Co-pretreatment of spruce and poplar for ethanol production

Effects of mixed feedstocks on pretreatment and enzymatic hydrolysis efficiency

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Department of Chemical Engineering
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Abstract

Oil is very important for today's society and the transportation sector is almost completely dependent on this material. Unfortunately, it affects the level of greenhouse gases negatively which in turn contribute to global warming. Therefore, it is necessary to reduce greenhouse gas emissions, especially in the sectors that emit the most, as transportation sector. The European Parliament has decided that by year 2020, 20 % of all fuels used in European transportation sector should be based on renewable energy sources. However, it is not easy to find an energy source that will replace oil, but one of the leading alternatives is ethanol produced from biomass.

There are many different raw materials that can serve as feedstock in ethanol production process, both first generation and second generation. However, within the same plant, usually the feedstock is not mixed and the ethanol process is optimized for one material. Diversifying feedstock base may lead to improved supply efficiency and, thus improved profitability. However, the heterogeneity of feedstock mixtures makes the concurrent processing of multiple feedstocks more challenging, which requires further investigations. This study concentrates on exploring the possibility to co-pretreat spruce (softwood) and poplar (hardwood) to enable to utilize mixed feedstock blends for ethanol production in a second generation process.

In this study it has been showed that there are no huge effects or synergies by mixing poplar and spruce on enzymatic hydrolysis. Although, if a 50% blend of spruce and poplar not possible to separate is to be pretreated, there is a wide range of conditions that can be applied and still obtain the same glucose yield after enzymatic hydrolysis.

Also, the study has showed that glucan amount was increasing and the concentrations of monomeric glucose were decreasing with higher percentage of poplar in the feedstock mixtures. From the pretreatment step it was concluded that poplar needs more severe pretreatment than spruce in order to dissolve hemicelluloses to the same extent.

The main conclusion from the study is that it is not effective to mix poplar and spruce considering sugar recoveries after enzymatic hydrolysis. When poplar and spruce are mixed, lower glucose recoveries than for pure materials are obtained after enzymatic hydrolysis. The mixtures have to be further test for fermentability in order to draw any conclusion on ethanol yield.

Populärvetenskaplig sammanfattning

Fossila bränslen är mycket viktiga för dagens samhälle och transportsektorn är nästan helt beroende av dem. Tyvärr påverkar de miljön negativt och bidrar till att växthusgasnivå ökar. Jordens medeltemperatur har ökat med 0.8°C sedan början av industrialiseringen. Den globala uppvärmningen är ett naturligt förlopp, men har under de senaste åren varit en stor del av människors bidrag. Idag har atmosfärens kvalitet blivit irreversibelt förstörd och om man inte gör något åt det, kommer jordens framtid inte att vara så ljus.

Det är därför nödvändigt att minska utsläppen av växthusgaser, särskilt i de områden där de emitteras som mest. Ett av de viktigaste områdena är transportsektorn. Myndigheterna är medvetna om risken i samband med de negativa effekterna av växthusgaser på miljön. År 2007 har Europaparlamentet beslutat att 20 % av alla bränslen som används inom den europeiska transportsektorn ska vara baserade på förnybara energikällor år 2020. Målet i Sverige är att användningen av förnybar energi ska nå ända upp till 50 % av den totala energianvändningen år 2020.

Dock, är det inte så enkelt att hitta en ny energikälla som kommer att ersätta fossila bränslen i transportsektorns befintliga infrastruktur, men ett av de ledande alternativen är etanol. Valet av råmaterial som används till produktion av etanol kan variera från område till område och traditionellt är etanolprocessen optimerad för en typ av material. Men, en breddning av råmaterialbas kan leda till förbättrad leveranseffektivitet och därmed förbättrad lönsamhet. Bearbetning av flera råvaror är samtidigt en utmaning och leder till ytterligare undersökningar.

Denna studie har undersökt hur poppel, gran och blandningar av dessa två påverkar förbehandling och enzymatisk hydrolys med mål att ge en grund för ytterligare undersökningar om jäsning för framställning av etanol. Studien visar att det inte finns några större effekter på enzymatisk hydrolys av att blanda poppel och gran. Däremot, om man har 50 % blandning av poppel och gran som ska förbehandlas så finns det ett brett spektrum av betingelser som kan appliceras för förbehandlingssteget och som kan leda till samma resultat efter enzymatisk hydrolys när det gäller glukosutbyte.

Studien har även visat ett tydligt mönster gällande sockerhalter erhållna i flytande och fast fas efter förbehandling. Mängden glukan ökade och monomer glukos minskade med högre procentandel poppel i materialet. Från förbehandlingssteget kan man även dra slutsatsen att poppel behöver tuffare förbehandlingsförhållande än gran för att lösa upp hemicellulosan i samma utsträckning.

Med tanke på glukosåtervinning efter enzymatisk hydrolys är det inte effektivt att blanda poppel och gran. Blandningarna måste fermenteras för att dra slutsatser om hur de påverkar etanol.

Acknowledgments

This master thesis was done for 20 weeks at the Department of Chemical Engineering. Many students say that writing a master thesis is the most interesting time during the education, and this was truly even in my case. This was a wonderful experience and a pleasant time to remember supported by a strong group from the department.

I would like to thank Dr. Ola Wallberg for giving me the opportunity to do my master thesis at the department. I also want to thank my examiner Krisztina Kovacs and my supervisor Mats Galbe for their time, information and advices. A special thanks to my main supervisor Balázs Frankó that was always there for me when I needed help. Thank you for all inspiring discussions and knowledge you have provided me with.

Finally I would like to thank my family and friends for motivating me during challenging periods.

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Abbreviations

ASL= acid soluble lignin
AIL=acid insoluble lignin
DMC= direct microbial conversion
DM= dry matter
HPLC= High Performance liquid chromatography
NREL= National Renewable Energy Laboratory
SHF= separate hydrolysis and fermentation
SSF= simultaneous saccharification and fermentation
WIS= water insoluble solids

1. Introduction

1. 1 Background

Oil is a fantastic material because of its chemical composition and volumetric energy content that allows it to be used in so many different applications. It has been used since ancient times and had a positive impact for the development of automobile and aircraft industries during the 20th century. Therefore it is very important for today's society and the transportation sector is almost completely dependent on this material (Allain, 2015).

Unfortunately, oil has a negative impact on the environment. When burnt, it affects the level of greenhouse gases negatively which in turn contribute to global warming. Global warming is the continuing rise in the average temperature of Earth's climate system. The Earth's temperature has rapidly increased with 0.8°C since the beginning of the industrial age (NASA, 2017). Global warming is a natural phenomenon but has over the years been majorly contributed to by humans. Today the quality of the atmosphere has been irreversibly destroyed over time and if nothing is done about it, the future of planet Earth will not be bright.

Greenhouse emissions have to be reduced, especially in the sectors that emit the most. One of the major sectors is the transportation sector (Environmental Protection Agency, 2017). The authorities have been aware of the increased risk associated with the negative impact of greenhouse gases on the environment. In 2007, the European Parliament has decided that 20% of all fuels used in European transportation sector should be based on renewable energy sources by year 2020 (Directive, 2009). The goal in Sweden is that the use of renewable energy should reach 50 % of total energy use by the year 2020 (Energidepartementet, 2015).

Another problem with oil sources is that they are not endless. The usage of oil increases linearly with time (Roser M, 2017). Two main reasons for that are that world population increases and that more countries are becoming industrialized which in turn leads to higher oil demands (Roser M, 2017).

However, it is not easy to find an energy source that will replace oil in the existing infrastructure of the transport sector, but one of the leading alternatives is ethanol. Ethanol has a lower flame temperature and a higher octane number than petrol, which is favorable in efficiency perspective (Agency, 2017). Traditionally, first generation ethanol is produced from sugar or starch-containing materials. The limitations of the process is using food crops as raw material which could lead to higher food prices and shortages in some countries and is morally questionable. Therefore, second generation ethanol production process utilizing non edible lignocellulosic raw materials has been developed (Aditiya H.B, 2016).

Many types of lignocellulose may serve as feedstock for second-generation ethanol production. The choice of feedstock can vary regionally and depends on various factors. Usually in the same plant, the ethanol production process is optimized for only one feedstock. However, the quality and supply variation of raw materials calls for an expansion of feedstock

base. By diversifying feedstock base, as for instance mixing two different materials, consistent input quality and improved supply efficiency can be achieved which contributes to improved profitability. The challenge with mixing feedstock is the differences in raw materials as for example chemical composition depending on which materials are used. Also, one more important aspect is the conditions that are applied on the different steps in the ethanol process. One condition should suit both materials and still lead to the achievement of a desired ethanol yield. These challenges require further investigations on how feedstock mixtures affect ethanol process (Nielsen F, 2017).

1. 2 Aim

The aim of this study was to investigate the effects of blending hardwood (poplar) and softwood (spruce) in various ratios on pretreatment and enzymatic hydrolysis efficiency in order to give a base for further investigations on fermentation to obtain ethanol. The main focus was to determine the best conditions for steam pretreatment step for different mixtures. The steam pretreatment is evaluated on the chemicals composition and enzymatic hydrolysability of the pretreated materials.

The conversion of lignocellulosic material to ethanol contains multiple steps and it is an integrated process. If single process parameters are changed, the entire downstream process can be affected in different ways (Novy V, 2015). The pretreatment is an integral part of the conversion process, and therefore the challenge was to obtain as high sugar yields as possible after the enzymatic hydrolysis step when combining hardwood and softwood.

Beside the experimental work, an extensive literature review is done in order to determine which conditions for the steam pretreatment step have been previously tested. The purpose of the study was to get better understanding in how the bioethanol is produced and even learn what impact the blended feedstock of poplar and spruce have on the process.

2. Literature review

2.1 Wood

Wood has had an important role through history of time, from Stone Age to 21th century. The beginning of 21th century brought the consideration of the issues dealing with environment, sustainability, energy, recycling etc. In many ways, we are rediscovering wood as material. Wood has unique and useful properties and it is recyclable, renewable, and biodegradable. It can be converted into many useful industrial chemicals, as for example ethanol, anti-bacterial medical agents, glue applications. However, to understand how important wood and wood materials are for our ‘’modern society’’ and how they can serve as chemical feedstocks we first need to understand wood chemistry and wood material properties (Rowell, 2005).

The chemical structure of wood consists of an interconnected network of cellulose, hemicelluloses, and lignin with small amounts of inorganics and extractives. While a living tree mostly consists of water, the dry-weight basis contains mainly sugar-based polymers (carbohydrates, 65-75%) that are combined with lignin (18-35%). The elemental composition of dry wood is 50% carbon, 6% hydrogen, 44% oxygen, and the small amount of inorganics (Rowell, 2005).

There are fundamental differences between different kinds of wood in types, sizes, proportions and arrangements of different cells that build up the wood. There are two types of wood, softwoods produced from gymnosperm trees and hardwoods produced from angiosperm woods. These two types can be distinguished by simple chemical analysis to show how their chemical composition differs. ***Table 1*** represents the summary of the carbohydrates, lignin and ash compositions of hardwoods and softwoods (Rowell, 2005) . The properties of hardwood and softwood are presented in the sections below.

Table 1. *Carbohydrates, lignin and ash content in hardwoods and softwoods*

Type of wood	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractives (%)	Ash (%)
Hardwoods	42.0±2.0	27.0±2.0	22.0±2.5	3.0±2.0	0.5±0.3
Softwoods	45.0±2.0	30.0±5.0	29.0±3.0	5.0±3.0	0.3±0.1

2.1.1 Softwood

The structure of softwood is simpler than the structure of hardwood. The axial or vertical system is composed of axial tracheids. Tracheids are very long cells and are taking up to 90% of the softwood material. This component gives softwood its conductive and mechanical function. The radial or horizontal system or in other words the rays are composed of ray parenchyma cells. These cells are barely visible and can be brick-shaped (Rowell, 2005). Some examples of softwood are spruce, pine and fir.

2.1.2 Hardwood

The structure of hardwood is more complicated than the structure of softwood. The radial or horizontal system contains of rays as in the softwood. The difference is that ray parenchyma cells can have different sizes and shapes. The axial or vertical system contains different

fibrous elements as for example vessel elements. The cells in the vessels are stacked one on another which forms vessels. The porosity of the hardwood is decided on the size and the arrangement of the vessels. For example if the vessels have the same size and are scattered throughout the growth ring, the wood is diffuse porous. On the other hand, if the vessels are much larger, the wood is ring porous. The fibers in hardwood are shorter than in the softwood and only function as support. Density and strength depends on the thickness of the fiber cell. If the fiber cell is thin, then the density is low (Rowell, 2005). Some examples of hardwood are poplar, birch and aspen.

Differences between softwood and hardwood are visible at both microscopic level and on the surface. ***Figure 1*** represents the view under the microscope of hardwood (above) and softwood (below) (Diffen, 2013).

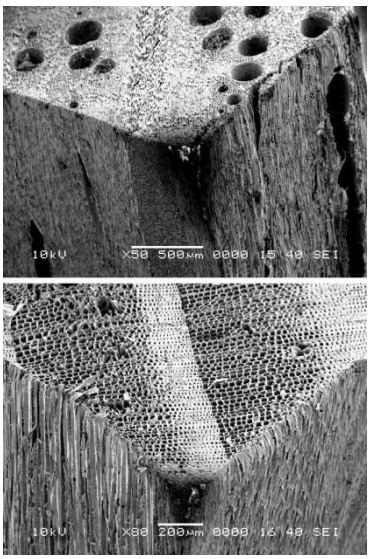

Figure 1. *Softwood (above) and Hardwood (below) under the microscope* (Diffen, 2013).

2.1.3 Carbohydrate polymers

Wood contains of two main carbohydrate polymers (cellulose and hemicellulose) which together with lignin build a strong network which provides trees with a transport system for water and nutrients. These carbohydrate polymers are described in the sections below.

2.1.3.1 Cellulose

Cellulose is an organic compound that consists of several hundred to many thousands β (1-4) linked D-glucose units which make linear chains. ***Figure 2*** represents the repeating unit cellobiose which make the structure of cellulose. Strong hydrogen bonds between the linear chains make the cellulose structure highly ordered. Cellulose is strong and resistant to hydrolysis (Rowell, 2005). It exists in the secondary cell wall and constitutes up to 50% of the dry substance in most wood species. Cellulose is insoluble in water (Hoyer, 2013).

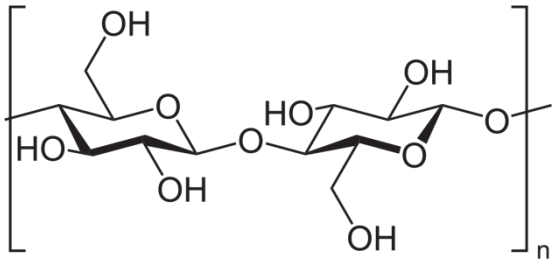

Figure 2. *The repeating unit cellobiose in cellulose* (Wikipedia, 2017).

2.1.3.2 Hemicellulose

The structure of hemicellulose is more complex than the structure of cellulose. Hemicellulose is made of polysaccharides that contain many different sugar monomers, as well as sugar acids. The structure of hemicellulose is presented in ***Figure 3***. Xylan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan are all part of the hemicellulose. The structure of hemicellulose is random, amorphous with little strength (Rowell, 2005). Together with cellulose, it makes a network for a structural backbone of the wood cell wall.

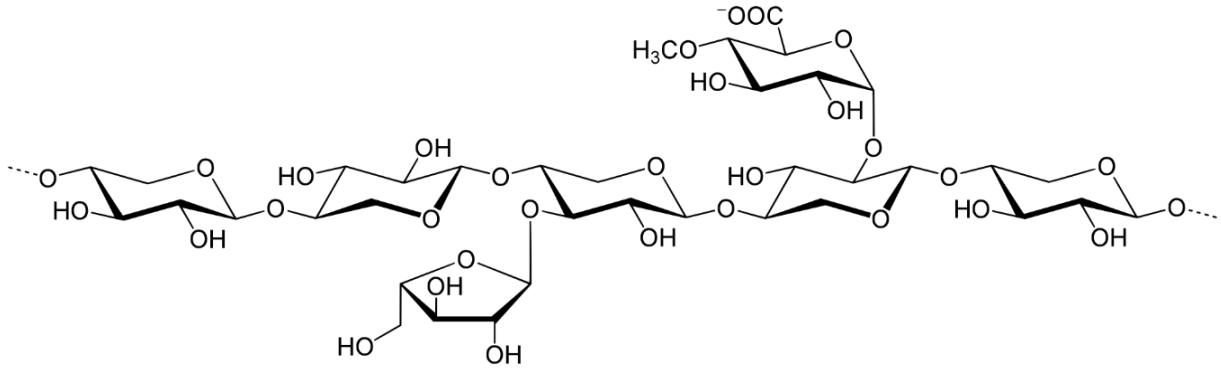

Figure 3. *The segment of hemicellulose structure (Wikimedia, 2015).*

The amounts of hemicellulose type differ depending if we have hardwood or softwood (Rowell, 2005). Major hemicelluloses in hardwoods are presented in ***Table 2.*** Major hemicelluloses in softwoods are presented in ***Table 3.***

Table 2. *Hardwood hemicelluloses* (Rowell, 2005).

