cited in [6]).

Enzymatic hydrolysis has high advantages because enzymes are highly specific and can work in mild conditions. However, the use of enzymes is still limited by the cost of enzyme isolation and purification. Factors affecting the hydrolysis of cellulose are substrate concentration, cellulase activity and other controlled environment such as temperature and pH. [6]

### 3.7 Fermentation

Fermentation is anaerobic metabolism using different microorganism to convert hexose and pentoses into ethanol [16]. Among them, yeast, *Saccharomyces cerevisiae* is most commonly used for the fermentation of hexoses. As mentioned in the above literature, fermentation can be done together with hydrolysis (SSF) or separately (SHF). Fermentable free sugars after hydrolysis are converted to ethanol under controlled temperature and anaerobic condition. Since there is a great fraction of pentose from hemicelluloses in lignocellulosic biomass, microorganisms capable of converting both hex-

oses and pentoses are beneficial for the fermentation process. In SSF process, microorganism should be capable of tolerating ethanol and other inhibitory outcomes from hydrolysis. In order to solve this problem there have been extensive studies on genetically modified microorganisms which can convert both pentose and hexose, and tolerate ethanol and other inhibitory compounds formed during SSF [16]. Studies have been done for genetically modified *Saccharomyces cerevisiae*, to make both pentose- and hexose-fermenting yeast [4].

The efficiency of the fermentation depends on the choice of organism, raw material, pretreatment, hydrolysis, pH, temperature, substrate, and ethanol concentration. The controlled conditions required by *Saccharomyces cerevisiae* are a temperature of approximately 37 °C and pH of approximately 5 [4]. Finally, distillation is done to purify the ethanol up to 96% [16]. Ethanol has a lower boiling point than water and evaporates before water. Ethanol is collected by condensation. Purified ethanol can be feed into the combustion engine as a separate fuel or as a blend with fossils fuel. Ethanol can be further purified by further distillation. However, distillation requires high cost and has limited separation capacity. To overwhelm this problem, there are several purification technologies such as non-heating fractional distillation by ultrasonic irradiation, oxidation of impurities by ozone, and adsorption of impurities by activated carbon or zeolite [22].

## 4 Experimental Part

### 4.1 Extraction System

The experiments were carried out at the Finnish Forest Research Institute, Jokiniemenkuja 1 Vantaa, in a laboratory scale flow-through extraction as shown in Figure 8. The extraction system consists of a pump (Water 510 HPLC), an oven (Varian 3400 GC), a capsule, steel capillaries with an inner and an outer diameter of 1/16 inch and 1 mm, respectively, a manometer and a control valve. The extraction system is illustrated in figure 8 and 10. The extraction capsule with an inner diameter of 21.2 mm, and 150 mm length, had a volume of 50 ml. During extraction, the capsule was placed inside the gas chromatography oven, in order to heat and control the extraction temperature.

In PHWE, water was pumped into the capsule through capillary by HPLC pump. In alkaline extraction, water was pumped into the cylinder (Figure 9) containing an alkaline

solution and the exact same flow of alkaline solution was forced out from the cylinder into the capsule. The water and the alkaline solution were preheated inside the oven before entering into the capsule.

For all the extractions, 10 g of dry spruce sawdust was loaded into the capsule. Average size distribution of the spruce saw dust is given in Appendix 4. TDS of the spruce saw dust was 47.67%. The amount of 10 g of dry weight saw dust was calculated as below:

$$\text{Mass of spruce taken (g)} = \frac{10\text{g}}{47.67} * 100 = 20.98 \text{ g} \approx 21 \text{ g}$$

The extraction capsule containing sawdust was completely filled with deionised water before each PHWE. In alkaline extraction, the capsule was filled with alkaline solution (0.55 M NaOH). The alkaline solution was prepared before the extraction in 500 ml volumetric flask. Temperature and flow were set manually. The system pressure was measured by manometer and adjusted by a valve. During extraction, the system was pressurised to 30-50 bar. The purpose of pressure was to ensure that the water stays in liquid form inside the capsule. The extract was cooled in cold tap water and collected to measure pH, brix and mass.

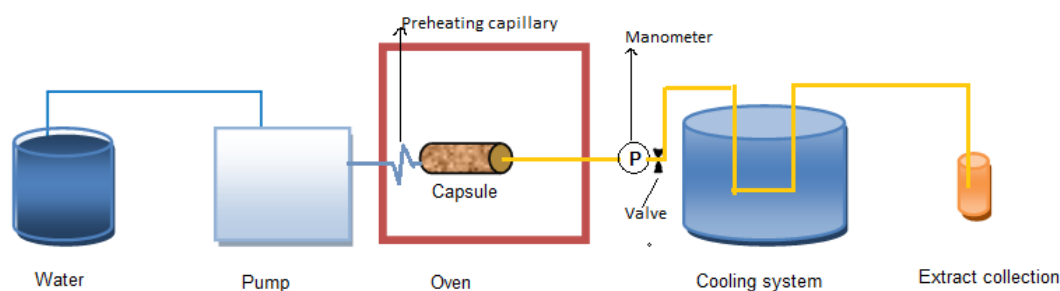


Figure 8. Extraction system consisting of water, pump, oven, capsule, valve, cooling unit and extract collection point.

For the alkaline extraction, the flow-through extraction system was redesigned with addition of an extra cylinder (Figure 9). The purpose of the cylinder was to pump the required amount of alkaline solution into the chamber without contaminating the pump.

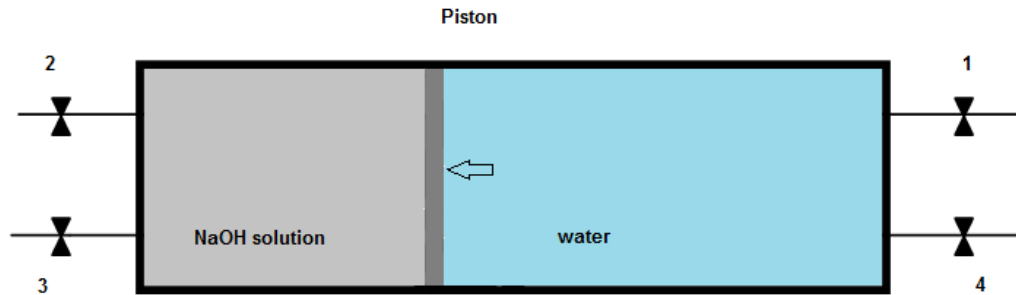


Figure 9. Diagram of the cylinder containing alkaline solution, used during alkaline extraction.

As shown in the Figure 9, the cylinder consists of one piston and four valves making two distinct chambers with two valves in each. The left chamber consists of the alkaline solution while the right chamber contains deionised water from the pump. At initial stage, Valve 1 and Valve 2 are closed and the alkaline solution is injected through Valve 3. As the alkaline chamber starts to be full, pressure increases inside the left chamber and the piston moves towards the water chamber. Since Valve 4 is open, water in the water chamber, goes out through Valve 4, while the piston moves towards Valve 1 and Valve 4. In this way, the left chamber is totally filled with the alkaline solution. The filling of alkaline solution moves the piston to the end of the water chamber. Then all the valves are closed.

Before the extraction, Valve 2 and Valve 1 are opened, and the water is feed into the water chamber through Valve 1. As a result, the alkaline solution is pushed to the extraction line through Valve 2, with the same flow rate as water is being pumped into the cylinder.

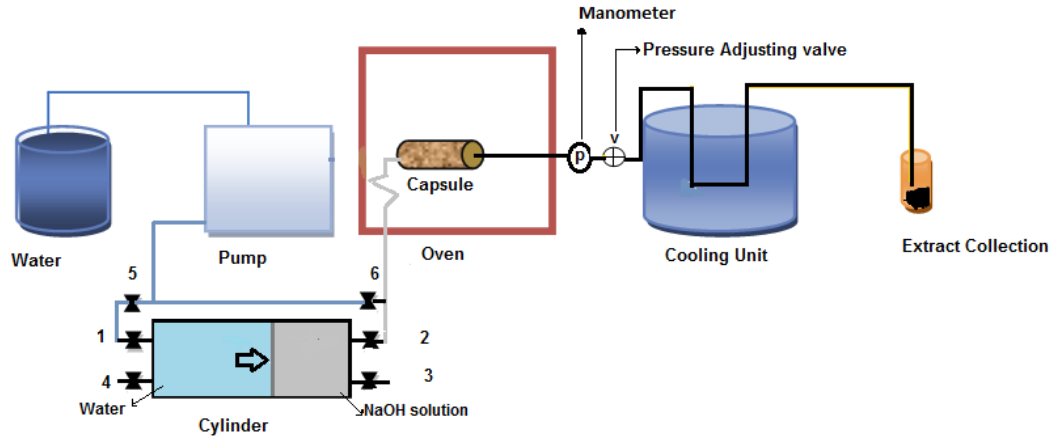


Figure 10. Extraction system with the addition of cylinder. Valve 5 and Valve 6 change solvent between alkaline solution and water.

#### 4.2 Pressurised hot water extraction

PHWEs were carried out at 180 °C and 210 °C. After the preparation procedure, as described above, the oven was heated to the required temperature. After the temperature was achieved, the pressure valve was closed, and the water was pumped at the rate of 4 ml/min into the capsule. When the system pressure reached 50 bar, control valve was adjusted to maintain the pressure between 30 to 50 bar throughout the extraction. During 60-minute extraction, extract was collected in 10 min fractions. Brix and pH were measured using a hand refractometer and a pH meter, respectively. The extract turned from light brown to dark brown during the first 30 min, and gradually faded out again into a light brown liquid (Figure 11).



Figure 11. Extract collected during PHWE. From left to right: 10th, 20th, 30th, 40th, and 60th minute extracts.

After 60 min, the pump was closed and the capsule was left to cool down at room temperature. The extracted spruce was transferred to the plastic bag, weighed and stored in a freezer for further analysis.

### 4.3 Alkaline Extraction

Alkaline extractions were carried out at 150 °C and 170 °C. The extraction period lasted for three 3 h and 40 min. Extraction was divided into *filling* phase, *extraction* phase, *displacement* phase and *washing* phase. The conditions for each phase are listed in Table 3 below:

Table 3. Phases during alkaline extraction

| S.N | Phase               | Solvent | Flow (ml/min) | Temperature (State) | Pressure (bar) | Time (min) |
|-----|---------------------|---------|---------------|---------------------|----------------|------------|
| 1   | <i>Filling</i>      | NaOH    | 0.7           | Not Applied         | Not Applied    | 60         |
| 2   | <i>Extraction</i>   | NaOH    | 0.8           | Applied             | 30-50          | 90         |
| 3   | <i>Displacement</i> | Water   | 0.8           | Applied             | 30-50          | 60         |
| 4   | <i>Washing</i>      | Water   | 5.0           | Not Applied         | Not Applied    | 10         |

The *Filling* phase was used to distribute 0.55 M NaOH solution throughout spruce sawdust inside the capsule without applying any pressure and temperature. *Filling* phase lasted for an hour and by the end of it, solvent NaOH appeared at the collection point. At that time, the valve was closed, flow was increased to 0.8 ml/min and heat was applied. The system was pressurised between 30 to 50 bar in both the *extraction* and *displacement* phase. Due to the small flow rate, it took much more time than in PHWE to reach to 30 bar pressure. During the *extraction* phase, black liquor was collected. In the *displacement* phase alkaline solution was replaced by deionised water. The Same flow rate and temperature as in *extraction* phase were used. At this phase the extract was clear and thin as compared to the *extraction* phase. *Displacement* phase was followed by *washing* phase, which had a water flow rate of 5 ml/min without pressure and heating.