Type of Hemicellulose	**Percent in Wood (%)**
Glucuronoxylan	15-30
Glucomannan	2-5

Table 3. *Softwood hemicelluloses* (Rowell, 2005).

Type of Hemicellulose	**Percent in Wood (%)**
Galactoglucomannan	15-23
Galactoglucomannan with Acetyl	10-15
Arabinoglucuronoxylan	7-10
Arabinogalactan	5-35

2.1.4 Lignin

Lignin is a three-dimensional polymer that can be found in plant cell walls between cellulose and hemicellulose and it is one of the most abundant polymeric substances (Hoyer, 2013). It is amorphous, highly complex with C-O-C (carbon- oxygen- carbon) and C-C (carbon- carbon) links in the molecules (Rowell, 2005). Chemical composition of lignin is represented in ***Figure 4.*** The difference between softwood and hardwood with respect to lignin is that the chemical structure of the monomers and linkages which make the lignin network differs (Wolfgang G. Glasser, 1989). The other difference is the amount, which can be seen in ***Table 1.*** In trees, the highest amount of lignin exists in the middle lamella. In softwood, it is even present in the secondary cell walls because of softwoods thickness (Hoyer, 2013).

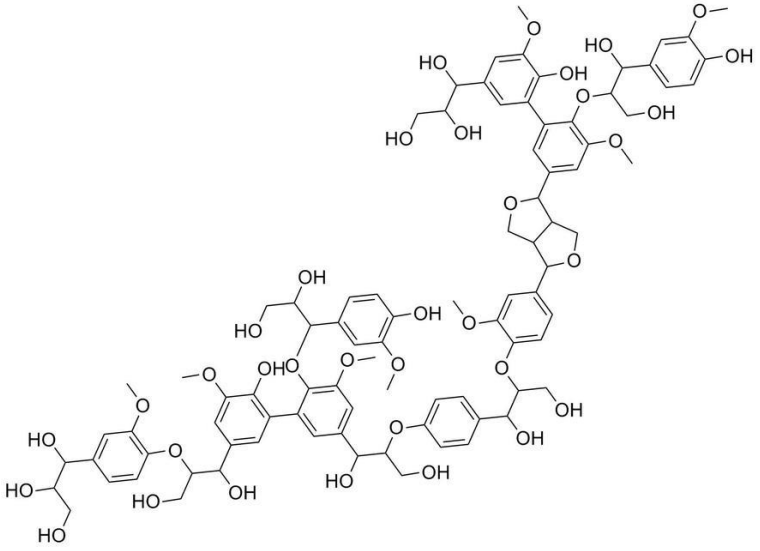

Figure 4. *A possible example of the chemical composition of lignin* (Paul-Scherrer-Institut, 2017).

2.1.5 Extractives

The components in the wood that can be extracted using solvents are called extractives. They can be classified by the solvent that extracts them, as for example there is water- soluble or toluene-ethanol soluble extractives. They consist of different organic compounds such as fats, fatty acids, fatty alcohols, phenols etc. Hardwoods have less extractive than softwoods as it can be seen in ***Table 1*** (Rowell, 2005). Their concentration is the highest in tree bark (Hoyer, 2013).

2.1.6 Other components

Beside the components mentioned in previous sections, wood contains also proteins, starch and pectin substances (Hoyer, 2013).

2.2 Bioethanol production

Currently, worldwide production of fuel ethanol is carried out from sugar- or starch-containing materials such as sugarcane, wheat and corn. This process is called first generation ethanol process. However, the conflict ‘’food versus fuel’’ lead to search for alternative biomass sources and the second generation ethanol process has been developed. The second generation ethanol process uses lignocellulosic material as feedstock. The lignocellulosic materials for ethanol production are agricultural residues (wheat straw, corn stover and sugarcane bagasse), hardwoods and softwoods. The hemicellulose in the agricultural materials and hardwoods consists mostly of the pentose sugar xylose. On the other hand, the hemicellulose in softwood consists mainly of the hexose sugar galactoglucomannan. These differences between the materials influence the ability to produce ethanol from different materials, especially when blending materials (Hoyer, 2013).

A simplified second generation process flow chart is represented in ***Figure 5.*** The process begins with the storage and preparation of the feedstock and is followed by pretreatment, enzymatic hydrolysis, fermentation and product recovery. The efficiency of the pretreatment step and enzymatic hydrolysis is dependent on the distribution of different polymers that exist in the materials. Woody biomass is more recalcitrant to microbial and enzymatic degradation than agricultural residues because it is physically stronger and has higher lignin content (Hoyer, 2013).

All process steps are described in the following sections.

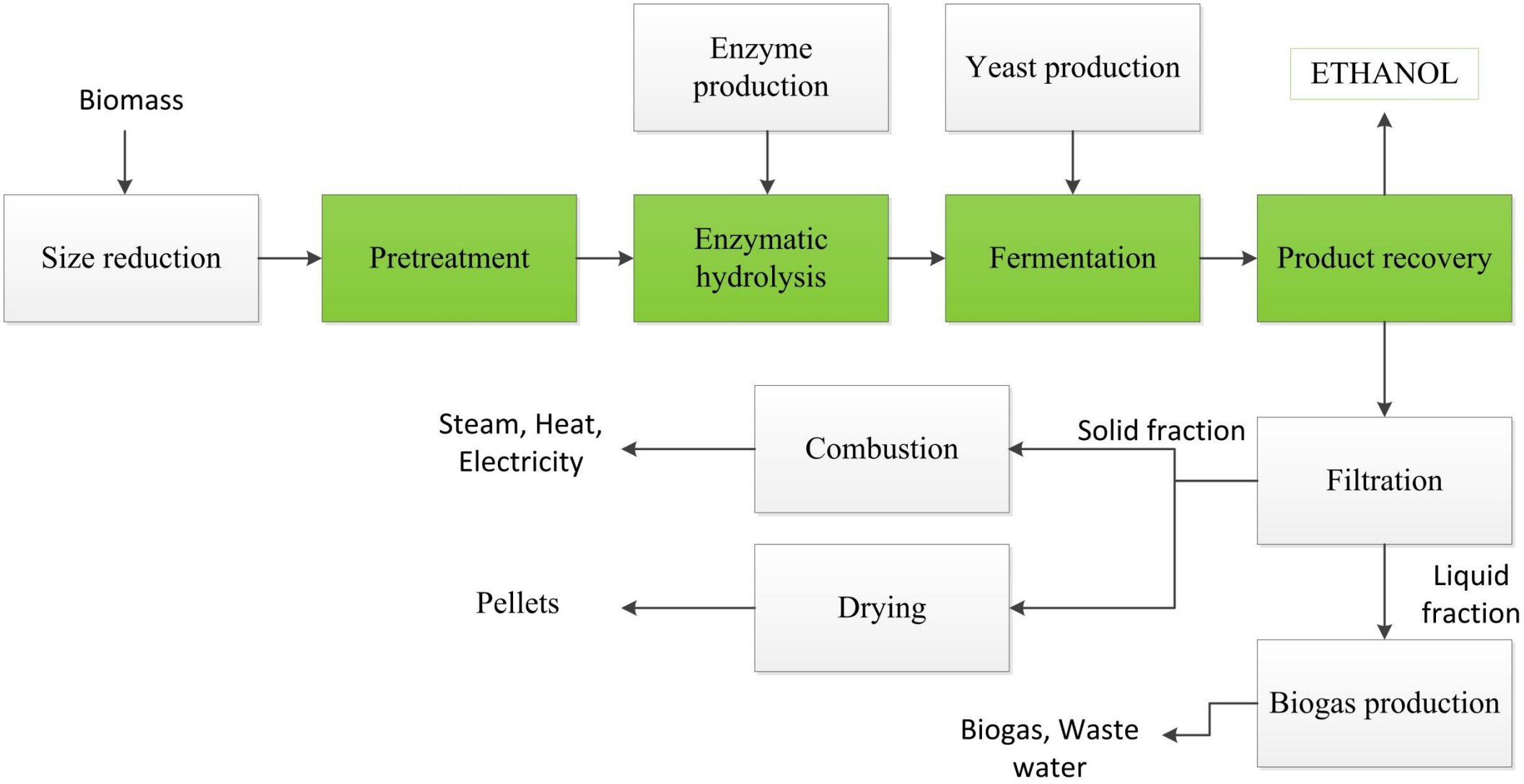

Figure 5. *Example of simplified process flowchart for the production of second generation ethanol.*

2.2.1 Pretreatment of lignocellulosic biomass

Lignocellulosic material can be used as feedstock in a wide range of applications, not only for ethanol production but also for example for fermentation of food additives, industrial chemicals etc. (Rowell, 2005). In order to use biomass for these applications, the feedstock first has to be pretreated (Gutierrez C.A, 2010).

In the previous sections, the complexity of the structure of lignocellulosic material was discussed. Lignin and hemicellulose cover the cellulose as presented in ***Figure 6.*** As it was mentioned the most part of the cellulose in biomass has a crystalline structure because of the strong hydrogen bonds between the linear chains, but there is even a part that is amorphous. Hemicellulose and cellulose make together a network because of the hydrogen bonds which is strengthened by lignin. Because of the strong bonds between cellulose, hemicellulose and lignin, the biomass cannot be hydrolyzed directly with enzymes, but must be pretreated first (Gutierrez C.A, 2010).

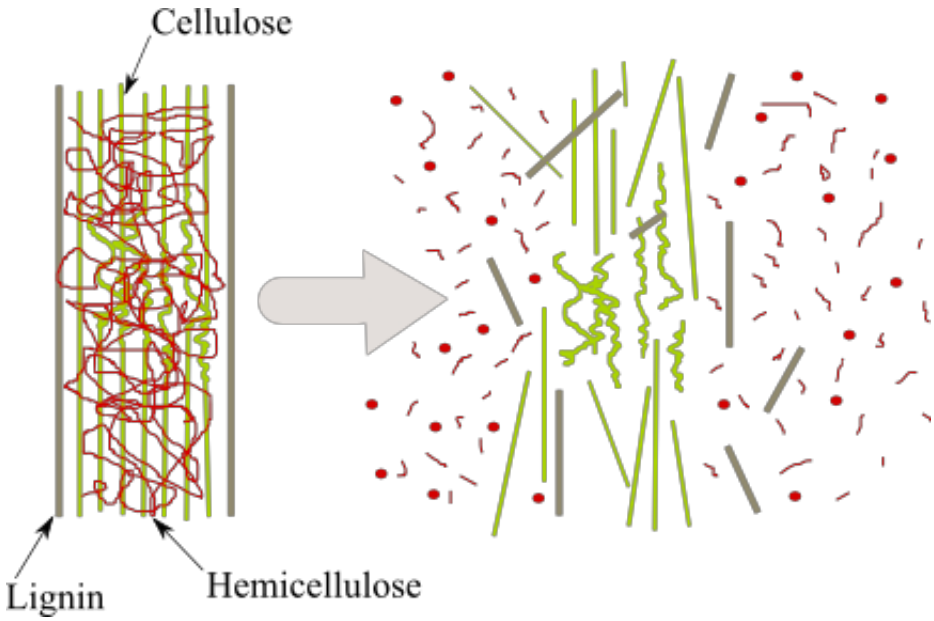

Figure 6. *Pretreatment process of lignocellulosic material* (Bondesson, 2016)

The goals of the pretreatment step are to solubilize hemicellulose and break down the strong network between the hemicellulose and cellulose, release and partly degrade lignin, reduce the crystallinity of cellulose and increase the amorphous part. Another important aspect is to avoid the formation of byproducts, which can be inhibitory for the subsequent process steps and decrease the yield of sugars obtained after the pretreatment. In addition, the pretreatment step should lead to the formation of sugars. If biomass is not pretreated, the yields of sugars in cellulose hydrolysis steps are very low compared to the yields that are obtained when biomass is pretreated. Therefore, the pretreatment step is necessary (Gutierrez C.A, 2010).

In the sections below, different methods for pretreatment of lignocellulosic material are briefly described.

2.2.1.1 Physical Methods of Pretreatment

Grinding, chipping and milling are all types of physical methods of pretreatment. The result is reduction in cellulose crystallinity which in turn increases the conversion of cellulose into glucose (Gutierrez C.A, 2010). The conversion of biomass into glucose after using wet or dry crushing as a pretreatment step has in some cases been reached to 50-60%. The energy need for grinding, chipping and milling is dependent on the desired particle size and biomass properties (Gutierrez C.A, 2010).

Even pyrolysis as physical method has been tested in different studies. The examples of tested materials are wood, waste cotton and corn stover. Volatile products and char were formed in these cases (Gutierrez C.A, 2010).

2.2.1.2 Physical-Chemical Methods of Pretreatment

Generally, physical-chemical methods are more effective than physical methods. The most implemented methods are steam explosion, liquid hot water, ammonia fiber explosion and CO_2 explosion (Gutierrez C.A, 2010).

Steam explosion is the most commonly used method and it is a process where biomass is treated with saturated steam at high pressure in a reaction called autohydrolysis. Here, hemicellulose and part of the lignin are converted into soluble oligomers which occur with help of small amount of acids that are released from the biomass itself under the process. Saturated steam can be in the range of 160-290°C, and a pressure of 0.69-4.85 MPa. Residence time can vary from several seconds to several minutes. Afterwards, decompression is done until atmospheric pressure which leads to rupture and opening of the fibers in the biomass (Gutierrez C.A, 2010).

Temperature, residence time, chip size and moisture content of the material affect the steam explosion. A severity factor can be used for the optimization of steam explosion process. The severity factor can be described with a function of time t (minutes) and temperature T (degrees Celsius) and reference temperature T_{ref} of 100°C:

$$logR_0 = \log\left(t\ exp\left(\frac{T - T_{ref}}{14.75}\right)\right)$$

The function does not take the moisture content and particle size of the raw material into consideration. The moisture content and particle size have both strong impacts on the steam explosion, as mentioned before (Gutierrez C.A, 2010).

If wood is taken for example, different conditions can give everything from small cracks in the wood structure to total defibrillation of the wood. Studies have shown that steam explosion is one of the most efficient methods for hardwood (poplar, oak, birch, maple), but less efficient for softwood (pine, cedar) (Gutierrez C.A, 2010). It has been reported that the efficiency of enzymatic hydrolysis has increased by 90% when poplar chips were steam exploded prior to the enzymatic hydrolysis. When poplar chips were not pretreated the efficiency was only 15 % (Gutierrez C.A, 2010).

When pretreatment is performed with liquid hot water, there is low or no formation of inhibitors. Also cellulose depolymerization occurs only at a certain degree. The procedure is done with liquid hot water, with a temperature range of 170-230°C and a residence time of 1-46 minutes. Another physical- chemical method is ammonia explosion which is similar to steam explosion. During this method, no inhibitors are built so washing with water before subsequent biological processes is not needed (Gutierrez C.A, 2010).

2.2.1.3 Chemical Methods of Pretreatment

Some common chemical methods of pretreatment are ozonolysis, dilute- acid hydrolysis, and organosolv process. Different chemical agents are used such as ozone, acids, alkalis, peroxide etc. Ozone is used for ozonolysis at room temperature and pressure. In this procedure no inhibitors are formed. For organosolv process, organic solvents as methanol, ethanol and acetone are used. Typical temperature range is 185-198°C and time 30-60 minutes. Almost total lignin solubilization and breakdown of internal lignin and hemicellulose bonds are obtained (Gutierrez C.A, 2010).

2.2.1.4 Biological Methods of Pretreatment

Biological processes are too slow and cannot be used at an industrial level. However, they have low impact on the environment and have low energy requirements. Some examples of biological methods of pretreatment are fungal pretreatment and bioorgonosolv pretreatment (Gutierrez C.A, 2010).

2.2.2 Assessment of pretreatment

The pretreatment step can be assessed in different ways. The recovery of sugars after the pretreatment step is obtained by the analysis of the material before and after the pretreatment step. Enzymatic hydrolysis after the pretreatment can be done in order to assess the digestibility of the pretreated material. During the pretreatment of lignocellulosic material, inhibitors are formed. Inhibitors are substances that in a significant amount can seriously inhibit the subsequent fermentation process (Gutierrez C.A, 2010). Main inhibitors that are formed are represented in ***Figure 7.***

The assessment of the pretreatment step is complex, and different pretreatment methods and conditions affect the rest of the process steps in the ethanol production process. Therefore, all the steps in the process should be optimized simultaneously under real process conditions (Hoyer, 2013).

The above described pretreatment methods work well on the materials that contain lower amount of lignin. Because woody biomass contain higher amount of lignin, the only suitable pretreatment methods are dilute acid pretreatment and steam pretreatment. The steam pretreatment with acid catalyst is considered as the best pretreatment method for woody biomass. Nowadays, this pretreatment method is used at industrial scale in different countries around the world (Hoyer, 2013).

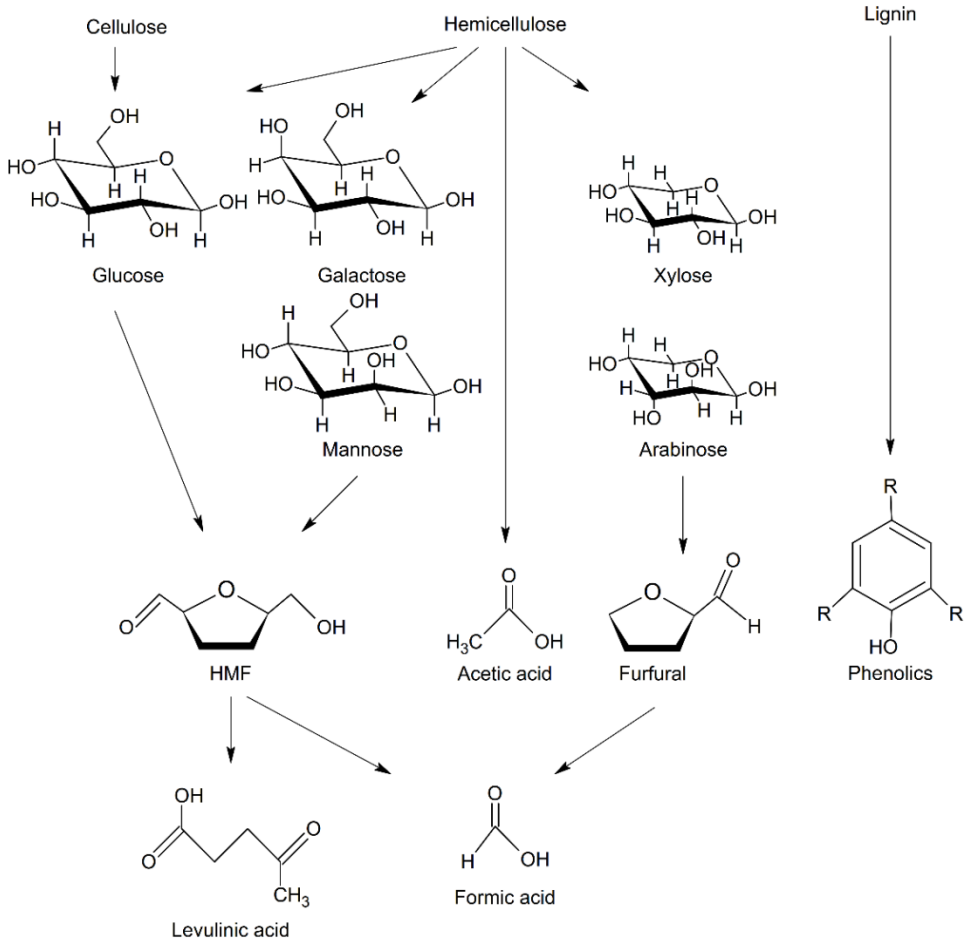

Figure 7. *Inhibitors that can be formed during the pretreatment of lignocellulosic material* (Erdei, 2013).

2.2.4 Hydrolysis

There are several types of hydrolysis and some of them are acid hydrolysis and enzymatic hydrolysis.

Acid hydrolysis is performed using acid. The typical conditions for acid hydrolysis are: an acid concentration of 1-5%, a temperature of 160-230°C and a high pressure of approx. 10 atm. The retention time for the process is from several seconds to minutes. This process only hydrolyses small amounts of cellulose, but is efficient for hemicellulose hydrolysis. Concentrated acid hydrolysis is efficient even for cellulose and is performed with higher acid concentrations up to 30% (Hoyer, 2013).

Enzymatic hydrolysis is a process where enzymes perform a chemical reaction during which the enzymes break the cellulose and hemicellulose chains into monomeric sugars. The cellulose chains are broken down into glucose and the hemicellulose chains into its constitutes, mainly xylose or mannose, depending on which raw material is used. For instance, the hemicellulose in spruce contains mainly mannose, while in poplar, the hemicellulose consists mainly of xylose. Monomeric sugars can later be used for fermentation to produce for example ethanol (Gutierrez C.A, 2010).

Compared to acid hydrolysis, enzymatic hydrolysis conditions are considered as mild. The process is usually performed at pH 4.8 and in a temperature range of 318-323 K (Balat, 2011). An ideal enzymatic hydrolysis process gives maximum conversion of substrate to sugars with least enzymatic input. (Columbia, 2017). The negative aspect of the enzymatic hydrolysis is long retention time compared to acid hydrolysis (Hoyer, 2013).

2.2.4.1 Factors affecting enzymatic hydrolysis of lignocellulose

Two factors that affect the enzymatic hydrolysis the most are the properties of substrate used and the process parameters. When lignocellulosic material is enzymatically hydrolyzed, the hydrolysis rate is high in the beginning, but decreases with the retention time. After a certain time the product formation will reach a maximum (Hoyer, 2013).

The important property of the substrate used is its total available surface area because it is important that it can access the glycosidic bonds in the cellulose of the pretreated material. However, the pores in the cellulose are big enough to accommodate a cellulolytic enzyme (Hoyer, 2013). The lignin amount in the pretreated material also affects the hydrolyzability because it blocks cellulases from accessing the cellulose (Hoyer, 2013).

Another important aspect that influences enzymatic hydrolysis is the pretreatment condition prior to enzymatic hydrolysis step. When the pretreatment severity is low, less hemicellulose is solubilized and hemicellulases together with cellulases are needed to break down the lignocellulosic structure. The process parameters also affect enzymatic hydrolysis. (Hoyer, 2013).

2.2.5 Fermentation

During the fermentation process the mixture of monomeric sugars is inoculated with microbes, such as yeast or bacteria. The monomeric sugars are converted into cellular energy, producing ethanol and carbon dioxide as a side-effect (Gutierrez C.A, 2010). *Saccharomyces cerevisiae* and *Zymomonas mobilis* are traditionally used microorganisms for ethanol production. The disadvantage with these microorganisms is that they cannot ferment xylose, but can ferment glucose to bioethanol (Balat, 2011).

Fermentation can be performed as a batch, fed-batch or continuous process. Batch fermentation process is considered as a very simple method. An initial, limited amount of substrates is added to the system. The substrate is inoculated with microorganisms so that the fermentation can start. During the fermentation, acid or alkali are possibly added for pH control. During fed-batch fermentation process, substrate is supplied to the reactor during cultivation. All products stay in the reactor until the fermentation is finished. This process is commonly used in industrial applications because it combines the advantages from both continuous and batch processes. The continuous fermentation process is a procedure where feed with required substrate is pumped continuously into an agitated vessel where the microorganisms are active. The choice for fermentation type depends on kinetic properties of microorganisms and type of lignocellulosic hydrolysate (Balat, 2011).

2.2.6 Hydrolysis and fermentation strategies

Enzymatic hydrolysis and fermentation can be performed as separate steps in a process called separate hydrolysis and fermentation (SHF). Liquid flow from hydrolysis reactor enters the glucose fermentation reactor. When performing SHF, there is a need for lower solids loadings and higher enzyme loadings due to inhibition of cellulase and β-glucosidase enzymes. That is the minor disadvantage of the process. On other hand, SHF gives a possibility to run each step under optimal conditions which are 318-323 K for enzymatic hydrolysis and 303K for fermentation. One of disadvantages for this method is that cellulose can be inhibited by glucose released during hydrolyses (Balat, 2011).

Enzymatic hydrolysis and fermentation can be performed simultaneously in a process called simultaneous saccharification and fermentation (SSF) which is commonly used when producing ethanol from lignocellulosic materials. The process takes between 5-7 days and the time depends on the accessibility of the cellulose and initial solids loading of the fermentation. The advantages of the process are no loss of sugars and the usage of degradation by-products that can be inhibitory for enzymes.

Direct microbial conversion (DMC) is a process which combine cellulose production, cellulose hydrolysis and glucose fermentation. The advantage of the process is that it simplifies operation and reduces the number of reactors, which in turn leads to lower cost of chemicals. On the other hand low ethanol yield is obtained due low tolerance of the microorganism to bioethanol and formation of byproducts acetate and lactate. (Balat, 2011).

2.2.7 Product recovery

In order to use ethanol for ethanol-gasoline blends, it is necessary to concentrate the ethyl alcohol up to 99%. Lower concentration can lead to failures in the engine during the combustion (Gutierrez C.A, 2010).

After the fermentation, the mixture contains two main components which are water and ethyl alcohol. Ethanol has higher volatility and lower boiling point. Taking this into consideration, ethanol and water can be separated with conventional distillation at a pressure equal to or higher than atmospheric pressure. However, ethanol concentrations higher than 95.6% are impossible to obtain with the conventional distillation due to azeotropic mixture of water and ethanol. To obtain concentrations higher than 99.5%, nonconventional separation technologies are used such as ethanol dehydration (Gutierrez C.A, 2010).

The conventional distillation is performed in two distillation columns. The first distillation column is called concentration or beer column, which can have different types of plates. The ethanol concentration of 35-50% comes from the first column and is sent to the next one. . In the second distillation column, ethanol concentration of maximal 95.6% is obtained (Gutierrez C.A, 2010).

The distillate is then sent to dehydration step where the desired ethanol concentration can be obtained in processes such as pressure-swing distillation, azeotropic distillation, extractive distillation and adsorption. (Gutierrez C.A, 2010)

3. Materials and Methods

3.1 Raw materials

3.1.1 Spruce

Spruce with a dry matter of 72% has been provided by Södra Skogsägarna, Växjö, Sweden. The material has been chipped by a hammer mill (GmbH & Co.KG, Germany) and sieved in order to obtain a chip size of 2-10 mm. The chips of the wood have then been stored at 4°C before further experimentation.

3.1.2 Poplar

Poplar with a dry matter of 40% has been provided by Södra Skogsägarna, Växjö, Sweden. The material has firstly been whittled and cut into smaller pieces with Turbine Cut System (Bosch AXT 25 TC). Then the wood pieces have been chipped by a hammer mill (GmbH & Co.KG, Germany) and sieved in order to obtain a chip size of 2-10 mm. The chips of the wood have then been stored at 4°C before further experimentation.

3.2 Pretreatment

Dry matter content of spruce and poplar was adjusted to 50% prior to pretreatment. In order to do that, the spruce was soaked in water at room temperature and then pressed in a 5 L filter press (HP5M, Fischer Maschinenfabrik, GbmH, Germany) at 200 bars in order to remove access liquid. The poplar, on other hand, was air dried for 12 hours.

The steam explosion pretreatment was performed at three different conditions that are presented in ***Table 4***. The parameters for each condition were determined and based on reported optima in previous studies (Franko B, 2015), (Stenberg K, 1998) and (Schutt, 2011).

Table 4. *Temperature, retention time and catalyst amount for condition 1, 2 and 3.*

Condition	Temperature [°C]	Retention time	Catalyst amount [w/w moisture]
Condition 1	210	5	2,5% SO_2
Condition 2	200	5	2,5% SO_2
Condition 3	210	5	1,25% SO_2

The same ratios of feedstock and feedstock blends were used for the three conditions. The ratios are presented in ***Table 5*** and denoted as P0 to P100 depending on the percentage of poplar dry matter in the raw material that was used.

Table 5. *The amount of poplar and spruce in the pretreated materials (P0-P100).*

Name of the pretreated material	Percentage of Spruce [%]	Percentage of Poplar [%]
P0	100	0
P10	10	90
P50	50	50
P100	0	100

For each condition, the same steam explosion equipment was used and it consisted of a 10 L reactor (Process- & Industriteknik AB, Kristianstad, Sweden) as described previously by Bondesson *et al* (Bondesson, 2016). The steam explosion unit is presented in ***Figure 8.*** The same procedure has been applied to all three conditions. An amount of the material was loaded into the reactor. When the desired retention time has eclipsed, the pressure was released to atmospheric and the pretreated material has been collected in a tank.

The impregnation has been performed in tightly closed plastic bags for 20 min and at room temperature. In order to determine how much of SO_2 was absorbed, the bags with the material have been weight before and after the impregnation. The size of the reactor allowed 600 g of dry matter impregnated material to be pretreated at a time and 2 shots of each blend has been pretreated.

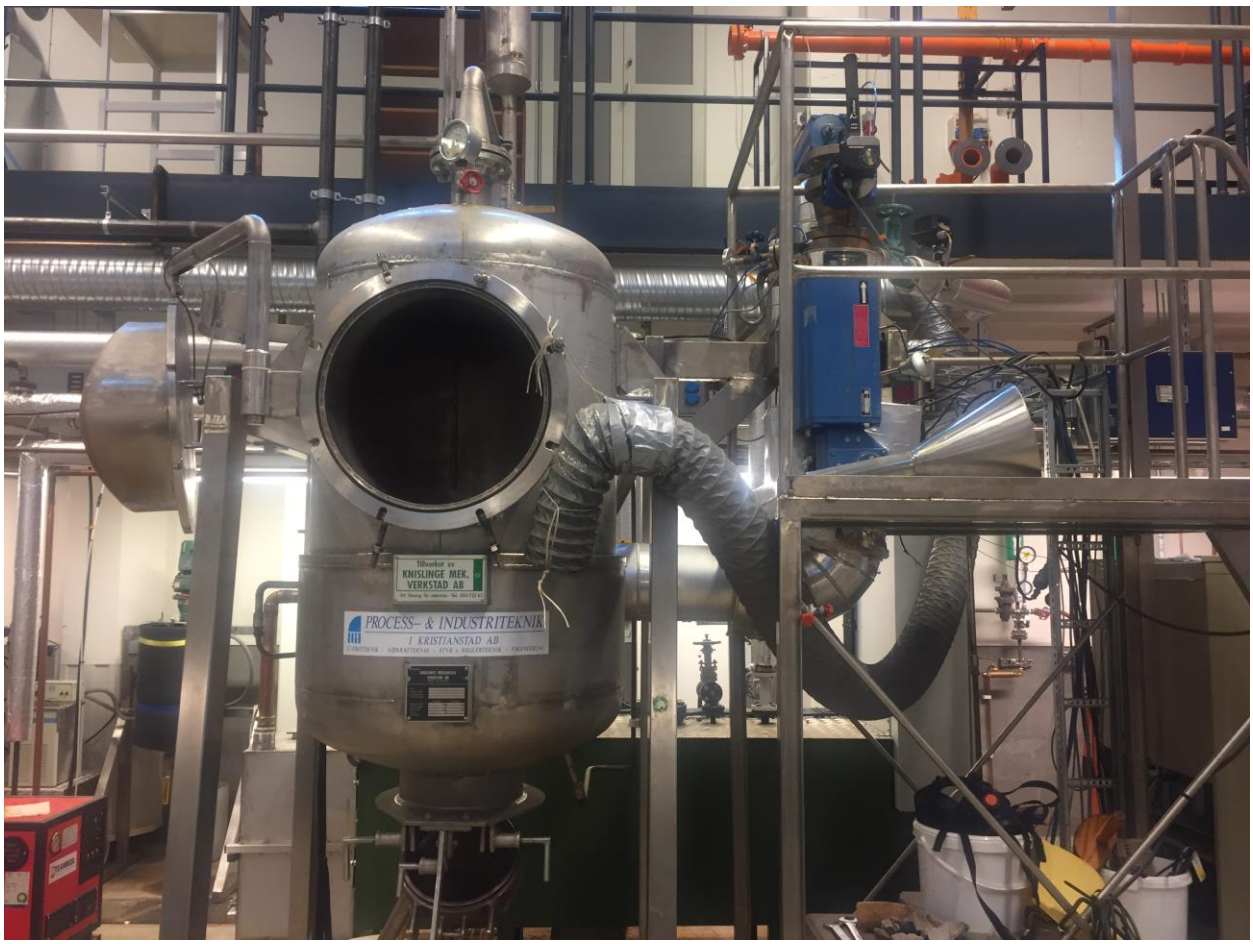

Figure 8. *The steam pretreatment unit at the department of chemical engineering, LTH.*

3.3 Enzymatic Hydrolysis

The enzymatic hydrolysis has been performed on unwashed slurries (P0, P10, P50 and P100) obtained from the three conditions. The purpose of the enzymatic hydrolysis was to determine the potential sugar yield.