Figure 12. Extracts collected during different phases of alkaline extraction. From left to right: *filling*, *extraction*, *displacement* and *washing* phase.

After the extraction, the capsule was cooled and extracted wood was washed with tap water to remove the remaining sodium hydroxide. The wood residual was weighted and stored in the freezer for further analysis. The extract was collected separately for each phase, and pH and brix values of the extracts were measured.

#### 4.4 Combined PHWE and Alkaline Extraction

In combined extraction, both PHWE and alkaline extraction were carried out in conjugative manner, first PHWE then alkaline extraction. 10 g of spruce sawdust was used in each extraction. The total time of the combined extraction was 4 h and 40 min, an hour of PHWE extraction and 3 h and 40 min for alkaline extraction. Four sets of extraction were carried out with and without cooling. In all the extractions with cooling, the capsule was cooled for about 40 min between PHWE and alkaline extraction. After cooling, the *filling* phase of alkaline extraction was started. On the other hand, in combined extraction without cooling, alkaline *filling* phase was directly started after PHWE was finished. Figure 13 shows the extracts collected during the different phases of alkaline extraction of a combined extraction.



Figure 13. Extracts collected during different phases of alkaline extraction. From right to left: filling, extraction, displacement and washing phase.

Extraction conditions (temperature, flow and pressure) were similar to separate extractions. All performed combined extractions are shown in Table 4.

Table 4. Combined PHWE and alkaline extractions.

| S.N | Extractions                                | Short name | Cooling |
|-----|--|------------|---------|
| 1   | PHWE 180 °C and alkaline extraction 150 °C | 180/150C   | Yes     |
| 2   | PHWE 180 °C and alkaline extraction 150 °C | 180/150    | No      |
| 3   | PHWE 180 °C and alkaline extraction 170 °C | 180/170C   | Yes     |
| 4   | PHWE 180 °C and alkaline extraction 170 °C | 180/170    | No      |
| 5   | PHWE 210 °C and alkaline extraction 150 °C | 210/150C   | Yes     |
| 6   | PHWE 210 °C and alkaline extraction 150 °C | 210/150    | No      |
| 7   | PHWE 210 °C and alkaline extraction 170 °C | 210/170C   | Yes     |
| 8   | PHWE 210 °C and alkaline extraction 170 °C | 210/170    | No      |

*\*C refers to cooling inbetween combined extraction*

Brix and pH were measured from the extract and both the extract and the wood were stored in a freezer for further analysis.

#### 4.5 Wood yield

After each experiment, the extraction capsule was emptied into a filter bag, washed, dewatered and transfer to the plastic bag. The residue was freeze-dried for cellulose, hemicellulose, lignin and ash analysis. Wood yield was calculated after drying the residue in a vacuum drier for 2-3 days.

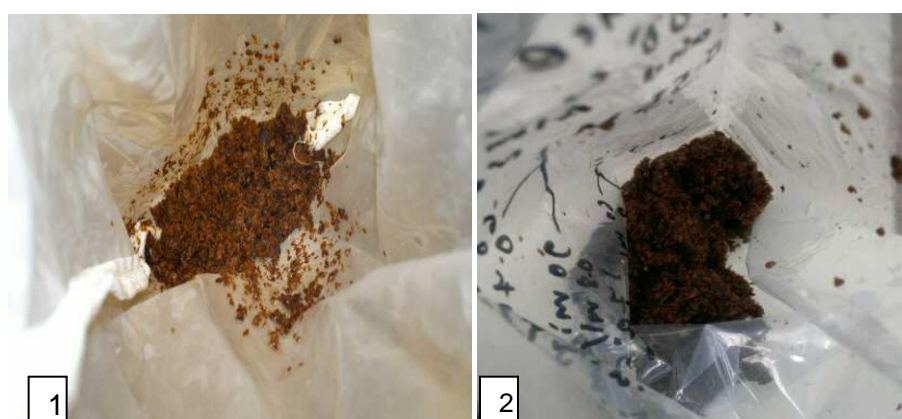


Figure 14. Wood residual from (1) PHWE 180 °C and (2) combined extraction 180/170

## 4.6 Chemical Analysis

### 4.6.1 Preparation before Analysis

Freeze-dried samples were milled before the analysis. Grinding of sample increases surface area and thus helps efficient chemical reaction during chemical analysis. Large size of sample might not facilitate the chemical distribution throughout the wood sample and hence results might not be reliable.

Dry weight of all milled samples (extracted and original spruce saw dust) was determined by oven drying of the samples at 105 °C. Table 5 shows the dry weight of each sample.

Table 5. Dry weight of extracted samples.

| S.N | Samples                                      | Dry weight (%) |
|-----|--|----------------|
| 1   | PHWE 180 °C                                  | 95.62          |
| 2   | PHWE 210 °C                                  | 96.43          |
| 3   | Alkaline Extraction 150 °C                   | 94.85          |
| 4   | Alkaline Extraction 170 °C                   | 95.48          |
| 5   | PHWE180/alkaline extraction 170- no cooling  | 95.62          |
| 6   | PHWE180/alkaline extraction170-withcooling   | 95.41          |
| 7   | PHWE180/alkaline extraction150-nocooling     | 95.57          |
| 8   | PHWE180/alkaline extraction150-withcooling   | 94.47          |
| 9   | PHWE 210 /alkaline extraction 170-no cooling | 94.48          |
| 10  | PHWE210/alkaline extraction170-withcooling   | 94.60          |
| 11  | PHWE210/alkaline extraction150-nocooling     | 93.36          |
| 12  | PHWE210/alkaline extraction150-withcooling   | 92.79          |
| 13  | Spruce Original                              | 95.34          |

All the analysed samples in the later analysis phase contain respective TDS, as shown in the table above.

### 4.6.2 Cellulose Analysis

The amount of cellulose in original and extracted samples was determined using acid hydrolysis, according to Sunderg et al. (2003) [18]. About 10 mg of the sample and a cotton linters calibration standard (Sigma Aldrich) were hydrolysed with 0.2 ml of 72% sulphuric acid, in 25 ml test tube. Hydrolysed samples were neutralised with barium carbonate. After that, 1 ml of 5 mg/ml sorbitol and resorsinol (internal standard) were

added to the samples after neutralization. Samples were then silylated by adding 200  $\mu\text{L}$  of pyridine, 150  $\mu\text{L}$  of hexamethyldisilazane (HMDS) and 70  $\mu\text{L}$  of trimethylchlorosilane (TMCS) and left overnight. Silylated samples were analysed using a gas chromatograph (GC) with a flame ionization detector (FID).

#### 4.6.3 Hemicellulose analysis

Composition of the hemicellulose in original and extracted spruce saw dust was determined using acid methanolysis according to Willför et al. (2009) [17]. About 10 mg of the extracted samples were depolymerised with acid methanolysis (2 M HCL in anhydrous MeOH). Afterwards, only the extracted wood samples were placed in the oven at 100  $^{\circ}\text{C}$  for five hours. Two calibration samples, first dried in a nitrogen hood and mixed with 2 ml of 2 M HCL in anhydrous methanol, were placed in the oven at 100  $^{\circ}\text{C}$  for three hour. After methanolysis, samples and standards were treated similarly.

After cooling, samples were neutralised with pyridine. An internal standard (0.1 mg/ml of sorbitol and resorsinol) was added to the neutralised samples. The samples were then dried and silylated with 100  $\mu\text{L}$  of pyridine, 150  $\mu\text{L}$  of hexamethyldisilazane (HMDS) and 70  $\mu\text{L}$  of trimethylchlorosilane (TMCS). Samples were left overnight for silylation and later analysed using GC-FID (Figure 15).

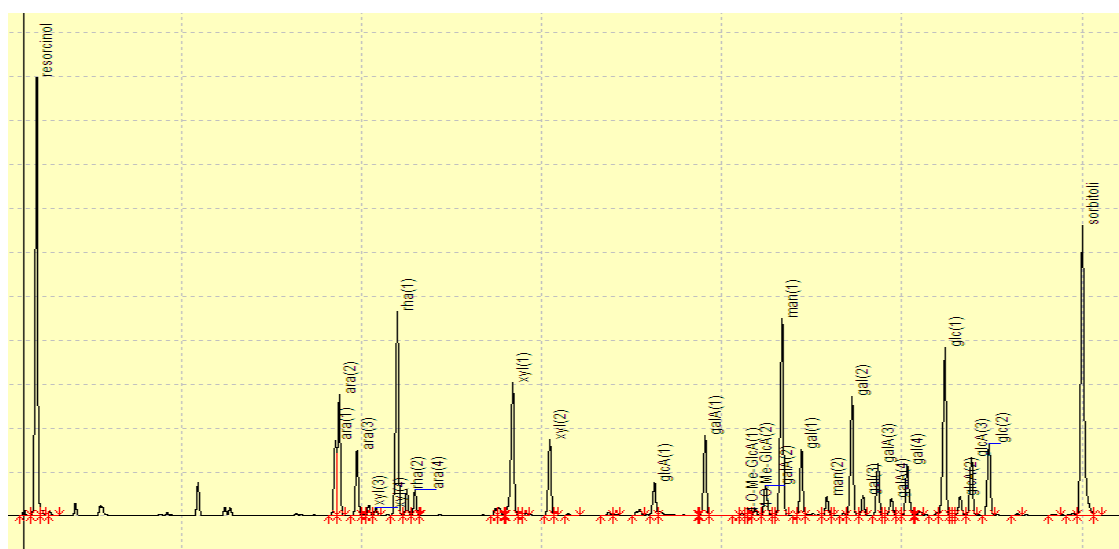


Figure 15. Hemicellulose peaks in gas chromatography obtained from a sample analysed by Methanolysis-gas chromatography.