2 L sterilized bioreactors (Infors AG, Bottmingen, Switzerland) equipped with a pitched 6-blade turbine and an anchor impeller were used. The amount of working slurry was 1 kg and the percentage of water-insoluble solids (WIS) was 10%. The enzyme cocktail used was Cellic CTec3 (Novozymes A/S). The enzyme cocktail was diluted in water and added to the reactors when the reactor temperature reached 45°C. The parameters for the enzymatic hydrolysis are presented in ***Table 6.*** The pH was continuously adjusted to 5 by manual addition of 50% sodium hydroxide (NaOH) solution or 72% sulphuric acid (H_2SO_4) solution. Samples were taken after 2, 4, 7, 10, 24, 48, 72 and 96 h and analyzed regarding monomeric sugar content.

Table 6. *The conditions for enzymatic hydrolysis step*.

Working weight [g]	1000
WIS content [%]	10
Enzyme dosage [w/w% of the WIS]	5
Temperature [°C]	45
Agitation [rpm]	400
Residence time [h]	96
pH	5

3.4 Analytical procedures

All analyses were performed in duplicates. Dry matter content was determined by automatic infrared moisture analyzer and on materials that have been dried in 105°C oven until constant weight was obtained. The composition of raw and pretreated materials regarding carbohydrates and lignin was determined using the laboratory analytical procedure (LAP) for determination of structural carbohydrates and lignin in biomass from National Renewable Energy Laboratory (NREL) (Sluiter A H. B., 2012). The extractives, ash content of the solid fractions and the composition of liquid fractions were determined using standardized laboratory procedures from NREL (Sluiter A H. B., 2008), (Sluiter A H. B., 2006) and (Sluiter A R. R., 2005)

All samples from compositional analysis and other experiments were centrifuged and filtered through 0,2 µl filters (GVS Filter Technology Inc., Indiana, United States) in order to eliminate particles. The samples were then stored at -20°C prior high-performance liquid chromatography (HPLC) analysis. The HPLC system consisted of an Aminex HPX-87H column with a De-Ashing Bio-Rad micro guard column (Hercules, California, United States) with a mobile phase of 5mM sulfuric acid at a flow rate of 0.5 ml/min, an injection volume of 20 µl and a temperature of 50°C. The samples were diluted prior to HPLC. Degradation products in the liquid fraction of the pretreated materials were analyzed by high performance liquid chromatography (Shimadzu Corporation, Kyoto, Japan).

3.5 Calculations

The calculations for recoveries over the pretreatment step were based on sugar measurements in both water-insoluble solids and liquid phase, before and after pretreatment.

The glucose recoveries over the enzymatic hydrolysis step were calculated based on the total available glucose in the liquid and the solid phase of steam pretreated materials and the amount of monomeric glucose after the hydrolysis. Because of the water added during the hydrolysis step, it was taking into account that the theoretical amount of glucose obtained after the hydrolysis was 1.111 times the amount of glucan in the solid fraction of the steam pretreated materials.

4. Results and Discussion

4.1 Composition analysis of raw materials

The composition of raw materials, spruce and poplar, is presented in ***Table 7.*** The content of carbohydrates was slightly lower in the spruce than in poplar. The biggest difference in the individual carbohydrates, when comparing the two materials, is in the amounts of xylan and mannan. The percentage of xylan is almost three times higher in poplar than in spruce. On the other hand, the amount of mannan was 3.5% in poplar and 11.1% in spruce, which is not surprising as the major hemicellulose component is different for softwood and hardwood. There was no significant difference in the amount of the extractives between the materials. Ash content for poplar was slightly higher, but not with a significant difference.

The same pattern for carbohydrates and lignin amount of spruce and poplar were shown in previous studies (Bura R, 2009), (Hoyer, 2013). They are also presented in ***Table 7.***

Table 7. *Composition of the spruce and poplar feedstocks as a percentage of dry matter (% of DM)*

Feedstock	Carbohydrates						Lignin		Extractives	Ash
	Glucan	Xylan	Galactan	Arabinan	Mannan	Sum of carbohydrates	ASL	AIL		
Spruce	40.9	5.8	2.2	1.4	11.1	61.4	4.4	28.4	4.2	0.3
Poplar	41.8	14.4	1.3	0.8	3.5	61.8	6.1	19.2	6.3	0.6
Spruce[1]	45.0	5.0	1.8	1.0	12.6	65.4	33.4			
Poplar[2]	43.8	14.9	1.0	0.6	3.9	64.2	29.1			

1 From (Hoyer, 2013), 2 from (Bura R, 2009).

4. 2 Pretreatment

4.2.1 Composition of the water insoluble fraction of pretreated materials

Composition of the water insoluble fraction of steam pretreated materials (P0-P100) for conditions 1, 2 and 3 is presented in ***Table 8.*** The same pattern for all three conditions was observed regarding the amount of carbohydrates. As it can be seen, the amount of glucan in the solid fraction was increasing with higher percentage of poplar in the feedstock mixtures. The same pattern for the amounts of xylan was observed. This is because of higher glucan and xylan content in raw poplar, see ***Table 7*** and also because poplar is easier to break down during the pretreatment step so more glucose is formed whit higher amount of poplar in the material.

For condition 2 (200°C, 5min, 2.5% SO_2) all sugars in the water insoluble fraction of pretreated P100 were detectable and higher than 0.1 %. In condition 1 (210°C, 5min, 2.5% SO_2), where the pretreatment conditions were more severe, not all sugars were detectable or higher than 0.1% for P100, see ***Table 8.*** This can be explained with the different composition of hemicelluloses in poplar and spruce. Also, since the proportions are so small, it can be assumed that there was a noise on the line obtained from the HPLC during the analysis.

The same pattern for ash content for all three conditions was observed. The ash content was increasing with the poplar amount, but only a slightly difference was observed which depends on the composition of the raw materials where no significant difference in ash content was obtained, see ***Table 7.***

The acid insoluble and soluble lignin content are also presented in ***Table 8.*** For all three conditions, AIL is decreasing with higher amount of poplar in the pretreated materials even because the acid insoluble lignin content was lower in the raw poplar than in raw spruce, see ***Table 7.***

The total composition of P100 after condition 2 is 99.8%, which means that analytical procedure in that case was accurate. The total composition of all other materials is over 100%, see ***Table 8,*** which means that results are slightly overestimated.

Table 8. *Composition of the water insoluble fraction of steam pretreated spruce, poplar and blends thereof as a percentage of dry matter (%DM) for conditions 1, 2 and 3.*

Pretreated materials	Carbohydrates						Lignin		Ash	Total
	Glucan	Xylan	Galactan	Arabinan	Mannan	Sum of carbohydrates	ASL	AIL		
CONDITION 1										
P0	52.5	0.2	0.3	0.1	0.1	53.2	2.6	45.0	0.3	101.1
P10	54.8	0.3	<0.1	<0.1	<0.1	55.1	2.7	42.8	0.4	101.0
P50	59.5	0.6	<0.1	<0.1	<0.1	60.1	2.5	37.5	0.6	101.0
P100	70.2	1.1	<0.1	n.d	0.2	71.5	3.1	26.4	0.7	102.0
CONDITION 2										
P0	54.6	0.5	0.6	<0.1	0.1	55.8	2.2	43.8	0.2	102.0
P10	55.5	0.4	0.5	0.2	0.1	56.7	2.5	41.9	0.3	101.4
P50	62.5	0.9	0.4	<0.1	0.2	64.0	2.4	33.6	0.4	100.4
P100	68.1	1.6	0.2	0.2	0.2	70.3	3.0	26.1	0.4	99.8
CONDITION 3										
P0	56.4	0.3	0.1	<0.1	<0.1	56.8	2.9	44.3	0.2	104.2
P10	58.2	0.3	<0.1	<0.1	<0.1	58.5	3.1	43.7	0.2	105.5
P50	63.0	0.5	<0.1	<0.1	<0.1	63.5	2.9	38.5	0.3	105.2
P100	72.5	1.0	0.1	n.d	0.1	73.7	3.4	26.6	0.4	104.1

n.d=not detected; P0=100%spruce; P10=10% poplar and 90 % spruce; P50= 50% poplar and 50% spruce; P100=100%poplar; Condition 1= 210ºC, 5 min, 2.5% SO2; Condition 2=200ºC, 5 min, 2.5% SO2 and Condition 3=210ºC, 5 min, 1.25% SO2

4.2.2 Composition of the liquid fraction for pretreated materials

Composition of the liquid fraction of steam pretreated materials (P0-P100) for conditions 1, 2 and 3 is presented in ***Table 9.***

In the pretreated material after condition 1, the concentrations of monomeric glucose were highest in the pretreated spruce (P0), 31.3 g/L and lowest in pretreated poplar (P100), 21.1 g/L even though the glucan content in raw spruce was lower than in raw poplar. The reason for that is that it is not possible to distinguish if the monomeric glucose obtained after pretreatment comes from the hemicelluloses or celluloses in the raw material. The pretreatment dissolves mostly hemicelluloses based on the severity of the pretreatment and since glucose is more abundant in the hemicellulose part in the raw spruce than in raw poplar, it is reasonable to obtain higher concentration of monomeric glucose from spruce than poplar. The same pattern was obtained for condition 2 and 3 as well.

Similarly, in the pretreated liquid material after condition 1, the concentration of monomeric mannose was higher in spruce (P0) than in poplar (P100) due to higher mannan content in raw spruce. The concentration of monomeric xylose was highest for pretreated poplar (P100), 31.7 g/L, due to higher xylan content in raw poplar, see ***Table 7.*** The same patter was obtained for condition 2 and 3 as well.

Table 9. *Composition of the liquid fraction of steam pretreated spruce, poplar and blends thereof for condition 1, 2 and 3.*

Pretreated material	Total sugars (expressed as monomeric sugar) (g/L)					Inhibitors (g/L)				
	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF*	Furfural	Formic Acid	Acetic Acid	Levulinic Acid
CONDITION 1										
P0	41.7	12.1	6.5	3.7	27.2	4.0	2.1	1.9	6.2	1.6
P10	33.1	12.7	5.5	3.2	22.8	3.3	2.3	1.4	6.7	1.3
P50	31.3	24.7	6.1	3.0	20.4	2.7	2.9	1.5	10.4	0.9
P100	25.3	33.7	5.2	2.0	8.7	2.1	4.0	1.5	14.9	0.7
CONDITION 2										
P0	28.8	13.4	6.2	3.9	27.6	2.6	1.6	1.7	6.3	0.9
P10	30.1	17.9	7.1	4.3	29.9	2.4	1.9	1.6	6.9	0.7
P50	27.7	32.0	7.4	3.9	24.1	1.8	2.2	1.7	10.6	0.6
P100	20.1	39.7	5.3	2.3	9.7	1.3	3.2	1.9	13.8	0.5
CONDITION 3										
P0	26.4	11.0	5.3	3.1	23.5	2.9	1.6	0.9	5.5	0.8
P10	28.7	12.5	5.2	2.9	22.8	3.1	1.9	0.9	6.8	0.8
P50	28.1	19.2	4.6	2.4	15.1	2.6	2.8	1.0	10.4	0.6
P100	18.2	31.9	3.8	1.6	7.8	1.3	3.1	0.9	14.1	0.3

*5-Hydroxymethylfurfural; P0=100%spruce; P10=10% poplar and 90 % spruce; P50= 50% poplar and 50% spruce; P100=100%poplar; Condition 1= 210ºC, 5 min, 2.5% SO2; Condition 2=200ºC, 5 min, 2.5% SO2 and Condition 3=210ºC, 5 min, 1.25% SO2

When comparing the conditions 1 and 2, higher concentration of glucose is observed after condition 1 in all pretreated materials except in P50. The 50% blend of spruce and poplar has not been affected by the temperature difference in the two conditions, with regard to glucose concentration. On the other hand, monomeric xylose concentration was higher in all pretreated materials after condition 2 compared to condition 1.

When comparing all three conditions regarding to monomeric glucose content, it can be observed that monomeric glucose content for P0 for condition 1 is much higher than for P0 for the other two conditions (condition 2 and 3). Although, the same pattern was observed. The monomeric glucose concentration did not differ significantly between the three conditions when comparing pretreated mixtures, P10 and P100. There was a significant difference for pretreated P50 and it was highest after condition 3.

Inhibitors formed during the steam pretreatment are also presented in ***Table 9.*** As it can be seen, for all three conditions, more acetic acid and furfural were formed with more poplar in the pretreated feedstock. On the other hand, HMF was found in higher concentrations with higher spruce blends. As it can be seen in ***Figure 7*** (theory section), furfural forms by the degradation of xylose and raw poplar contained more xylan than spruce did which explains the trend obtained for inhibitors after the pretreatement. Also, HMF forms from hexoses and the hemicellulose in spruce contains predominantly glucose. When comparing the conditions, highest amounts of inhibitors were formed after condition 1. One reason for that is that the pretreatment conditions were the most severe compared to the other two conditions. The formation of the byproducts can be inhibitory for the subsequent process steps.

4.2.3 Sugar recoveries

Overall sugar recovery for glucose for condition 1, 2 and 3 is presented in ***Figure 9.*** As it can be seen, the highest glucose recoveries are obtained in pretreated poplar (P100) for all three conditions.

Regarding condition 2, when comparing the mixtures, P10 and P50, the higher glucose recovery was obtained for P50. Interestingly, 10% poplar in the feedstock blend affected the recovery negatively, but 50% lead to a higher recovery compared to pretreated spruce P0. This leads to a theory that when mixing poplar and spruce, it is recommended to have at least 50% poplar in order to get higher glucose recovery than for P0, under pretreatment using condition 2. Although feedstock blends of 25% and 75% of poplar should be tested to see how that will affect glucose recovery and also to strengthen the theory.

There was no significant difference between the mixtures for condition 1 and 3 regarding glucose recovery.

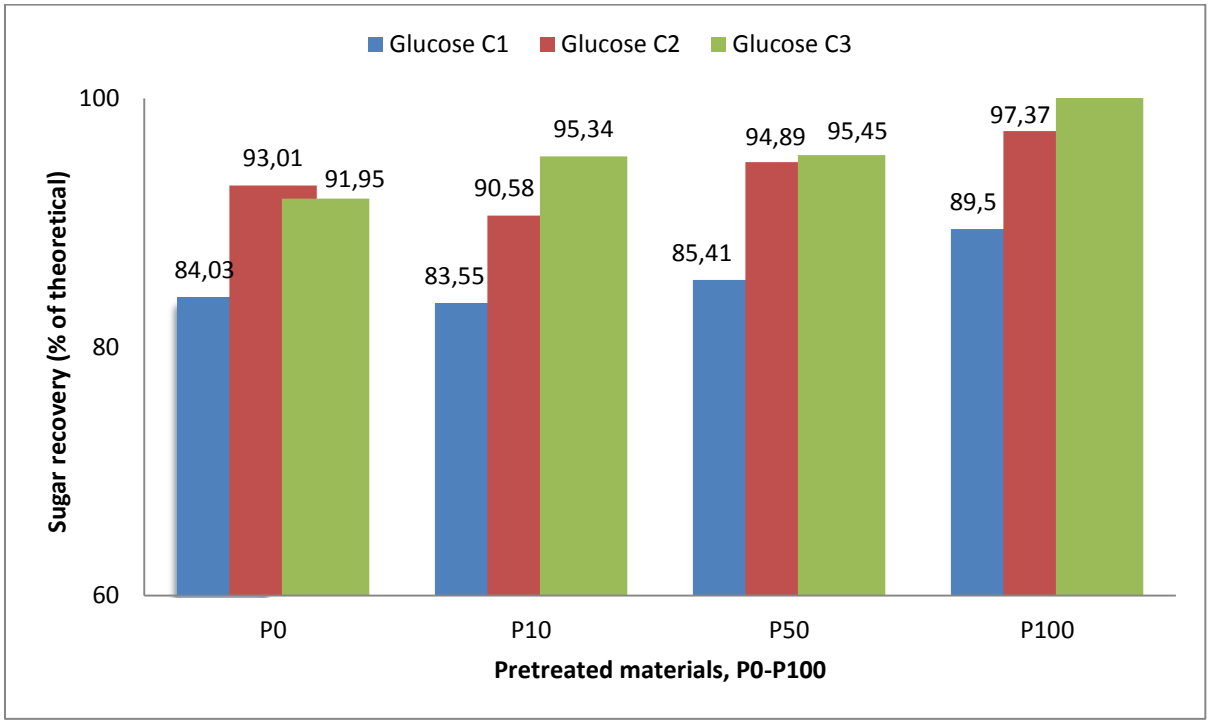

Figure 9. *Glucose recoveries over the steam pretreatment for spruce, poplar and blends thereof (P0-P100) for condition 1 (C1), condition 2 (C2) and condition 3 (C3). Recovery expressed as percentage of the theoretical based on sugars content of the raw material.*

Overall xylose recoveries for the three conditions are presented in ***Figure 10*** and as it can be seen the recovery is highest in condition 2 for all pretreated materials. This indicates that the condition 2 was least severe.