#### 4.6.4 Lignin Analysis

Klason lignin was determined according to Raiskila et al. (2007) [19]. About 300 mg of freeze-dried wood samples were added with 3 ml of 72% sulphuric acid and placed in an ultrasonic bath for an hour. Samples were mixed every ten minutes by using a vortex. Samples were then transferred with deionised water to Erlenmeyer flask and autoclaved at 121 °C for an hour. Aliquots with precipitates were filtered through sinters using suction bottles. Precipitates were washed with deionised water, and sinters with precipitation were placed overnight in an oven at 105 °C. Dried sinters were then cooled and weighed. The measured lignin contained extractives and ash. Soluble lignin in the filtrate was measured with UV-spectrophotometry (Shimadzu UV-2401PC, Shimadzu, Kyoto, Japan) at 203 nm using an extinction coefficient  $120 \text{ Lg}^{-1}\text{cm}^{-1}$ .

#### 4.6.5 Analysis of the extracts

This thesis mainly focuses on the analysis of solid fraction and the results are based on the wood analysis. However, extracts from each extraction were analysed for brix and pH. Brix gives the approximate measure of sugar content in the solution. In PHWE, the brix value is directly proportional to the hemicellulose content in the extract. A higher content of hemicellulose in the extract gives a higher brix value. In contrast, this is not applicable in the alkaline extraction as a high amount of lignin and other compounds contribute to the brix value. Thus, the brix value does not give the actual sugar content in the extract in alkaline extraction.

During the experiment, pH was measured by a pH meter and the brix value by a hand refractometer. Both the instruments were calibrated before the experiment. To measure the brix value, a couple of drops of extract was spread around the prism of the refractometer, and the brix was read from the inbuilt brix scale, through the eye piece lens.

#### 4.7 Ash Content

Ash content is the total amount of minerals present in the wood. Ash content was determined by the dry ashing method using a muffle furnace at 500 °C. All the pre-weighed samples were left in the muffle oven overnight. At high temperature, all the moisture and volatile materials vaporise and the organic content of the wood oxidises in the presence of air. Only the inorganic content (ash) stays as a residual. Ash content was determined as a percentage of original dry wood.

## 4.8 Energy analysis

Energy is consumed for different purposes, for example size reduction, heating, pumping, cooling, cold storing and drying. Among these, a large amount of energy was spent in the size reduction and heating process. Heating power required in order to produce 100 kg of cellulose from different extractions was calculated in this thesis project.

## 5 Result and Discussion

### 5.1 Wood yield

As shown in Figure 16, wood yield from separate PHWEs and alkaline extractions was much higher than the combined PHWE and alkaline extractions (Figure 16). Among the single extractions, alkaline extraction, both 150 °C and 170 °C, had a wood yield higher than 75%. In PHWE, the wood yield decreased by about 9% as the temperature increased from 180 °C to 210 °C. The result seemed reasonable, as the temperature increases, most of the hemicellulose, some of lignin and cellulose dissolves in the solution.

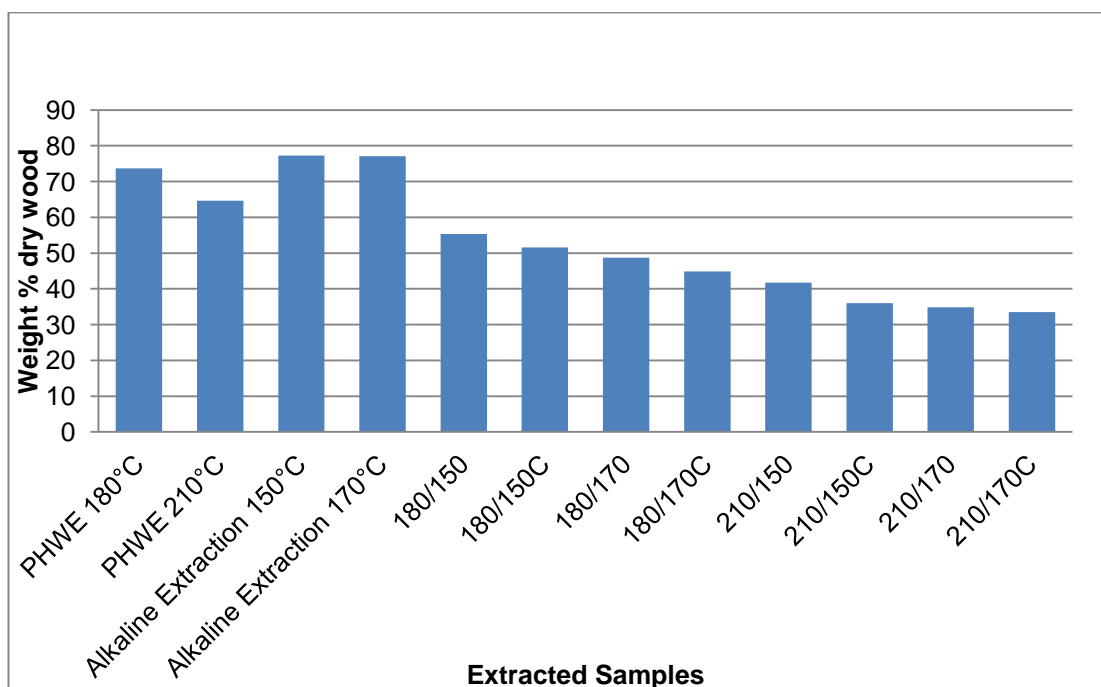


Figure 16. Wood yield after extraction, (C) refers to cooling before alkaline extraction.

In the combined extractions, at least 45% and up to 66% of the wood was dissolved during extraction. Long retention time and both PHWE and alkaline pretreatment for the same sample seemed to be the driving factors behind the low wood yield during the combined extractions. All the lower temperature combined extractions consisting PHWE 180 °C dissolved around 45 to 65% of the wood. Higher temperature extraction, consisting of PHWE 210 °C dissolved between 60 to 67% of the original wood. Cooling between the extractions decreased the amount of wood residual by about 5%, in all combined extractions except 210/170. Separate alkaline extractions at 150 °C and 170 °C dissolved only 23% of the wood, whereas about 64% of wood was dissolved during combined extraction 210/170C. High temperature favours the degradation of the lignocelluloses and hence high amount of wood is dissolved during high temperature extractions.

Cooling inbetween the combined extractions decreased wood yield, roughly by 1 to 5%. When the system was brought to cool down, temperature dropped gradually. This somehow delivered conditions to continue the extraction at low temperature and atmospheric pressure. The pH of the 60th minute extract from the PHWEs was around 3 (Figure 21). Some part of the extract might have remained inside the capsule. Thus, wood inside the capsule might have been in acidic conditions for 40 min during cooling. This might have made the lignocelluloses matrix more sensitive to the alkaline extraction after cooling. All this conditions seemed to favour the decrease in the wood yield due to cooling.

## 5.2 Cellulose yield

Cellulose yield for each extraction is shown in the Figure 17. Separate PHWE and alkaline extractions dissolved less than 6% of the original cellulose. Similarly, of all the combined extractions, 180/170 and 180/150 yielded 74% of the cellulose, which was highest yield of all combined extractions. Significant amount of cellulose was lost during combined extraction compared to separate extractions. In combined extractions, increasing the temperature of PHWE lowered the cellulose yield. In combined extractions 210 /150 and 210/170, almost 60% of the cellulose was dissolved in to the extract, which was 20% more than the combined extraction containing PHWE 180 °C. Combined extraction series containing PHWE 180 °C dissolved more cellulose than separate extractions, but the reduction in hemicelluloses and lignin yield was significant.

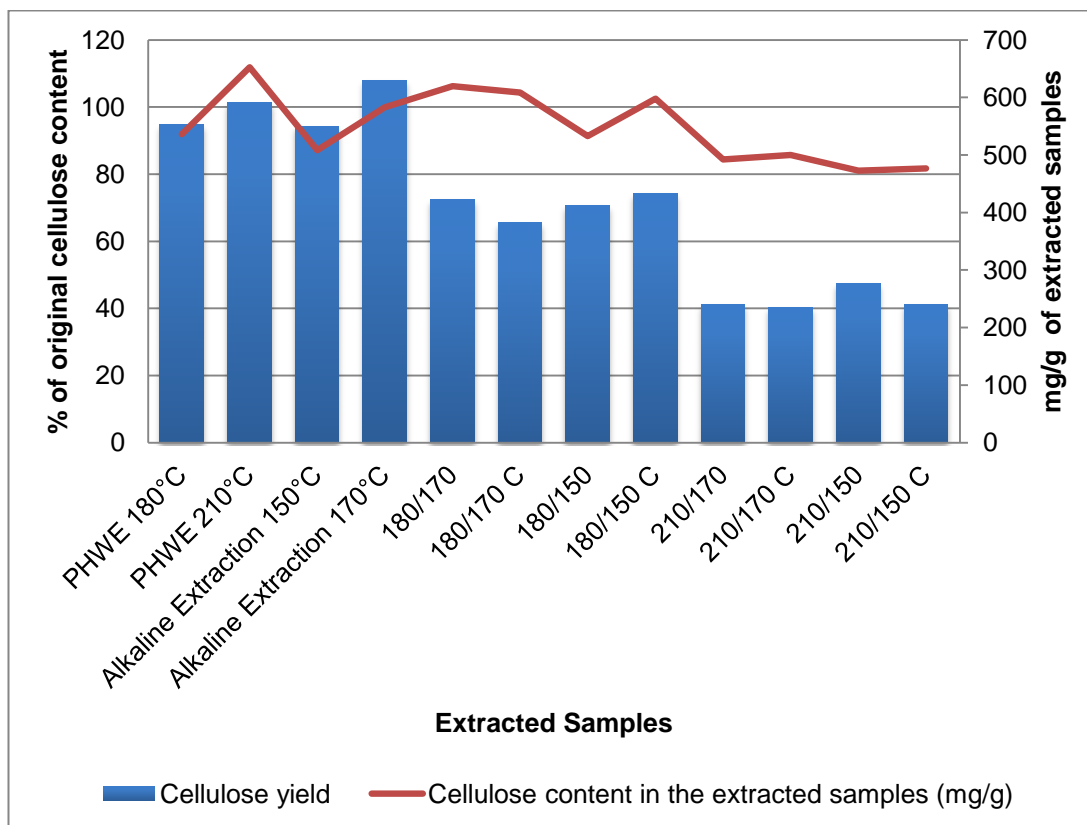


Figure 17. Cellulose yield and content after extraction, (C) refers to cooling before alkaline extraction

Introduction of cooling between the extractions lowered the cellulose yield in combined extractions 210/150, 180/170 and 180/170 by roughly 5%. The results were related to the lower wood yield during combined extraction. In contrast, cellulose yield slightly increased due to cooling in extraction 180/150.

The cellulose recovery over 100% in PHWE 210 °C and alkaline extraction 170 °C was due to error. The error comes through measurement errors or through others random errors during analysis. However, the most probable reason in chemical analysis is the method itself. It can happen, that even if the measurement is said to be total analysis, in real situation the treatments (like PHWE and alkaline extraction) can open the matrix so that the chemical group to be analysed is more prone to the analysis than the untreated biomass.