The highest recovery is obtained from P50 after condition 2. When comparing the mixtures, P10 and P50, the higher recovery is obtained in the 50% poplar mixture after pretreatments under condition 1 and 2. This means that there is a positive effect on the recovery of xylose when the materials are mixed equally. There was no significant difference for condition 3 when comparing the mixtures.

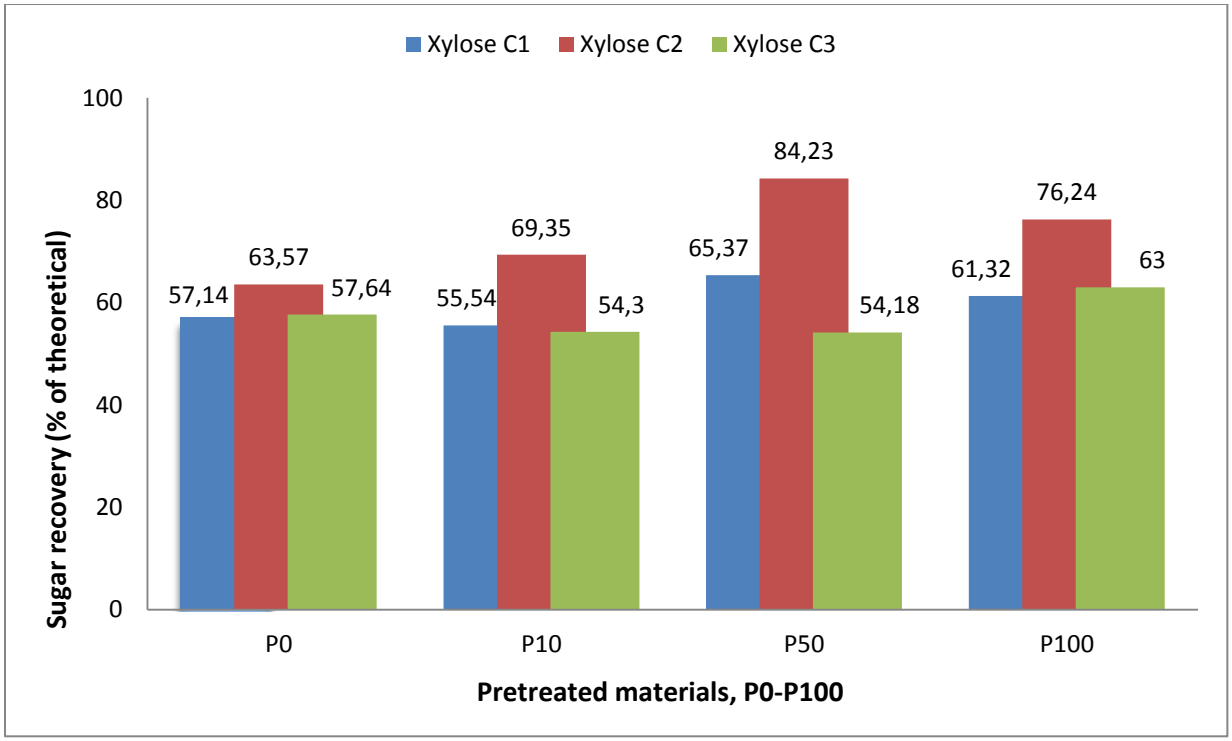

Figure 10. *Xylose recoveries over the steam pretreatment for spruce, poplar and blends thereof (P0-P100) for condition 1 (C1), condition 2 (C2) and condition 3 (C3). Recovery expressed as percentage of the theoretical based on sugars content of the raw material.*

Recovery of mannose after steam pretreatment for all three conditions is shown in ***Figure 11.*** The highest recovery for mannose is obtained for P50 after pretreatment under condition 2. The recovery for pretreated poplar, P100, is higher than for pretreated spruce, P0, in all three conditions. When comparing the mixtures, the higher recovery is obtained for the 50% mixture than for 10% mixture after condition 1 and 2.

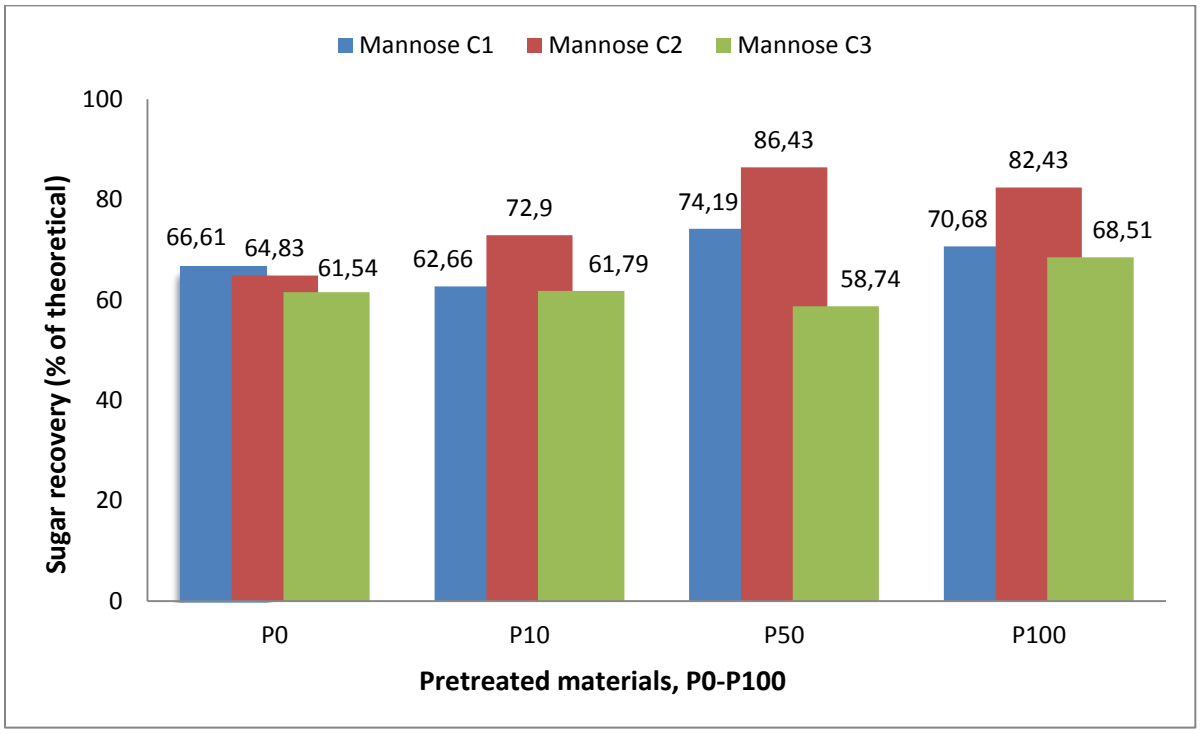

Figure 11. *Mannose recoveries over the steam pretreatment for spruce, poplar and blends thereof (P0-P100) for condition 1 (C1), condition 2 (C2) and condition 3 (C3). Recovery expressed as percentage of the theoretical based on sugars content of the raw material.*

As it can be seen in ***Appendix 1, 2 and 3***, the recovery for overall (from liquid and solid part) glucose was highest in all three conditions compared to all other sugars. This can be explained by the fact that it is harder to degrade cellulose than hemicellulose and there is much more glucose in the cellulose part than in the hemicellulose. The recovery is lower for the hemicelluloses than for the celluloses. The recovery for xylose was the lowest in all three conditions, compared to glucose and mannose.

The reason for decreased recoveries of glucose, xylose and mannose can be due the loss in mass in the retrieval of the pretreated biomass from the collection vessel and also to the formation of secondary degradation products.

4.3 Enzymatic Hydrolysis

Enzymatic hydrolysis has been performed in duplicates for each pretreated material coming from conditions 1, 2 & 3 and the results are presented in the sections below. The hydrolyzed material can further be used for the fermentation in order to obtain ethanol. Previous studies (Balat, 2011) show that the major advantage when enzymatic hydrolysis and fermentation are performed separately is that they can be carried out under their optimal conditions. The disadvantage is longer overall time.

The concentration profiles for glucose during enzymatic hydrolysis are presented in the sections below. The final glucose concentrations and yields from all three conditions are presented in ***Appendix 4.***

The concentration profiles for xylose and mannose for condition 1 are presented in ***Appendix 5***. It can be seen that the concentrations did not change during the enzymatic hydrolysis step. The same pattern is obtained for condition 2 and 3. This is because the enzyme cocktail used in the experiment was specialized for glucan and not other carbohydrates.

4.3.1 Condition 1

Figure 12 represents the concentration profiles for glucose during the enzymatic hydrolysis for condition 1 of steam pretreated feedstocks and feedstock blends (P0, P10, P50 and P100) and also final glucose yields. Standard deviations are also represented in the figure. Each line is separately plotted in ***Appendix 6*** where standard deviations can be seen more clearly.

The highest glucose concentration has been obtained after hydrolyzed P0 and the lowest after hydrolyzed P10. However, the difference between obtained glucose concentration from hydrolyzed P0 and hydrolyzed P100 was only 0.5 g/L. Even though the final glucose concentration is almost the same for hydrolyzed P0 and P100, the glucose yield was highest for P0 and lowest for P100. As it can be seen in ***Figure 12*** the glucose yields are decreasing with the amount of poplar. One explanation for that could be that the amount of inhibitors formed were higher in P100 than in P0.

Glucose concentration profile for P10 has a small dip after 72h. This is due to high standard deviation from analyses. Therefore it is recommended to repeat enzymatic hydrolysis for this material in order to get more consequent result.

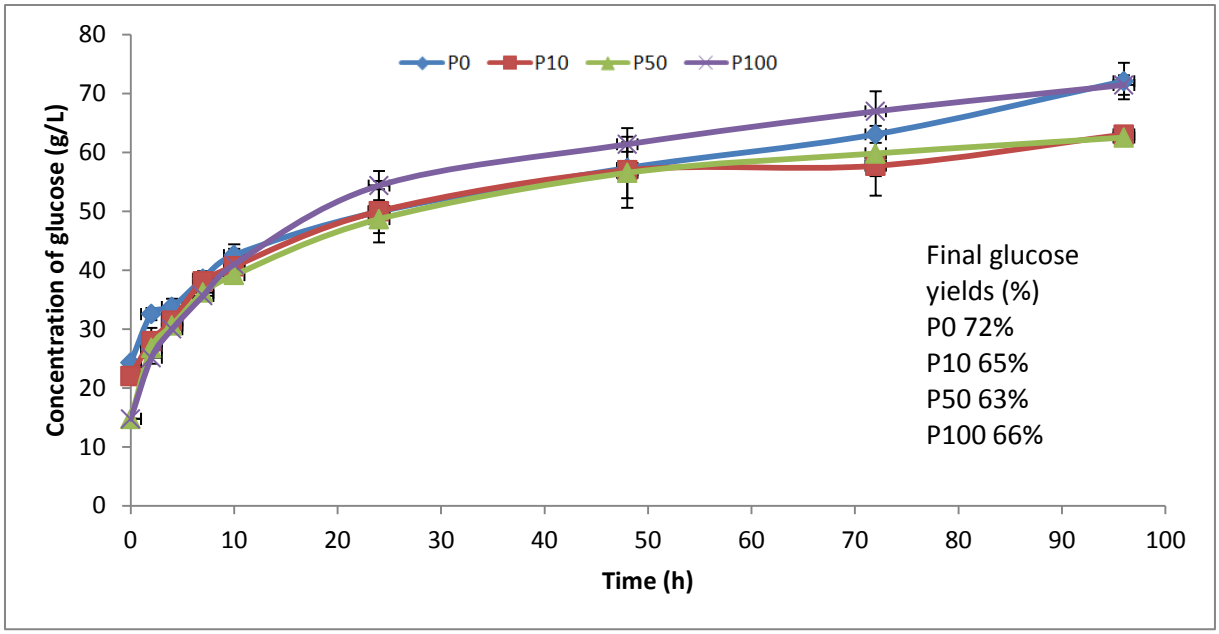

Figure 12. *The concentration profiles of glucose (g/L) during the enzymatic hydrolysis for condition 1. The final glucose concentrations after the enzymatic hydrolysis are shown. The enzymatic hydrolysis has been run for 96 hours.*

4.3.2 Condition 2

Figure 13 represents the concentration profiles for glucose during the enzymatic hydrolysis for condition 2 of steam pretreated feedstocks and feedstock blends (P0, P10, P50 and P100) and also final glucose yields. The highest glucose concentration has been obtained after hydrolyzed P100 and the lowest after hydrolyzed P10. Standard deviations are also represented in the figure. Each line is separately plotted in ***Appendix 7*** where standard deviations can be seen more clearly.

The same trend for glucose yields for the mixtures does not apply here as for the condition 1. The final glucose yield is highest for P100 and lowest for P0. Interestingly, the glucose yield here is not increasing with the amount of poplar, instead it decreases with 10% poplar and then decreases from P50. Although the same pattern for glucose recovery over the pretreatment step has been observed and can be the explanation for glucose yields over the enzymatic hydrolysis.

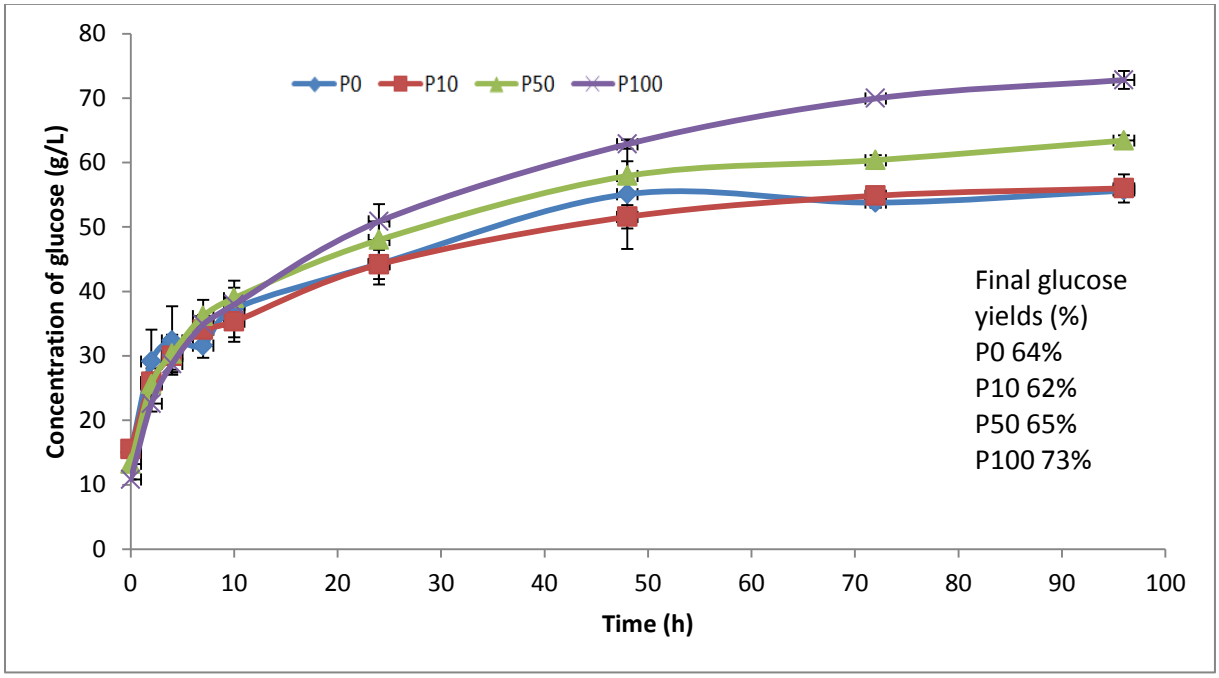

Figure 13. *The concentration profiles of glucose (g/L) during the enzymatic hydrolysis for condition 2. The final glucose concentrations after the enzymatic hydrolysis are shown. The enzymatic hydrolysis has been run for 96 hours.*

4.3.3 Condition 3

Figure 14 represents the concentration profiles for glucose during the enzymatic hydrolysis for condition 3 of steam pretreated feedstocks and feedstock blends (P0, P10, P50 and P100) and also final glucose yields. Standard deviations are also represented in the figure. Each line is separately plotted in ***Appendix 8*** where standard deviations can be seen more clearly.

The highest glucose concentration was obtained after hydrolyzed P100 and the lowest after hydrolyzed P0. Since glucose concentration depends on the available glucose in the pretreated material, see ***Table 10,*** and the glucose yield during enzymatic hydrolysis is this result reasonable.

The glucose yield after 96 h of hydrolyzed material was highest in P100 and lowest in P0 and it was not increasing with the amount poplar. Comparing the mixtures, there was no significant difference in the final glucose yield.

A dip in the concentration profile for P0 is obtained after 72h. Even this material should be hydrolyzed once more to obtain more consequent results.

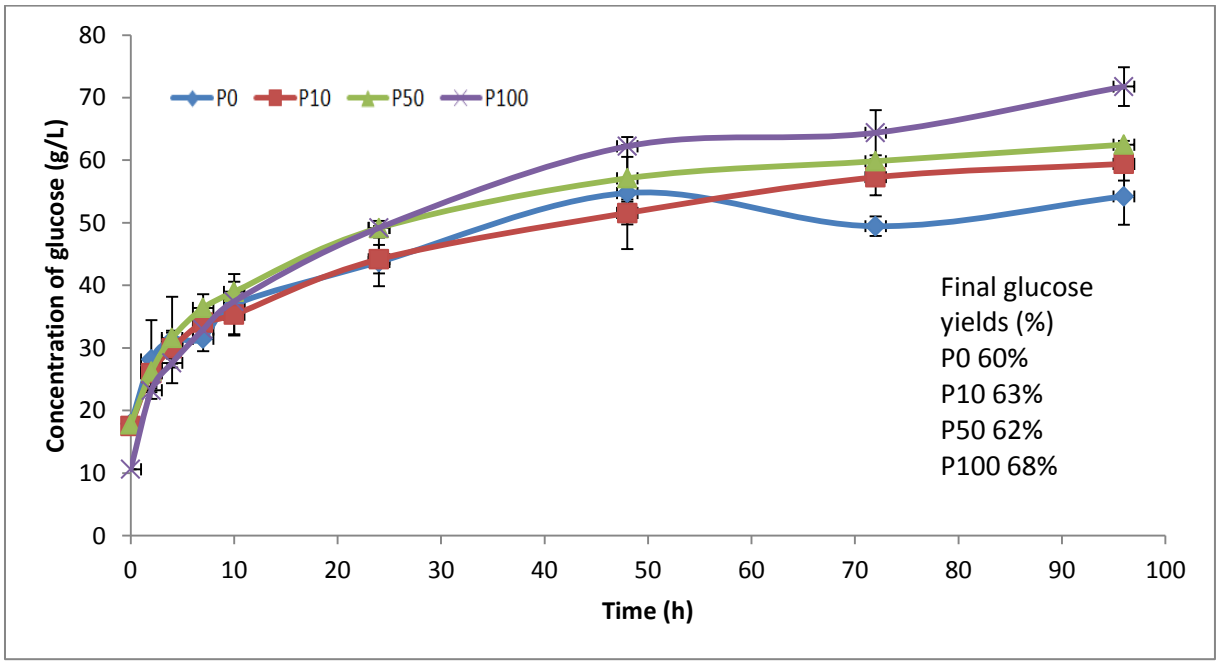

Figure 14. *The concentration profiles of glucose (g/L) during the enzymatic hydrolysis for condition 3. The final glucose concentrations after the enzymatic hydrolysis are shown. The enzymatic hydrolysis has been run for 96 hours.*

5. Conclusions

During the pretreatment step there was a clear pattern for sugar amounts in both liquid and solid phase obtained after the pretreatment. In the solid fractions of pretreated materials, glucan amount was increasing and in the liquid part glucan amount was decreasing with more poplar in feedstocks.

The pretreated poplar after all three conditions gave the highest overall glucose recovery. When considering the glucose recovery, for pretreatment 2, the study showed that 50% mixture of poplar and spruce affected glucose yield positively compared to 100% spruce material.

The lowest glucose recoveries for all materials were obtained after condition 1 (210ºC, 5 min, 2,5% SO_2). Therefore it can be concluded that the best conditions for the pretreatment step are conditions 2 and 3 if considering glucose yield.

Considering the glucose yields after enzymatic hydrolysis it can be concluded that enzymatic hydrolysis was effective for poplar after all three pretreatment conditions, but not for spruce. Comparing the mixtures, there was no significant difference in the final glucose yield after any enzymatic hydrolysis. Therefore it can be concluded that there are no huge effects or synergies by mixing poplar and spruce on enzymatic hydrolysis.

The highest glucose yield (76.8 %) after enzymatic hydrolysis was obtained for pure poplar after condition 1. Quite low glucose yields are obtained which can be explained with low enzyme dosage during the enzymatic hydrolysis step.

Since almost the same glucose yields for the mixtures after enzymatic hydrolysis were obtained, it can be concluded that a wide range of conditions for the pretreatment can be applied and still lead to the same results regarding glucose yields.

6. Future work

In this study, different parameters for pretreatment step were tested. To further investigate the pretreatment step, it would be of interest to test more conditions. One example is to run the pretreatment at even milder conditions than the condition 2 in the study and see how that will affect the sugar recoveries.

To strengthen the hypotheses in this study it is recommended to pretreat feedstock blends of 25% and 75% of poplar and see how those will affect sugar recoveries. Also it is recommended to repeat pretreatment to validate if the results are consequent.

The enzymatic hydrolysis can be further investigated by changing the parameters. In this study, the same parameters and WIS content have been used for all enzymatic cycles. It would be interesting to see how the parameters affect the yields of glucose after enzymatic hydrolysis.

Also it is interesting to test digestibility/hydrolysability at industrially relevant conditions. To some extent this was actually done in this study by choosing not so low WIS content. Of course higher WIS content could be eventually tested on the best pretreatment.

It can also be of interest to run enzymatic hydrolysis on washed substrate. This would give more information on digestibility without the interference with the liquid phase since degradation compounds, monomeric and oligomeric sugars can inhibit enzymes and give a negative effect.

The hydrolyzed material can further be used for the fermentation in order to obtain ethanol. Previous studies show that the major advantage when enzymatic hydrolysis and fermentation are performed separately is that they can be carried out under their optimal conditions. The disadvantage is longer overall time. The fermentability test would also give more information on whether it is effective to mix poplar and spruce. For instance, diluting poplar with spruce could possibly give less inhibitors. Also, if poplar that had higher amount of glucose yield is not possible to ferment, but the mixtures are than it would be effective to mix the mixtures from the fermentation view.

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8. Appendices

8. 1 Appendix 1

Overall sugar recoveries (glucose, xylose and mannose) over the pretreatment for condition 1 are presented in ***Figure 15***.

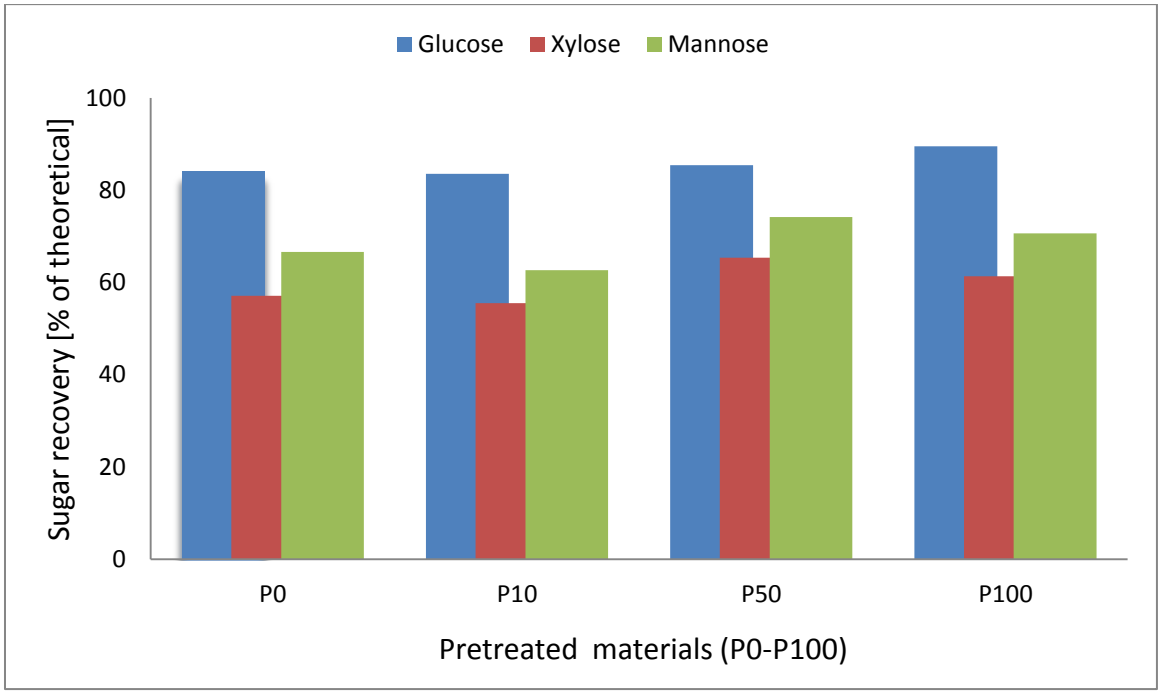

Figure 15. *Overall glucose, xylose and mannose recoveries over the steam pretreatment (condition 1) of spruce, poplar and blends thereof. Recovery expressed as percentage of the theoretical based on sugars content of the raw materials.*

8. 2 Appendix 2

Overall sugar recoveries (glucose, xylose and mannose) over the pretreatment for condition 2 are presented in ***Figure 16.***

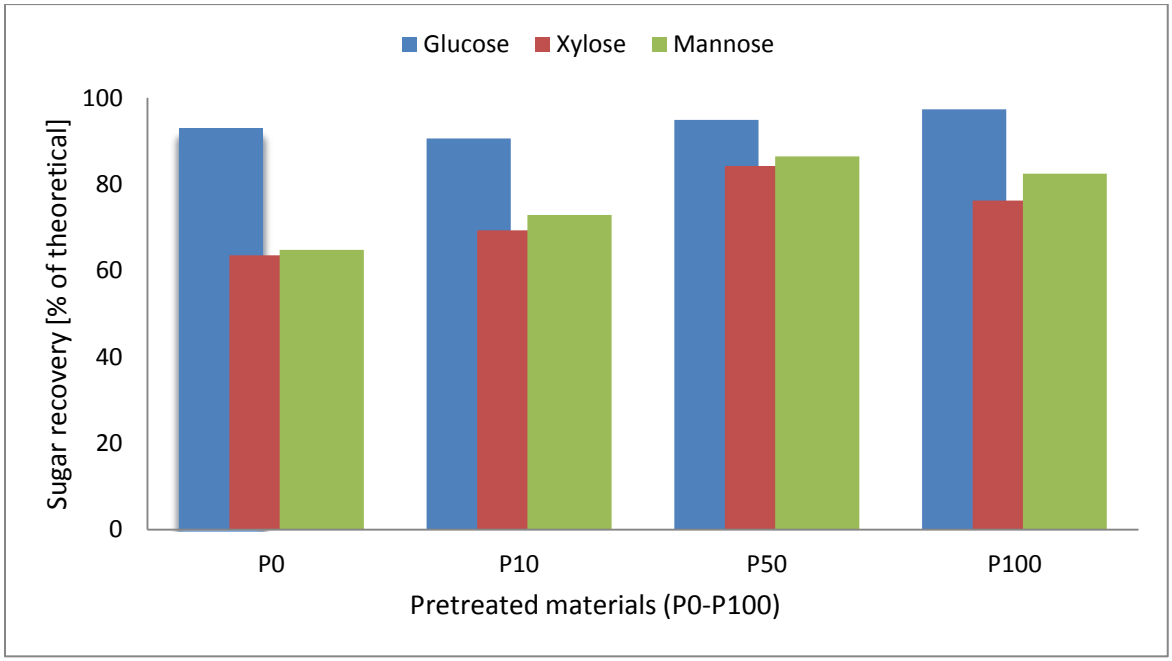

Figure 16. *Overall glucose, xylose and mannose recoveries over the steam pretreatment (condition 2) of spruce, poplar and blends thereof. Recovery expressed as percentage of the theoretical based on sugars content of the raw materials.*

8. 3 Appendix 3

Overall sugar recoveries (glucose, mannose and xylose) over the pretreatment for condition 3 are presented in ***Figure 17.***

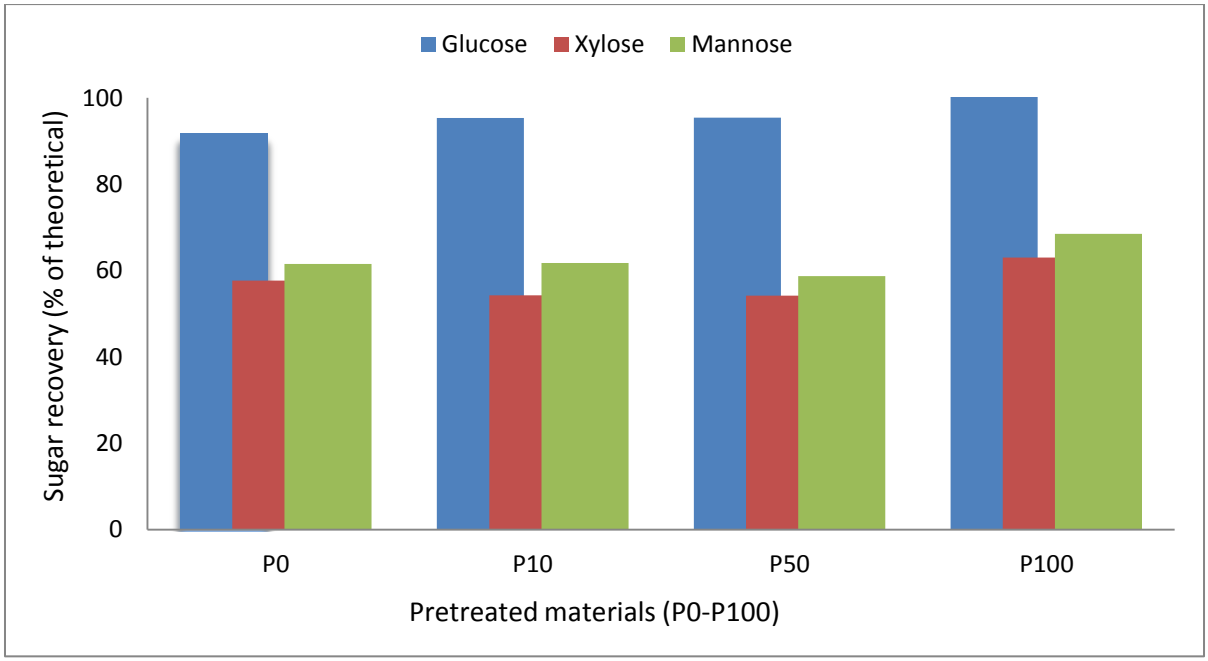

Figure 17. *Overall glucose, xylose and mannose recoveries over the steam pretreatment (condition 3) of spruce, poplar and blends thereof. Recovery expressed as percentage of the theoretical based on sugars content of the raw materials.*

8. 4 Appendix 4

Table 10. *Final glucose concentrations and yields after enzymatic hydrolysis for all hydrolyzed slurries (P0-P100) obtained from pretreatment*.

Conditions	**Slurries**	**Final glucose concentration [g/L]**		**Final glucose yield [%]**	
		EH 1	EH 2	EH 1	EH 2
Condition 1	P0	74.3	69.9	76.8	67.8
	P10	63.3	62.7	66.0	63.5
	P50	61.8	63.2	63.9	62.6
	P100	69.4	72.6	65.6	66.7
Condition 2	P0	55.7	55.6	63.8	64.4
	P10	57.5	54.4	63.7	61.1
	P50	62.8	63.9	64.9	65.5
	P100	71.8	73.8	72.7	74.1
Condition 3	P0	51.0	57.4	57.2	63.7
	P10	57.4	61.3	61.2	64.7
	P50	62.9	62.1	62.5	61.3
	P100	69.6	73.9	66.2	70.2

EH1=Enzymatic hydrolysis first run. EH2= Enzymatic hydrolysis second run.