### 5.3 Hemicellulose Content

Figure 18 shows the percentage of original hemicellulose left in the wood residual. Both separate PHWE 210 °C and 180 °C removed 70 and 85% of the hemicellulose, respectively. Alkaline extraction 170 °C and 150 °C seemed less effective in terms of hemicellulose removal and dissolved less than 55% of the original hemicellulose. All the combined extractions removed at least 85% of the original hemicelluloses. Similarly, in combined extractions starting with PHWE 210 °C, more than 90% of the hemicellulose from the original saw dust was extracted. In combined extractions, applied temperature in the alkaline extraction did not play a major role to dissolve more hemicelluloses, as most of the hemicellulose had been extracted during PHWE. All the combined series of PHWE 180 °C dissolved similar amount of hemicellulose regardless of different temperature during alkaline extractions and similar behaviour was observed during combined series of PHWE 210 °C.

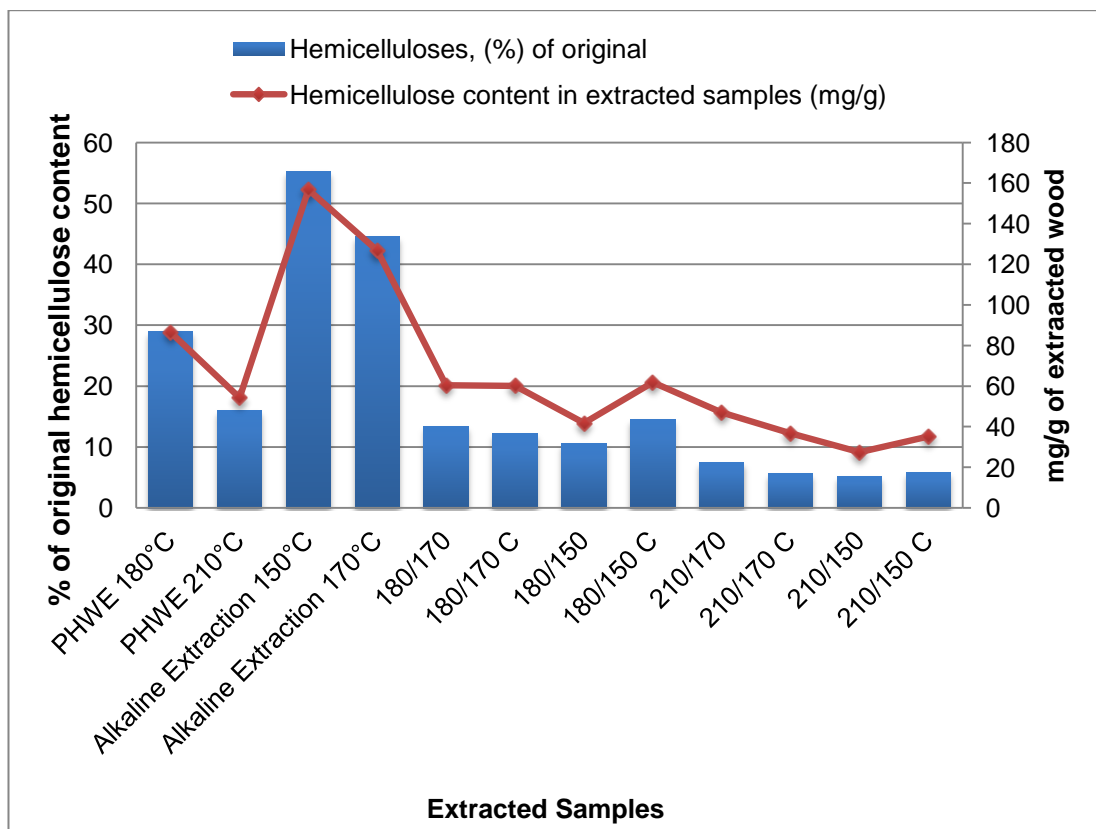


Figure 18. Hemicellulose content in the wood residual. (C) refers to cooling before alkaline extraction

Removal of hemicellulose due to cooling during combined extractions seemed to depend on the temperature applied during alkaline extraction. Cooling before higher tem-

perature alkaline extraction (170 °C) seemed to lower the hemicellulose yield roughly by 1%. On the other hand, cooling inbetween combined extractions containing alkaline extraction 150 °C, seemed to slightly increase the hemicellulose content in the wood residual.

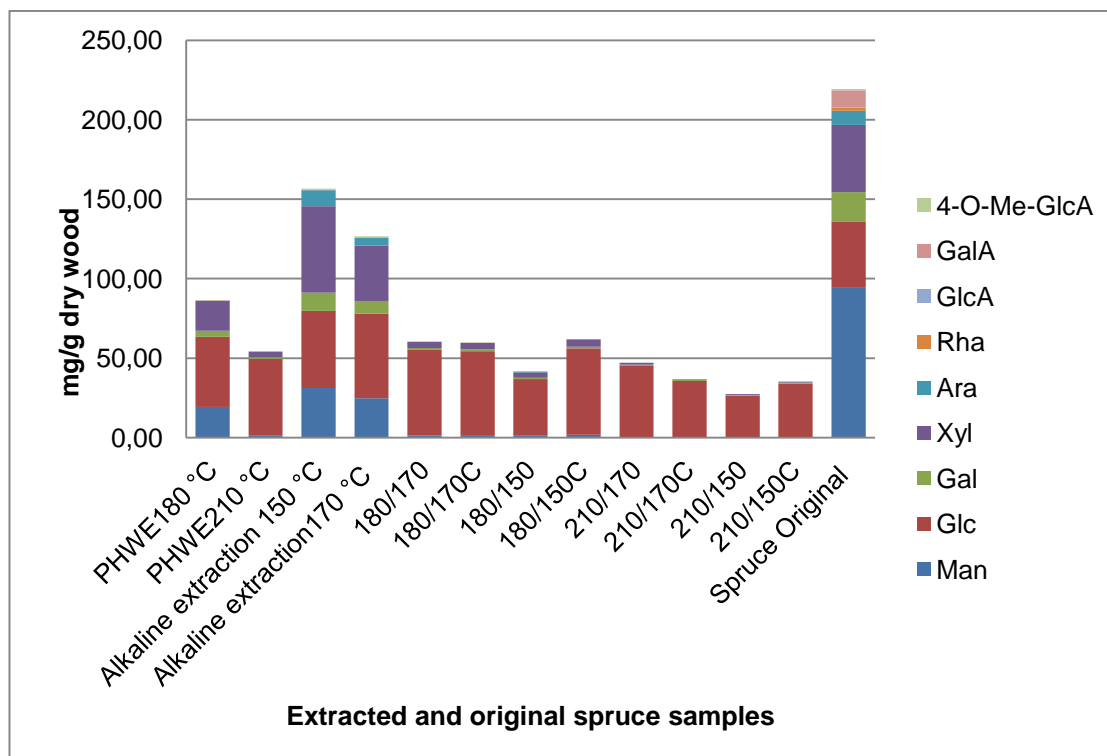


Figure 19. Hemicelluloses in extracted and original spruce sawdust. (C) refers to cooling before alkaline extraction

As shown in Figure 19, hemicellulose-based glucose was less affected by the extraction. Some fraction of the glucose might have disintegrated from the cellulose during severe pretreatment. Low temperature PHWE dissolved all the hemicelluloses except for glucose, mannose, xylose and glucuronic acid. At the high temperature most of the hemicelluloses except for glucose dissolved efficiently into the extract. Separate alkaline extractions did not seem to be effective to remove hemiculoses like galactose, xylose, arabinose and mannose. Combined extraction containing PHWE 180 °C removed most of the hemicelluloses other than glucose. Combined extraction with PHWE 210 °C seemed to be the most effective extraction to remove almost all the hemicelluloses other than glucose. Glucose along with galactose and mannose are hexoses and can be hydrolysed for the bioethanol production.

#### 5.4 Lignin content in the wood residual

Among the four separate extractions, both the alkaline extractions at 150 °C and 180 °C, removed more than 50% of the original lignin in the wood (Figure 20). Separate PHWEs were not as effective as alkaline extraction in terms of lignin removal, and removed just less than 45% of the lignin. At least one fourth of the lignin was present in every extracted sample. All the combined extractions dissolved between 54 to 73% of the original lignin, whilst combined extraction 210 /170C removed 73% of the lignin present in the 10 g of spruce sawdust.

In combined extraction, the effect of temperature in alkaline extraction had noticeable results. While increasing temperature of alkaline extraction from 150 to 170 °C, more amount of lignin was dissolved in the extract.

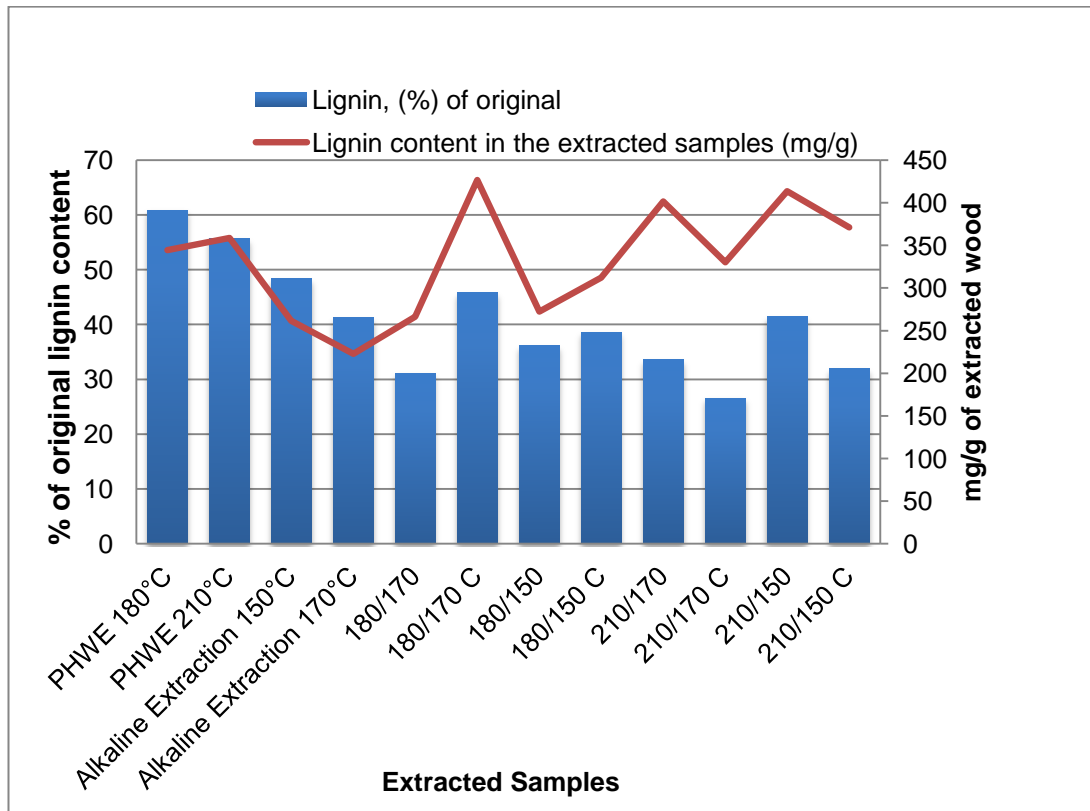


Figure 20. Lignin in the extracted wood residual, (C) refers to cooling before alkaline extraction

In combined extraction containing PHWE 210 °C, cooling yielded approximately 6% less lignin yield in the wood residual than without cooling. The result was reverse in the combined extractions containing PHWE 180 °C, as lignin yield due to cooling was increased by 2 and 15% for extractions 180/150 and 180/170, respectively. The results

can be explained by the fact that residual lignin in the wood is lower at high temperature PHWE and short extraction time [21]. It was found that increasing temperature of PHWE in combined did not favour the delignification. Higher temperature PHWE in combined extraction resulted in higher lignin content in the wood residual compared to combined extraction with lower temperature PHWE. Cooling increased the extraction time and more importantly favoured condensation of the lignin. Cooling might result in condensation of lignin onto the wood fibre resulting high residual lignin [21]. High molecular weight lignin may have condensed and stuck to the extraction capsule and capillaries. Therefore, at low temperature extractions residual lignin was higher due to cooling.