8. 5 Appendix 5

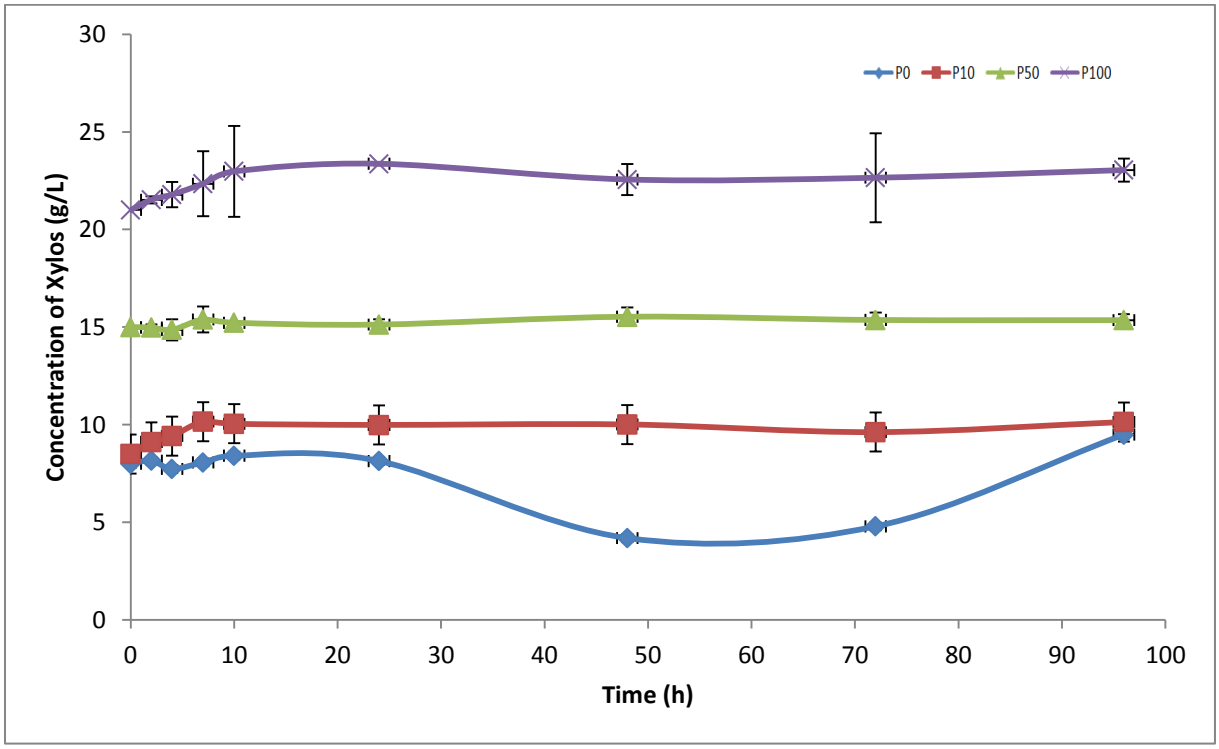

Figure 18. *The concentration profiles of xylose (g/L) for P0-P100 during the enzymatic hydrolysis for condition 1. The enzymatic hydrolysis has been run for 96 hours.*

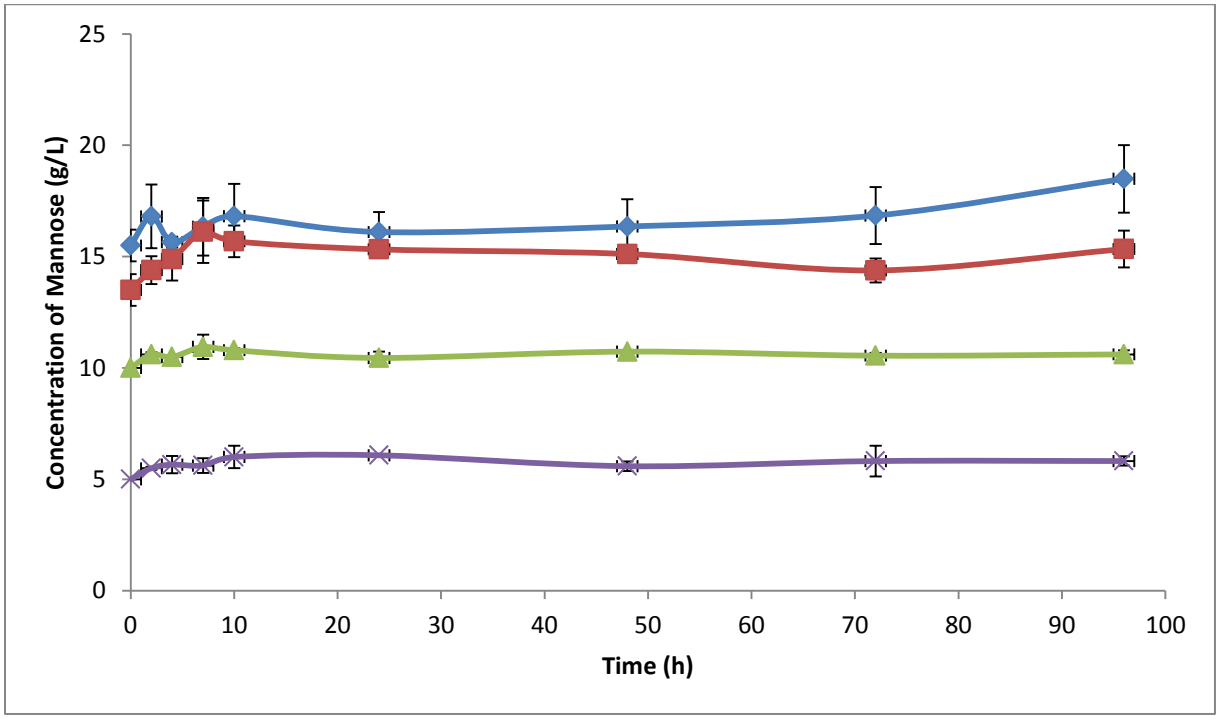

Figure 19. *The concentration profiles of mannose (g/L) for P0-P100 during the enzymatic hydrolysis for condition 1. The enzymatic hydrolysis has been run for 96 hours.*

8. 6 Appendix 6

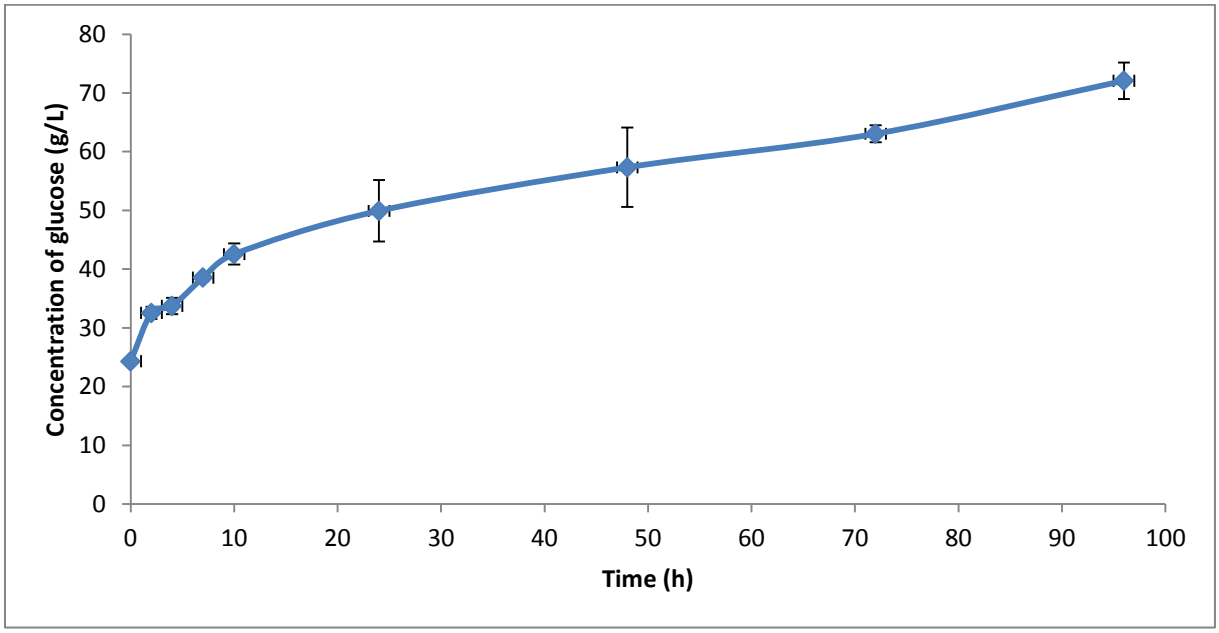

Figure 20. *The concentration profiles of glucose (g/L) for P0 (100% spruce) during the enzymatic hydrolysis for condition 1. The enzymatic hydrolysis has been run for 96 hours.*

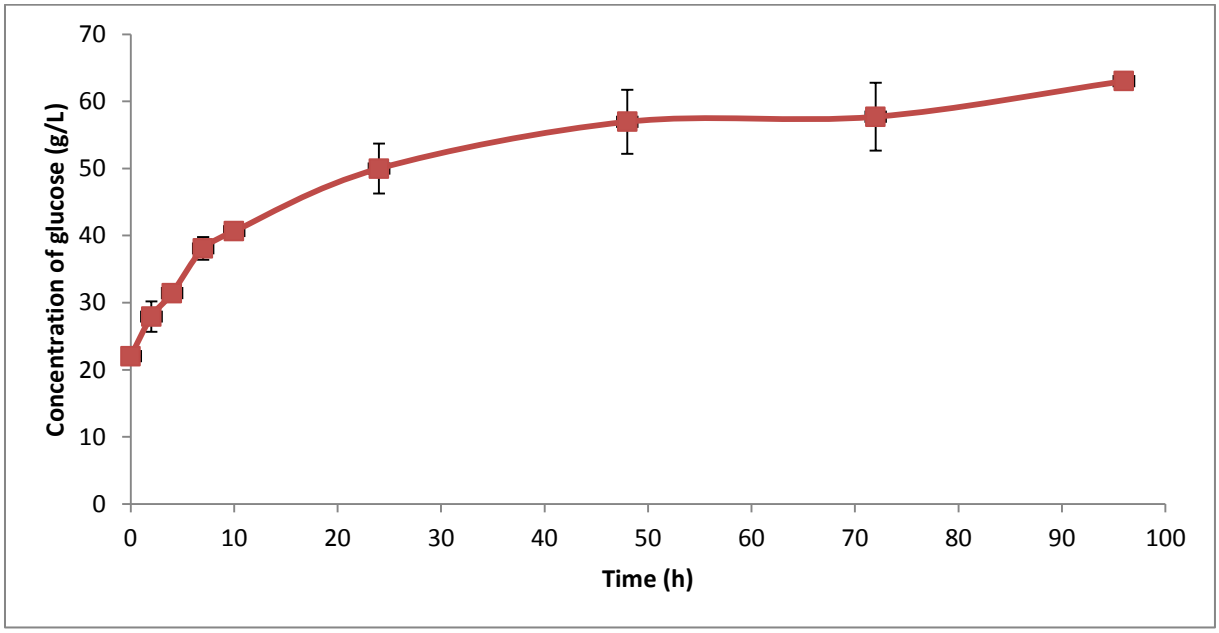

Figure 21. *The concentration profiles of glucose (g/L) for P10 (10% spruce and 90% poplar) during the enzymatic hydrolysis for condition 1. The enzymatic hydrolysis has been run for 96 hours.*

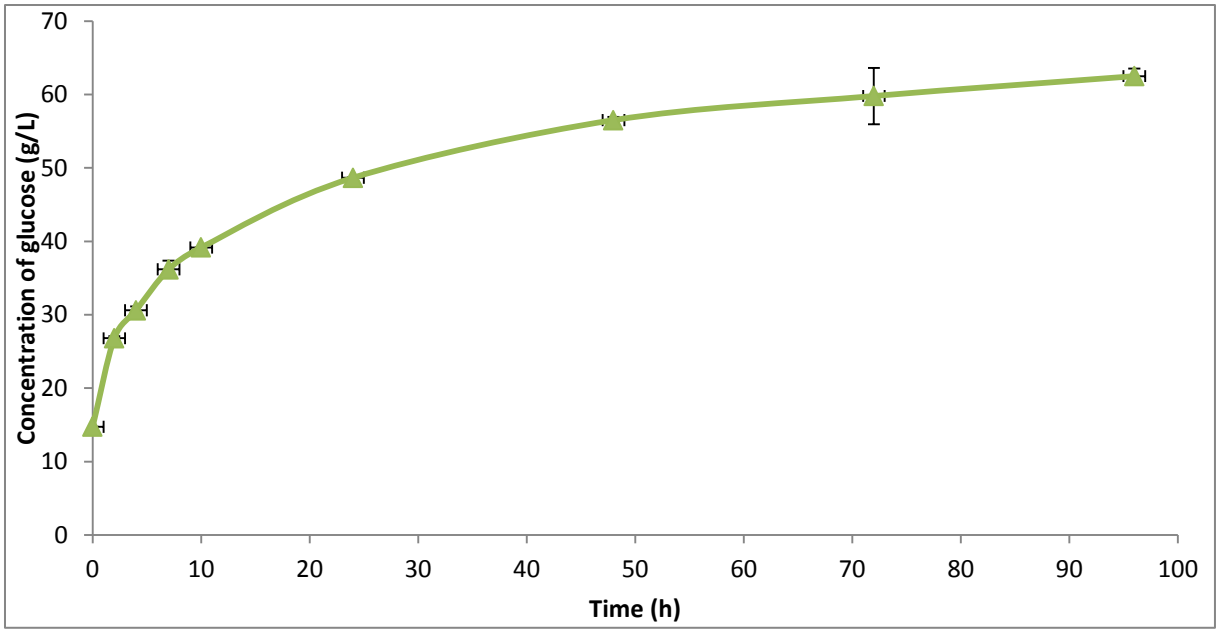

Figure 22. *The concentration profiles of glucose (g/L) for P50 (50% spruce and 50% poplar) during the enzymatic hydrolysis for condition 1. The enzymatic hydrolysis has been run for 96 hours.*

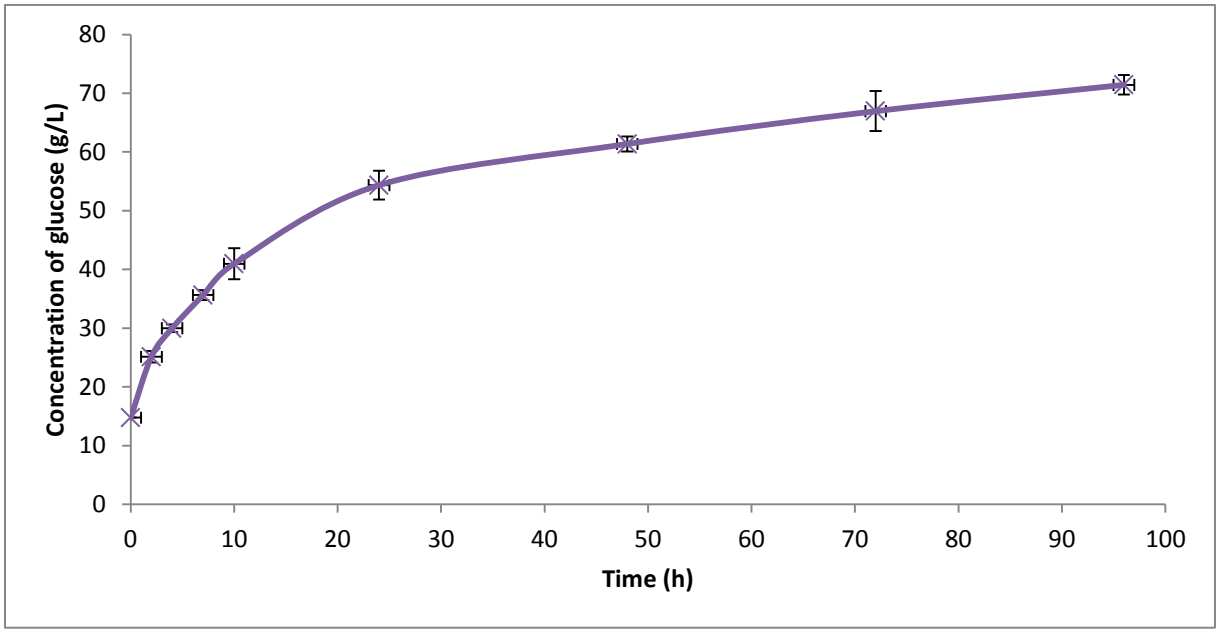

Figure 23. *The concentration profiles of glucose (g/L) for P100 (100% poplar) during the enzymatic hydrolysis for condition 1. The enzymatic hydrolysis has been run for 96 hours.*