### 5.5 Ash content

Ash content in all the extracted samples was below 3%. The ash content of the original spruce was about 0.27%.

### 5.6 Analysis of the extracts

In PHWE 210 °C, brix value rose rapidly to 3.5 between 10 to 20 min and stabilized till 30 min meaning that most of the hemicelluloses dissolved during that time (Figure 21). After 30 min, brix value of extract from PHWE 210 °C, gradually lowered and by the end of extraction was 0.5. In PHWE 180 °C, brix value rose slowly and steadily to 2.5 between 10 to 30 min before reaching 2.4 after 40 min. During PHWE 180 °C most of the hemicellulose was extracted between 20 to 40 min. Hemicelluloses in the extract from PHWE 180 °C slightly decreased in 40th minute and by the end of the extraction gradually fell down to about 0.5. Comparing the brix values in Figure 21 below with the hemicellulose yield in Figure 18 above, it was quite clear that after 30th minute of PHWE 210 °C, most of the hemicelluloses from the spruce sawdust had been already dissolved in the extract and the wood had less hemicellulose left. In case of PHWE 180 °C, temperature seemed to be the limiting factor, and all hemicelluloses were not extracted. High temperature breaks the lignocelluloses matrix and the removal of hemicellulose becomes easier as the temperature increases. Although the final brix of both low and high temperature PHWEs were similar, at the end of the extraction wood residual from PHWE 180 °C had the high amount of hemicelluloses than the wood residual from PHWE 210 °C.

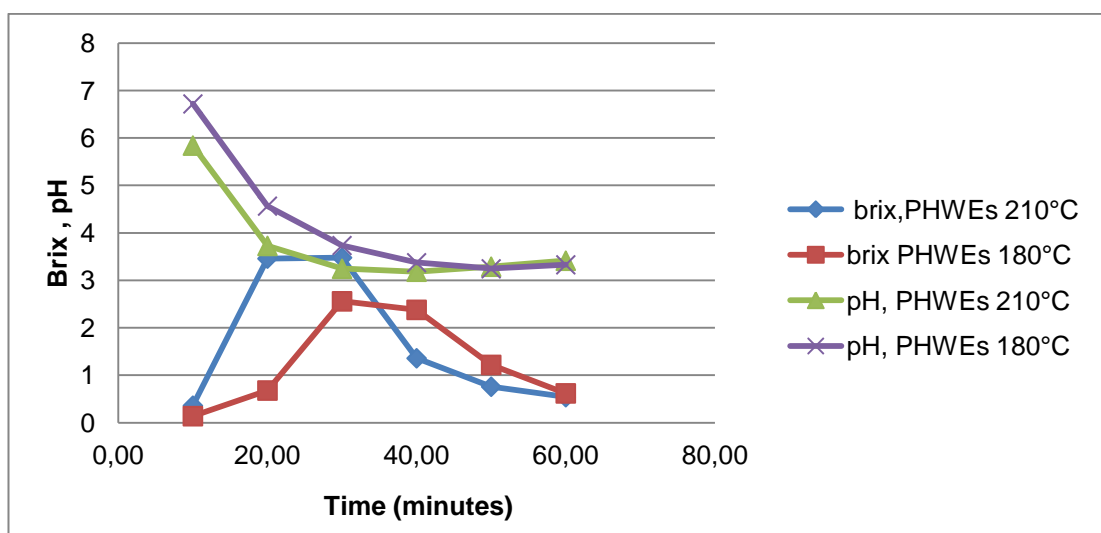


Figure 21. Average pH and brix of all PHWEs

Extracts from the PHWEs 210 °C was slightly more acidic compared to the extract from PHWEs 180 °C, however in both extractions, the same final pH of 3.5 was reached. High temperature might have liberated high amount of acetic acid from the hemicellulose chains and released excess amount of uronic acids from the pectins [10]. This has resulted in lower pH in higher temperature.

In alkaline extraction, pH was mainly affected by the alkaline solution (0.55 M NaOH). Most of the extract from the *filling* phase of alkaline extraction in combined extraction were slightly acidic. The alkaline solution (0.7 ml/min, 60 min) in the *filling* phase of combined extraction, might not have been enough to neutralise the acidic wood inside the capsule due to PHWE. While in separated alkaline extraction, pH was already above 7 in the *filling* phase. After the *filling* stage, pH of all the separate and combined alkaline extraction was between 12 and 13.

### 5.7 Composition after extraction

The composition of the extracted wood residual is shown in Figure 22. All the extractions produced wood residual containing between 47 to 65% of the cellulose. The composition of cellulose in the untreated spruce saw dust was 42% of the dry wood weight. Compared to original wood, cellulose composition in the extracted wood rose by 6 to 13%.

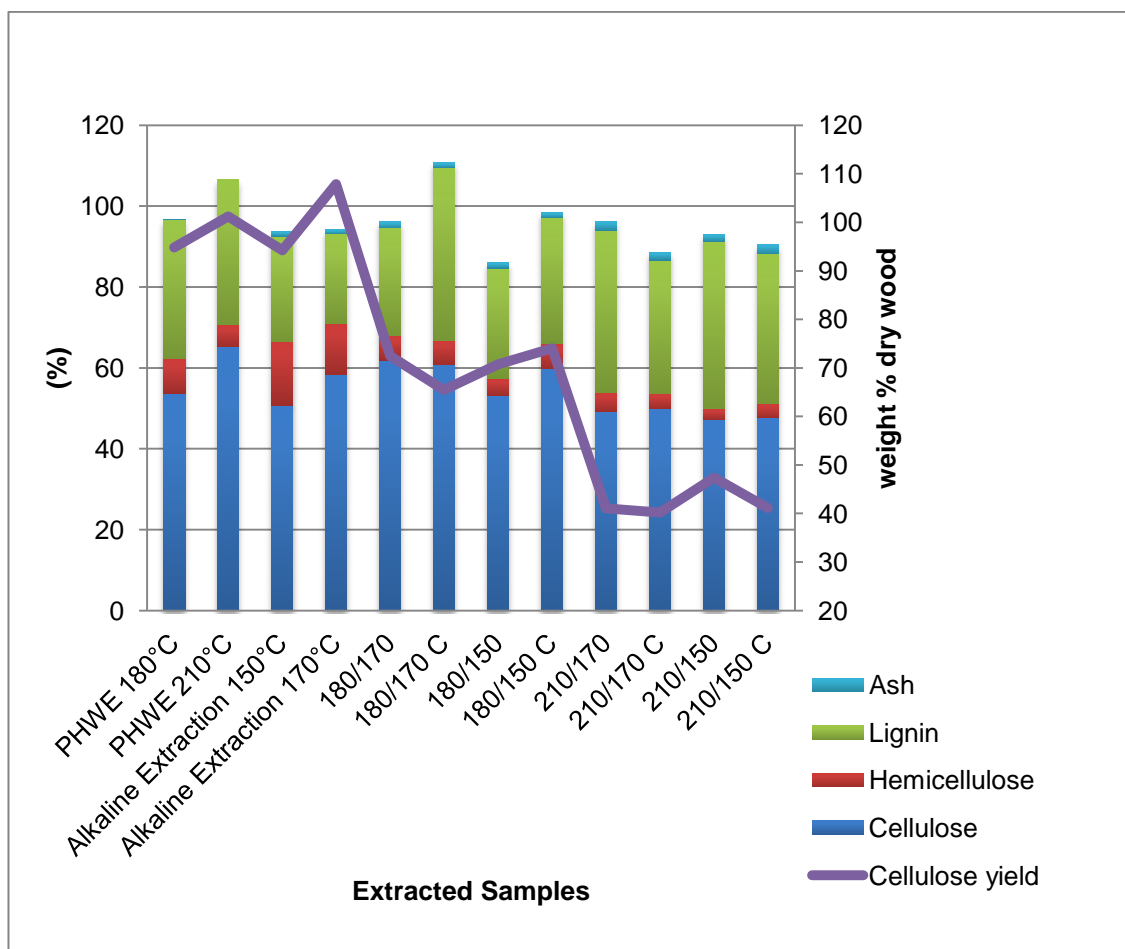


Figure 22. Composition of extracted samples and cellulose yield, (C) refers to cooling before alkaline extraction

Wood residual from combined extractions with PHWE 210 °C contained least cellulose of all extractions. Figure 21 shows that, during these extractions, approximately 60% of the cellulose was dissolved into the extract. Apart from cellulose, composition of lignin and ash were high in all combined extraction series 210/170(C) and 210/150(C). Wood residual from combined extractions 180/170(C) contained around 60% of cellulose. Lignin content in 180/170 was around 26%, which was comparatively less than the other combined extracted wood, but it rose to 42% in 180/170 °C due to condensation of lignin during cooling. Similarly, separate PHW extracted wood contained more than 53% of cellulose, less than 9% hemicellulose and more than 35% of lignin content. Alkaline extracted wood contained 12 to 15% of hemicelluloses which was highest hemicellulose composition of all the extracted wood residual. But the lignin content was less than 27%. In extractions, except for 180/150, cooling seemed to have smallest effect on cellulose composition. However, cooling seemed to have effected the composition of lignin in the wood residual. Lignin content in the wood residual from combined

extraction 180/170 and 180/150 increased significantly due to cooling. While cooling during combined extractions 210/150 and 210/170, decreased lignin content in the wood residual by 4 and 7%, respectively. The speculations were similar as mentioned in chapter 5.4. The composition of both PHWE 210 °C and 180/170C extracted saw-dust is over 100%, which is due to error as explained in chapter 5.2.

### 5.8 Heating power

Figure 23 shows the cellulose, hemicellulose and lignin yield in the residual wood and the heating power required to produce 100 kg on cellulose from spruce saw dust.

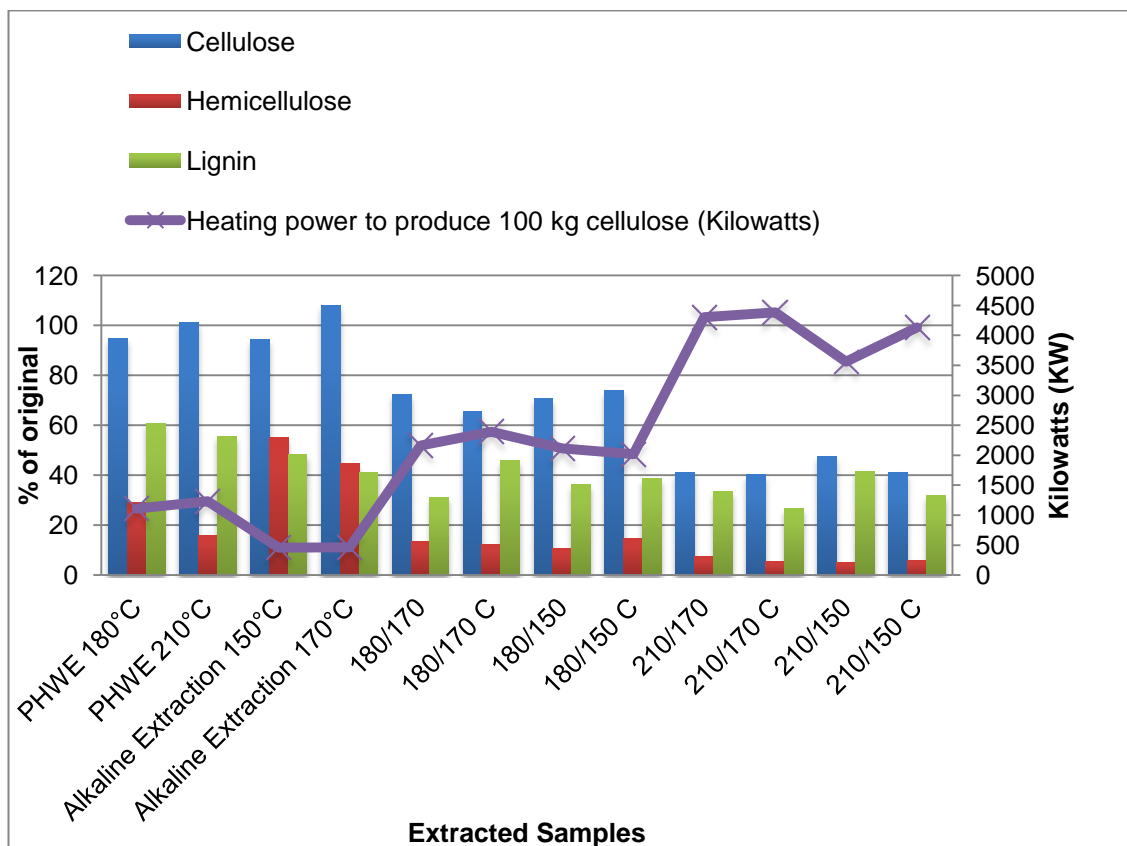


Figure 23. Cellulose, hemicellulose, lignin yield in the wood residual and heating power required to produce 100 kg of cellulose from spruce sawdust, (C) refers to cooling before alkaline extraction

Heating power required to produce 100 kg of cellulose was calculated based on the temperature applied during extraction, flow, retention time and cellulose content in the extracted samples. As a result, combined extraction consumed a large amount of energy as compared to separate extraction. Figure 23 shows that to produce 100 kg of

cellulose applying combined extraction 210/170, about 4 MW of energy is required for heating purpose. On the other hand, separate alkaline extraction required only about 500 kW of energy for heating. This value was significantly lower as compared with combined extraction 210/170. High cellulose yield, short retention time and low temperature during alkaline extraction may be the cause for low energy consumption. However, in large scale extraction, use of efficient heat exchangers could minimise the waste heat. In this case, heating power analysis may not yield conclusive results, but it is necessary to consider other factors, such as composition of extracted wood, hemicelluloses and lignin removal efficiency and efficiency of hydrolysis and fermentation.

### 5.9 Ethanol projection with complete hydrolysis

Figure 24 shows the estimated ethanol production from 1000 kg of extracted spruce saw dust, assuming complete hydrolysis.

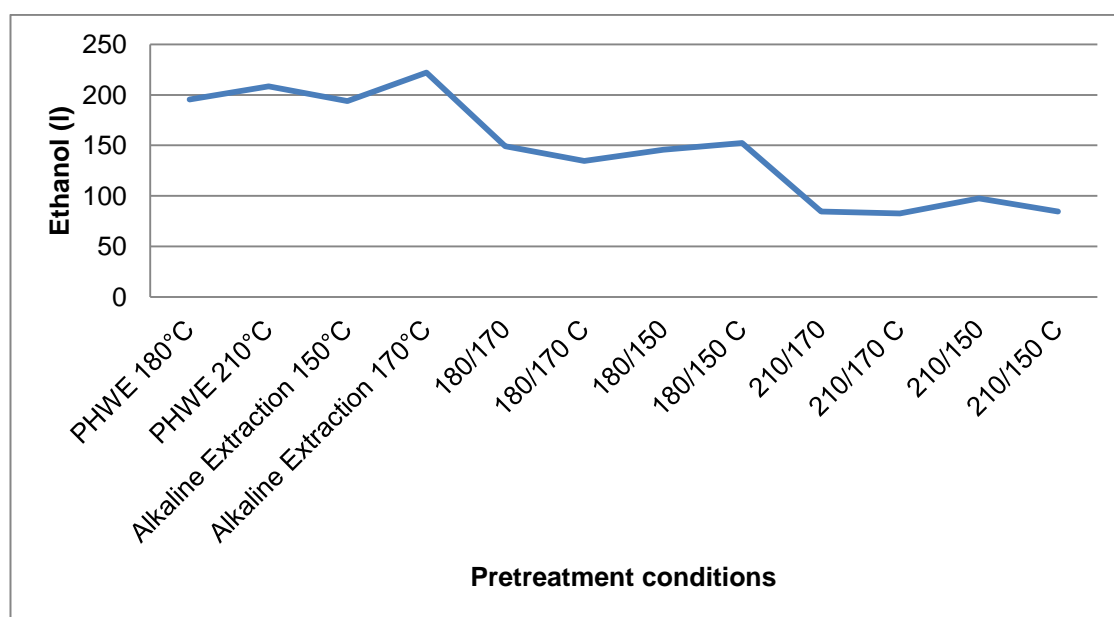


Figure 24. Ethanol production from 1000 kg raw spruce with complete hydrolysis, (C) refers to cooling before alkaline extraction

On the basis of cellulose yield during each extraction, the amount of cellulose yielded from 1000 kg of spruce was calculated. The amount of ethanol was estimated assuming complete hydrolysis, fermentation efficiency of 0.85, ethanol yield of 0.51 and process recovery at 0.90 [22]. With complete hydrolysis wood from the separate PHWEs and alkaline extractions can produce about 200 l of ethanol per 1000 kg of the spruce biomass. Similarly combined extractions 180(150,170) and 210(150,170) produced

about 150 and 100 l of ethanol, respectively. However, sugar recovery during hydrolysis cannot be 100%. Thus, the actual ethanol production would be lower than the projected value. For scientific results this experiment should be continued by hydrolysis and fermentation.

## 6 Discussion

The goal of extraction was to achieve extracted spruce, rich in cellulose and containing as small amount of lignin and hemicellulose as possible. Through the experiments it was concluded that the PHWE recovered most of the cellulose and removed almost two-third of the hemicellulose. However, PHWE turned out to be less effective method to remove considerable amount of lignin. The effect of increased temperature in PHWE was found to be effective for removing hemicelluloses, but several studies show that the increased temperature results in high content of inhibitory compounds, which later affect the hydrolysis-fermentation process. On the other hand, alkaline extraction dissolved more lignin than PHWE, but seemed less effective to remove hemicellulose. However, cellulose was less affected during separate alkaline and PHWEs. Cellulose yield in both cases were above 90%.

In order to understand the possible interaction between PHWE and alkaline extraction, combined extractions were designed. The idea was to use PHWE and alkaline extractions to dissolve most of the hemicelluloses and lignin from the raw material. Cellulose, hemicelluloses and lignin form complex matrix due to which cellulose cannot remain unaffected during combined PHWE and alkaline extraction. During fractionation of lignocelluloses some part of cellulose is torn out along with lignin and hemicelluloses. Figure 23 shows that significant amount of cellulose dissolved along with lignin during combined extraction. For example in 210/170C, to remove 73% of the original lignin, it cost three-fifth of the original cellulose. The effect of cooling inbetween the combined extractions produced noticeable difference but did not seem fruitful for the fractionation of lignocellulose. Cooling increased the extraction time and favoured the condensation of lignin. Condensation of lignin also increases the resistance to delignification. As a result lignin yield on the wood residual was higher in 180/150(C) and 180/170(C).

During the PHWE, most of the hemicellulose was dissolved into the extract. The release of the acidic groups of hemicellulose increased the acidity of the extract during

PHWEs. As a result, pH of the extract at the end of all PHWEs was measured to be near 3, which is shown in Figure 21. Most of the hemicellulose, i.e. about 70% during PHWE 180 °C and about 85% during PHWE 210 °C had been removed by the end of the PHWE. As shown in Figure 19, most of the acidic group in hemicellulose were extracted during PHWE. As a result, alkaline extraction became more severe to the spruce sawdust. In lignocelluloses, hemicellulose acts as the connector between cellulose and lignin giving rigidity to the whole structure. When most of the hemicellulose was dissolved from the solid wood fraction, cellulose became less resistive to the alkaline condition and a significant amount of cellulose was dissolved during alkaline extraction phase of the combined extraction.

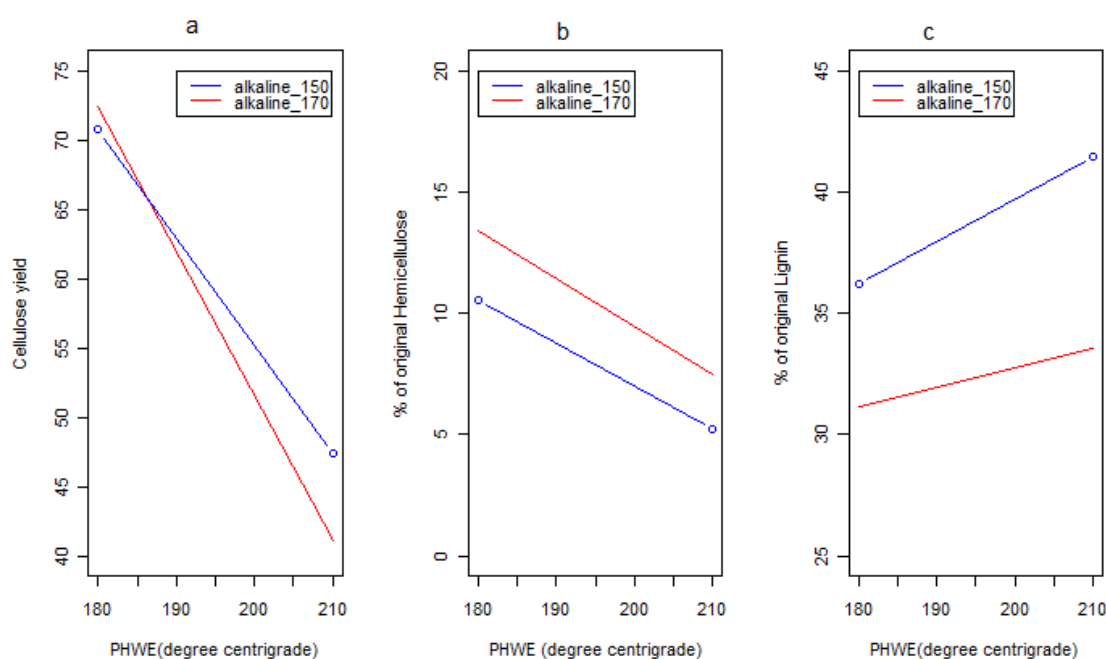


Figure 25. Percent of original cellulose, hemicellulose and lignin in wood residual, after extraction.

The interaction of PHWE and alkaline extraction on the cellulose, hemicellulose and lignin composition is shown in Figure 25. Figure 25(a) shows that, as the temperature in the hot water extraction increases, a large proportion of the cellulose degrades in the alkaline extraction. However high temperature PHWE combined with low temperature alkaline extraction favours the hemicellulose degradation. Figure 25(c) shows the interaction between alkaline extraction and PHWE on the lignin removal. High temperature PHWE has a negative impact in the lignin removal. As the temperature of the PHWE increased, it was more difficult to remove the lignin from the wood residual. It seems that the alkaline extraction in the combined extraction is mainly focused on cellulose

degradation. As a result, lignin becomes less affected. High temperature PHWE during combined extraction did not favour the fractionation of spruce sawdust to form cellulose rich wood residual. The degradation of cellulose could be minimized by lowering the temperature during PHWE. Other factors which might affect the fractionation are NaOH charge, flow rate and retention time.

## 7 Conclusions

There are various pretreatment methods which have been developed and implemented in the biorefinery sector. The choice of the pretreatment methods for particular type of biomass depends on composition of the biomass and the byproducts produced by pretreatment methods. One method that is efficient for a particular biomass might not deliver similar results to the other type of biomass. This thesis project was focused on the PHWE and alkaline extraction of Norway spruce. During the project, it was found that PHWE is very effective pretreatment method in terms of hemicellulose removal. Similarly, another advantage of the PHWE is the use of green solvent 'water', which is more environmentally friendly than other chemical solvents. Another pretreatment method, alkaline pretreatment, was found to be effective in terms of lignin removal. Alkaline pretreatment also causes less degradation of cellulose. Combining these two pretreatment methods could remove subsequent amount of hemicellulose and lignin from the lignocellulose matrix of spruce. However, temperature of the extraction was found to be crucial factor in terms of wood and cellulose recovery. High temperature PHWE and alkaline extraction resulted in low cellulose yield and low cellulose composition in the wood residual. Lower temperature PHWE combined with alkaline extraction could be beneficial for the bioethanol production as the cellulose degradation is smaller than in higher temperature combined extraction.

Bioethanol from biomass is a biorefinery concept. Biorefinery is used to produce fuels, chemicals and power through integrated process. Fractionation is crucial step for the production of biorefinery products, but each fraction obtained can be used for different purposes in order to minimise waste. From the fraction of cellulose and hemicellulose, chemicals like furfural and HMF can be made [2]. There are many valuable products which can be produced from the lignocellulose derived sugars. Lignin can be incinerated to generate power or thermo chemically converted to syngas [2]. The cost of bioethanol from various wood varies between 0.33-1.0 \$/L (Nystrom et al. 1985; Lynd et

al. 1996; von Sivers and Zacchi 1996 cited in [23]). Production cost of ethanol can be reduced in a refinery plant combining the production of fuel, chemicals and power from the different fractions.

Apart from pretreatment, there are several factors such as energy management, sustainable production and supply of biomass, cost of biomass, enzymes cost, cost of bio-ethanol purification, waste management and technology development which come along with biofuel production from the biomass. Several studies show that biomass could be a potential alternative energy source. Forest and agriculture residual and cellulose based waste could be utilised to produce high value chemicals and fuel, but the environmental impact assessment of different product chain is essential.

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## Appendix 1. Equipment used

- GC: Shimadzu GC-2010 (column Agilent HP-1, inner diameter 0.2 mm)
- Autoclave( Tuttnauer 3870 ELV)
- Autoclave ( Sturdy SA-232X)
- Vacuum oven( Heraeus VT 6025)
- Muffle oven ( Naher N 11)
- Centrifuge ( Heraeus Multifuge X3R)
- Ultrasonic bath ( Elma S 300)
- Extraction pump (Water 510 HPLC pump)
- Extraction oven ( Varian 3400 GC)
- UV-spectrophotometer (Shimadzu UV-2401PC, Shimadzu, Kyoto, Japan)
- Freeze dryer
- Hand refractometer
- pH meter

### GC-FID (Gas Chromatography- Flame Ionisation Detector)

GC-FID is commonly used GC detector which uses flame source to ionise compounds. It is commonly used for the analysis of organic compounds. It can detect all the compounds that ionise in hydrogen and air flame. Injected samples combusts in hydrogen/air flame and ionise. Due to large electric potential applied across the burner, current is produced by the ionised particle as they move toward the collector. Current produced is directly proportional to the sample being burned. The produced current is measured by the electrometer, converted to digital form and sent to an output device. FID is a mass sensitive detector and responses to the amount of material passing through the flame at a given time.

## Spectrophotometer

Spectrophotometer measures the absorbance of the sample, which later relate to the concentration on the absorbing molecules. Spectrophotometers are of different type according to the wavelength, at which they can measure absorbance. UV-VIS (ultraviolet visible) spectrophotometer can measure the absorbance in the ultraviolet and visible wavelength ranges of light (190 to 1100 nm). Light source is passed through diffraction grating or prism, which break into individual wavelength of light. Slit in front of sample is adjusted to let the selected wavelength to reach to the sample. A photoelectric tube, which measures the light passed through the sample (transmittance), is placed after sample, linearly to the light source, slit and the sample. Absorbance is calculated according to following equation.

$$\text{Absorbance} = \log \left( \frac{1}{\% \text{ transmittance}} \right)$$

Absorbance is unit less, however absorbance should mention with wavelength (e.g., absorbance at 203 nm).

## Autoclave

Autoclave is used to sterilised equipments and samples by placing under saturated steam at 121 °C.

## Ultrasonic bath

Ultrasonic bath produces vibration with frequency 18 kHz. Because of the high frequency vibration, smallest vacuum bubbles are formed in the liquid. It implodes during a high pressure phase creating a high pressure wave. This process is widely used for removing dirt particle from the object to be cleaned. In chemical analysis, it can be also used to mix samples, which otherwise would be very hard to do.

## Heating block with nitrogen hood

It is equipment used in analysis work to evaporate the moisture from the samples. Samples are loaded in a heating block and a nitrogen blowdown is introduced to the samples to speed up the evaporation process. Nitrogen is an inert gas and does not

take part in chemical reaction with the samples. Heating temperature and the nitrogen flow can be manually controlled.

#### Freeze dryer

It is used for freeze drying of different materials. Frozen materials are placed in the freeze dryer and the pressure is reduced so that the frozen water (moisture) is directly released into gas phase. Moisture can deteriorate the stored material, providing the condition for autolysis, or the growth of spoilage organism [20]. This procedure is widely used to store the materials for long period of time. In laboratory, it can be used for drying the samples in order to preserve the quality.

#### Vacuum oven

Vacuum oven is used in different fields, such as biochemistry, pharmacy, medicine and health, agricultural and scientific researches, for drying and sterilization. It is mainly designed for drying thermo-sensitive and oxidative material. Vacuum created inside the chamber reduces the boiling point of water and which facilitates the drying process at low temperature. This technology can be ideal for drying temperature-sensitive materials. In addition, drying process is shortened and efficient.

## Appendix 2. Chemical Analysis

### Cellulose Analysis

The amount of cellulose in the original and the extracted spruce sawdust was determined by the acid hydrolysis method, according to Sundberg et al. (2003). Two parallel samples were prepared during cellulose, hemicelluloses and lignin analysis.

Approximately 10 mg of the original and the extracted spruce samples and cotton linters calibration standards (sigma Aldrich) were taken into 25 ml test-tubes. A glass ball was added to each test tube. Samples were hydrolysed with 0.2 ml of 72% sulphuric acid and left in the fume hood for 2 h. After that, 0.5 ml of deionised water was added to each samples and left for another 4 h. All the samples were again diluted by adding 6 ml of deionised water and left overnight inside the fume hood.

Samples were placed into a autoclave at 121 °C for an hour. During that period, internal standard (250 µg of Sorbitol and 250 µg of Resorsinol) were diluted to 50 ml deionised water. Autoclaved samples were cooled down in room temperature. Two drops of bromocresol green (indicator) was added into each sample. In order to neutralize the samples, generous amount of barium carbonate was added into each samples and mixed until the solution turned blue. 1 ml of freshly prepared internal standard was added to the samples. After that, neutralized sample were centrifuged for 20 min at 1500 rpm.

0.5 ml of the clear aliquot from the centrifuged samples was transferred into individual 10 ml test tube. 1 ml of acetone was added and the samples were dried using a heating block at 60 °C with nitrogen hood. The samples were further dried in vacuum oven for 15 min.

For silylation, 200 µL of pyridine, 150 µL of hexamethyldisilazane (HMDS) and 70 µL of trimethylchlorosilane (TMCS) were added to the vacuum dried samples. Samples were mixed in the vortex and left overnight to silylate. The next day, sample were analysed with GC-FID.

### Hemicellulose Analysis

The amount of hemicellulose in the original and extracted spruce saw dust was determined using acid methanolysis. Two parallel samples were prepared during analysis. About 10 mg of sample in pear shaped flask were depolymerised with 2 ml of methanol reagent (2 M HCL in MeOH). After that, samples were placed in oven at 100 °C for 5 h. For standard preparation, 1 ml of sugar calibration standard was added to two pear shaped flask, and dried in a heating block at 60 °C with nitrogen hood. 2 ml of methanol reagent (2 M HCL in MeOH) was added to the standard and placed into oven, after two hours other samples had been in oven. That way, Sample got five hours and the standards got three hours into the oven, and both are removed at the same time from the oven.

After the samples were cooled and depressurised, 200 µL of pyridine was added to neutralise the samples. Again the samples were mixed in vortex. 1 ml and 4 ml of internal standard (0.1 mg/ml sorbital and 0.1 mg/ml resorcinol) was added to the standard and samples respectively. After that, the Sample and the standards were treated similarly. Sample were then heated and evaporated in a heating block at 60 °C, under nitrogen hood until all the methanol had evaporated. Samples were dried in vacuum oven at 40 °C for 15 min.

Samples were silylated with 100 µL of pyridine, 150 µL of hexamethyldisilazane (HMDS) and 70 µL of trimethylchlorosilane (TMCS). All the samples were mixed in vortex and left overnight for silylation. Silylated samples were analysed for non-cellulose carbohydrates using GC-FID.

### Lignin Analysis

Two parallel samples were prepared during analysis. Analysed samples were not free from extractives.

About 300 mg of the original and the extracted spruce sawdust were measured in 30 ml test tube. 3 ml of 72% sulphuric acid was added to the sample and mixed gently in vortex. Samples were further mixed in a ultrasonic bath for an hour. Samples were mixed in vortex, every ten minutes, to help sulphuric acid distribute thoroughly to the samples. Samples were transferred with deionised water (80 ml) to individual Erlenmeyer flask. Flasks were closed with aluminium foil and put in a autoclave at 121 °C for an hour.

Sinter classes with pores diameter 4 mm were put in oven at 105 °C for half an hour and cooled in desiccators, before measuring dry weight. Autoclaved sample were cooled and filtered through sinters with suction bottles. Precipitation on the sinter was washed with 30 ml of deionised water. Soluble lignin was collected as an aliquots and the insoluble lignin as a precipitation on the sinter classes. After filtration, the sinter classes were placed in oven at 105 °C for overnight. Sinter classes were cooled in desiccators and weighted. The mass in mostly lignin as there is very less ash and extractives in spruce.

Acid soluble lignin from the suction bottle was transferred into 250 ml volumetric flask and diluted to full, with deionised water. For calibration sample, 3 ml of 72% of sulphuric acid was diluted to 250 ml volumetric flask. Then the sample were analysed in UV-spectrophotometer (Shimadzu UV-2401PC, Shimadzu, Kyoto, Japan) at 203 nm using an extinction coefficient 120 Lg<sup>-1</sup>cm<sup>-1</sup>. Calibration sample was used to zero the absorbance, at wavelength 203 nm. Absorbance of the samples was measured between 0.2 - 0.7.

Dilute lignin was calculated from the absorbance using following formula.

$$\text{Soluble lignin} = \frac{A*(100-u)}{K*m4*L} - B$$

Where,

A = Absorbance at 203 nm

u = amount of extractive

m4= amount of dry extracted sample

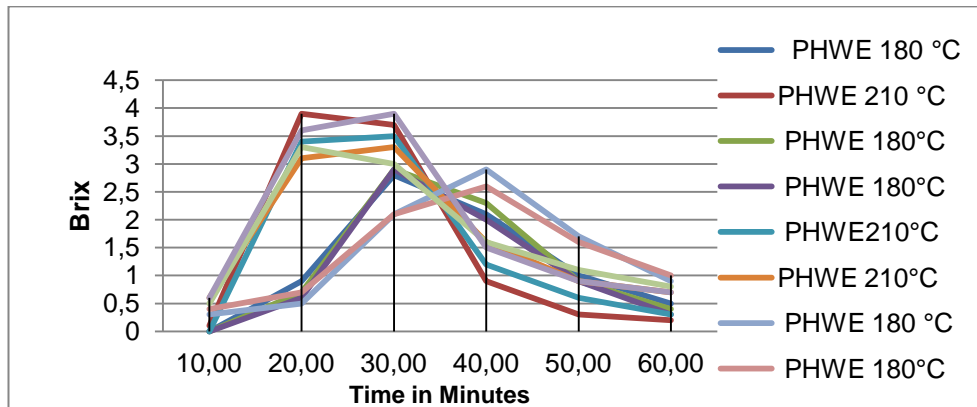
K = Extinction factor, 128 L/gcm for softwood and 110 L/gcm for hard wood

L= Dilution factor

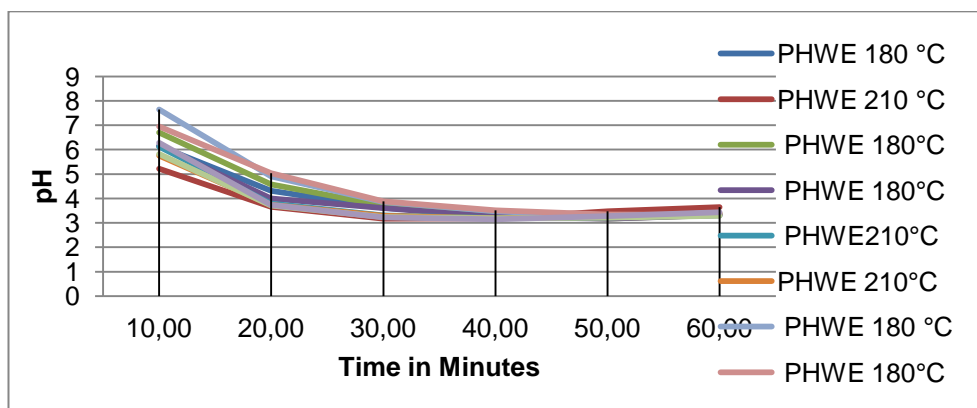
B carbohydrate correction factor, 0.3 for 0.5 g sample and 0.18 for 0.3 g sample

### Appendix 3. Individuals brix values of PHWE samples.

Individual Brix values of extract



Individual pH of extract



#### Appendix 4. Size distribution of the spruce saw dust.

Size distribution of the spruce saw dust used, is given below.

| Size (mm)   | pan  | 0,05 | 0,10 | 0,20 | 0,50  | 1,00  | 2,00  | 4,00  | 8,00 |
|-------------|------|------|------|------|-------|-------|-------|-------|------|
| Average (%) | 0,10 | 0,55 | 1,18 | 6,21 | 13,87 | 29,94 | 34,65 | 12,85 | 0,65 |

Where pan refers to size smaller than 0.05 mm

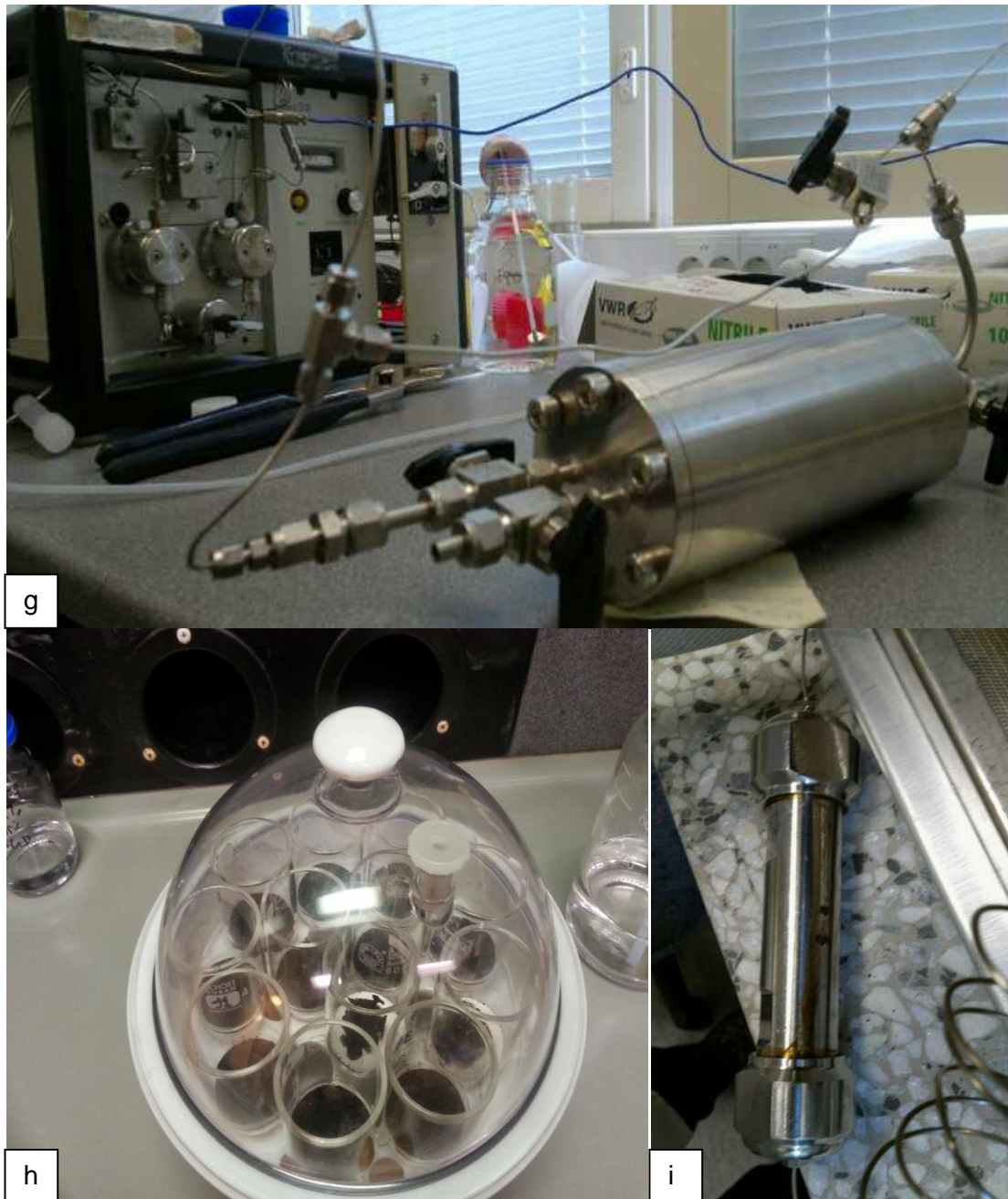
The size distribution is the average of different batches of saw dust from the same saw mill.

## Appendix 5. Pictures of the experiment set up



Pictures (a) Manometer showing pressure in the extraction line (b) Capsule inside oven (c) Extract collection during extraction phase of alkaline extraction (d) dried sample ready to be milled (e)

Mill used to reduce the size of the extracted sample (f) Sample on the heating block with nitrogen hood



Pictures (g) Cylinder used in alkaline extraction (h) Sinter classes containing lignin precipitation inside desiccators (i) Picture showing extract leaked during alkaline extraction, experiment was redone.