8. 7 Appendix 7

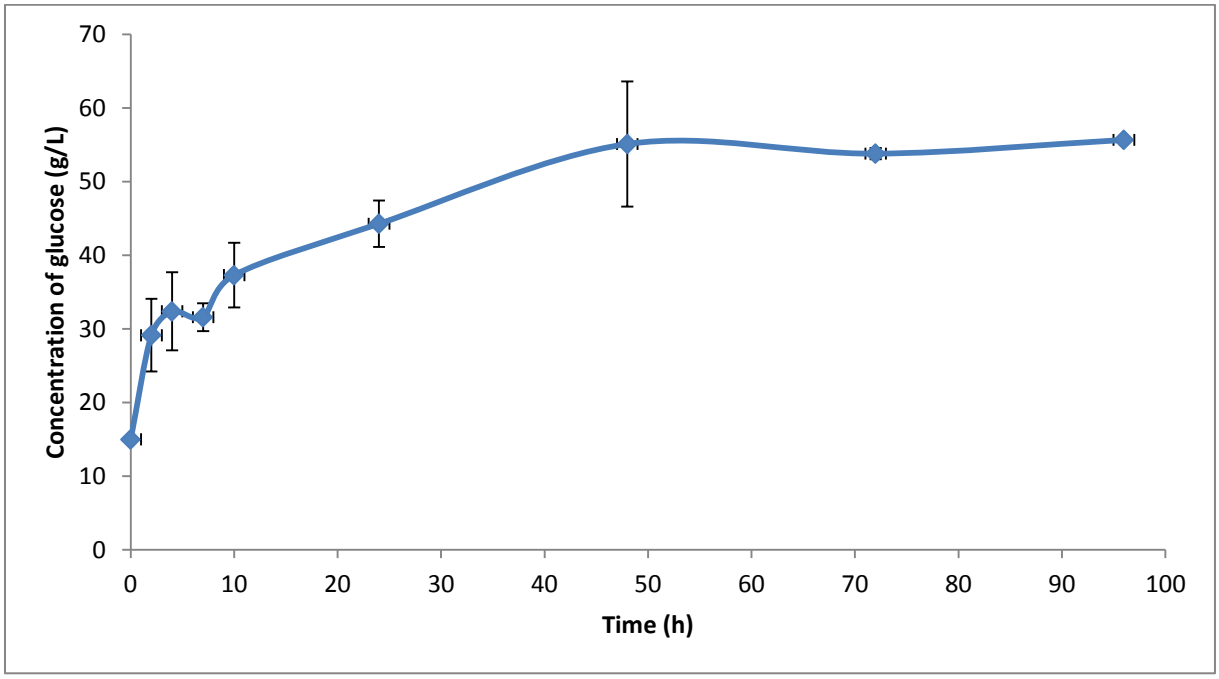

Figure 24. *The concentration profiles of glucose (g/L) for P0 (100% spruce) during the enzymatic hydrolysis for condition 2. The enzymatic hydrolysis has been run for 96 hours.*

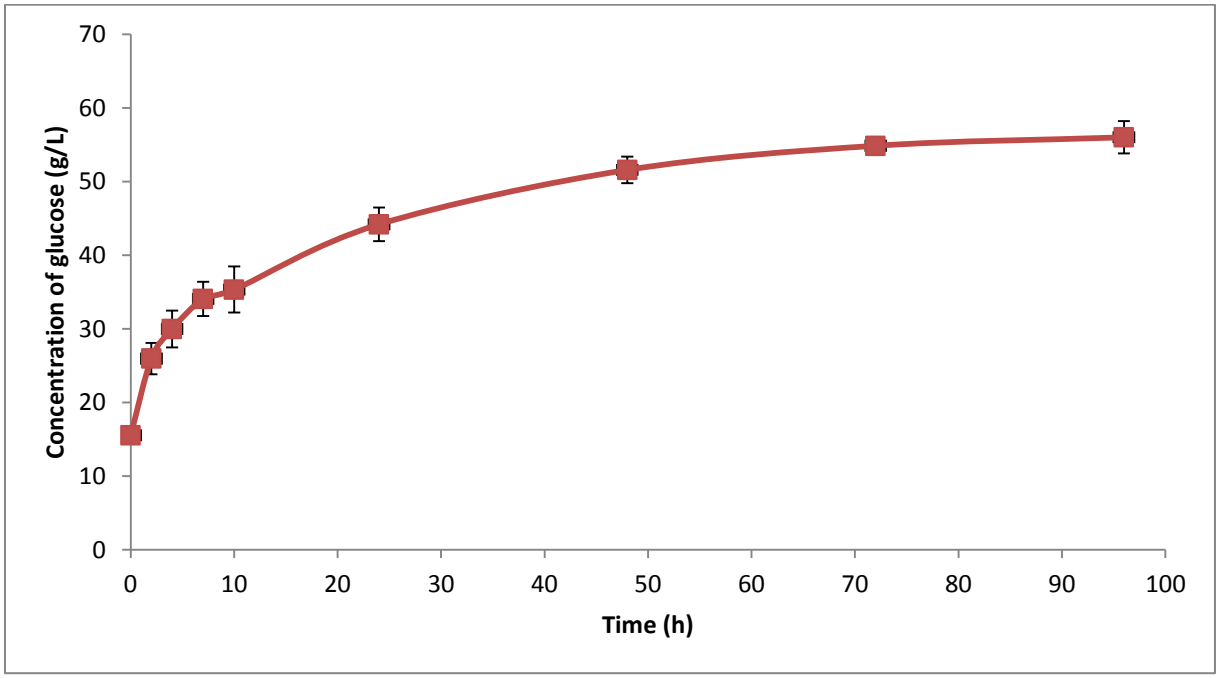

Figure 25. *The concentration profiles of glucose (g/L) for P10 (10% spruce and 90% poplar) during the enzymatic hydrolysis for condition 2. The enzymatic hydrolysis has been run for 96 hours.*

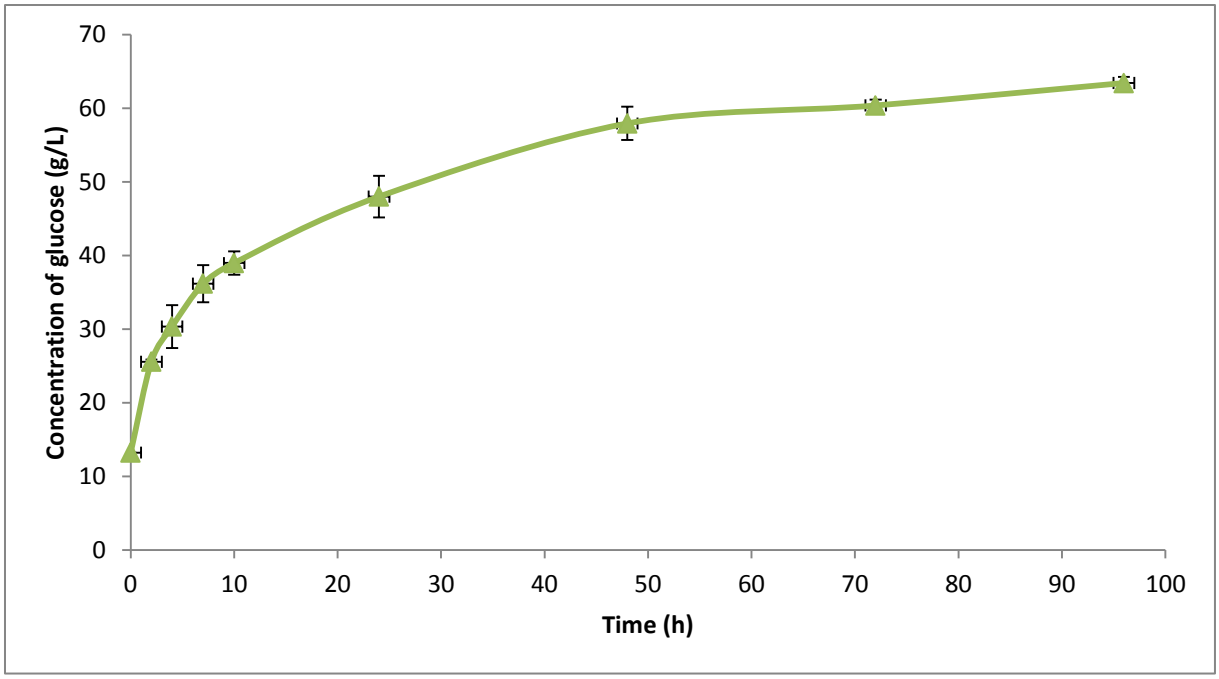

Figure 26. *The concentration profiles of glucose (g/L) for P50 (50% spruce and 50% poplar) during the enzymatic hydrolysis for condition 2. The enzymatic hydrolysis has been run for 96 hours.*

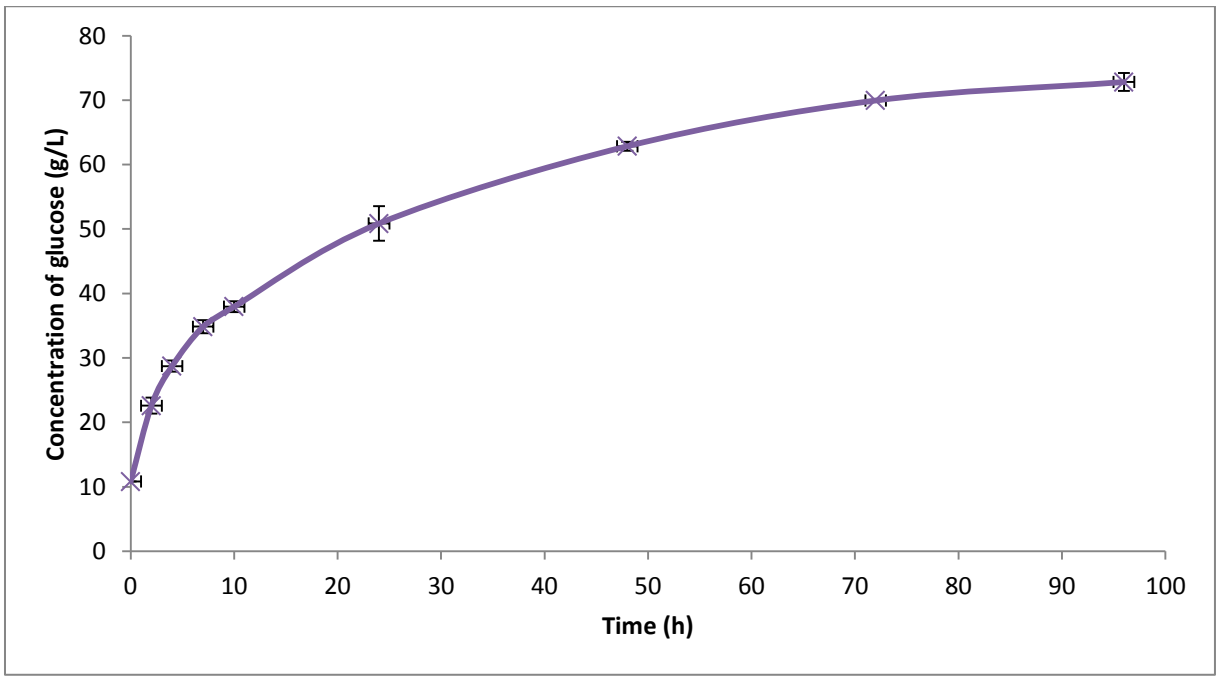

Figure 27. *The concentration profiles of glucose (g/L) for P100 (100% poplar) during the enzymatic hydrolysis for condition 2. The enzymatic hydrolysis has been run for 96 hours.*

8. 8 Appendix 8

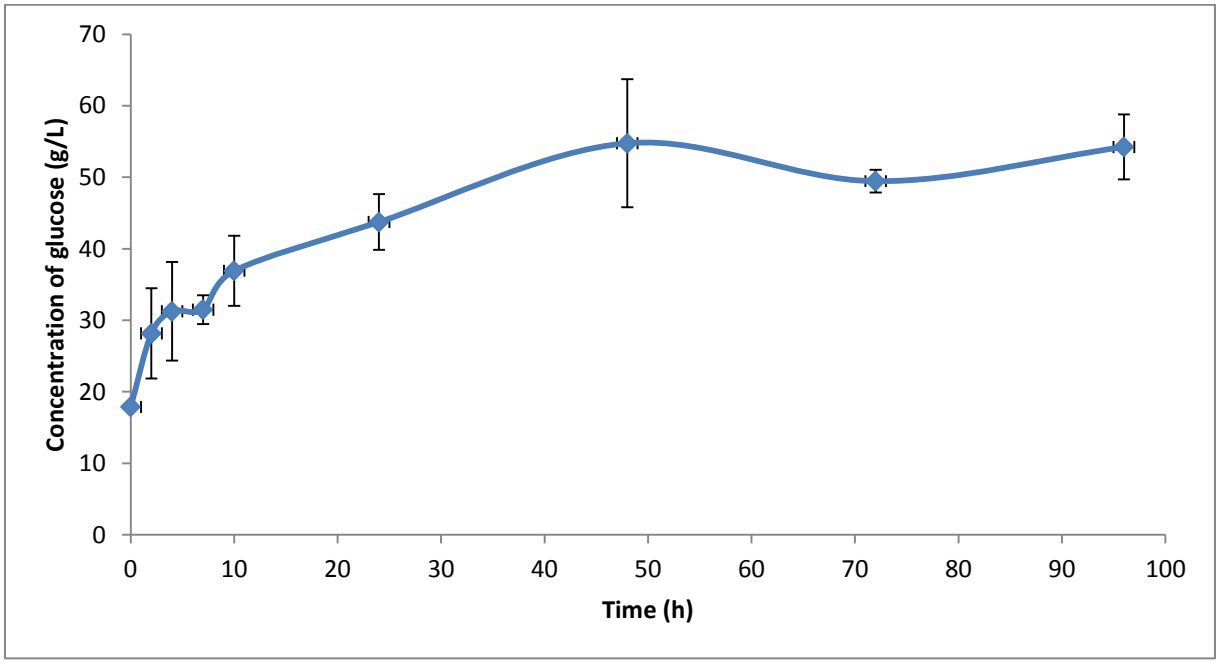

Figure 28. *The concentration profiles of glucose (g/L) for P0 (100% spruce) during the enzymatic hydrolysis for condition 3. The enzymatic hydrolysis has been run for 96 hours.*

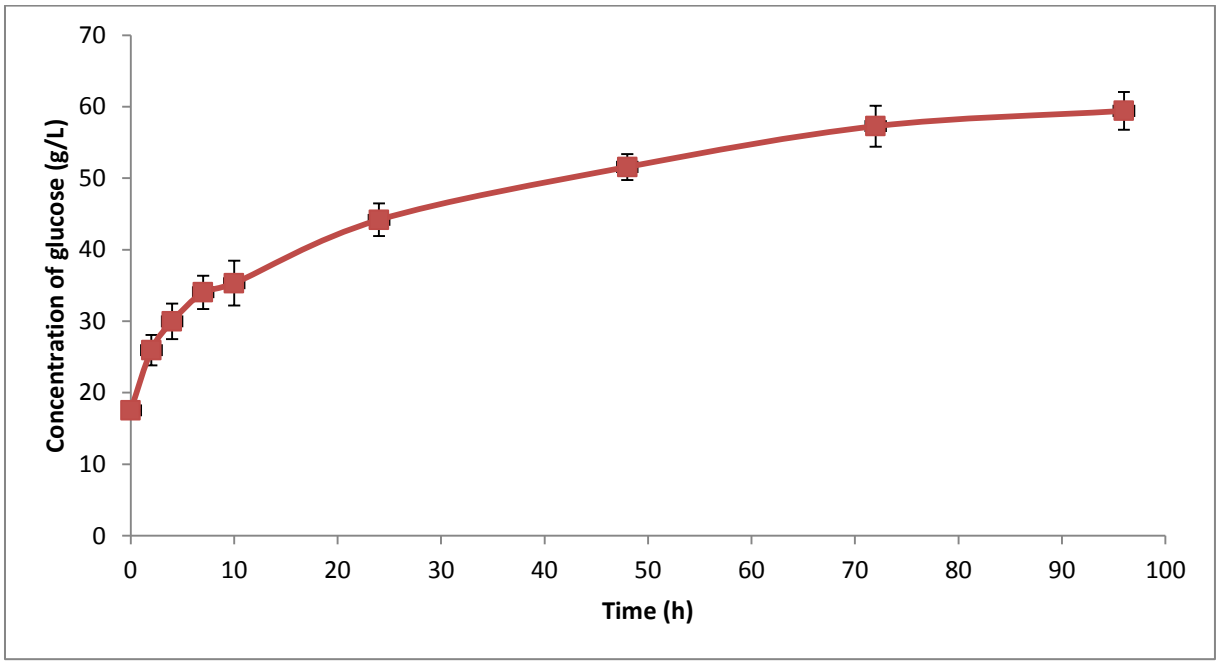

Figure 29. *The concentration profiles of glucose (g/L) for P10 (10% spruce and 90% poplar) during the enzymatic hydrolysis for condition 3. The enzymatic hydrolysis has been run for 96 hours.*

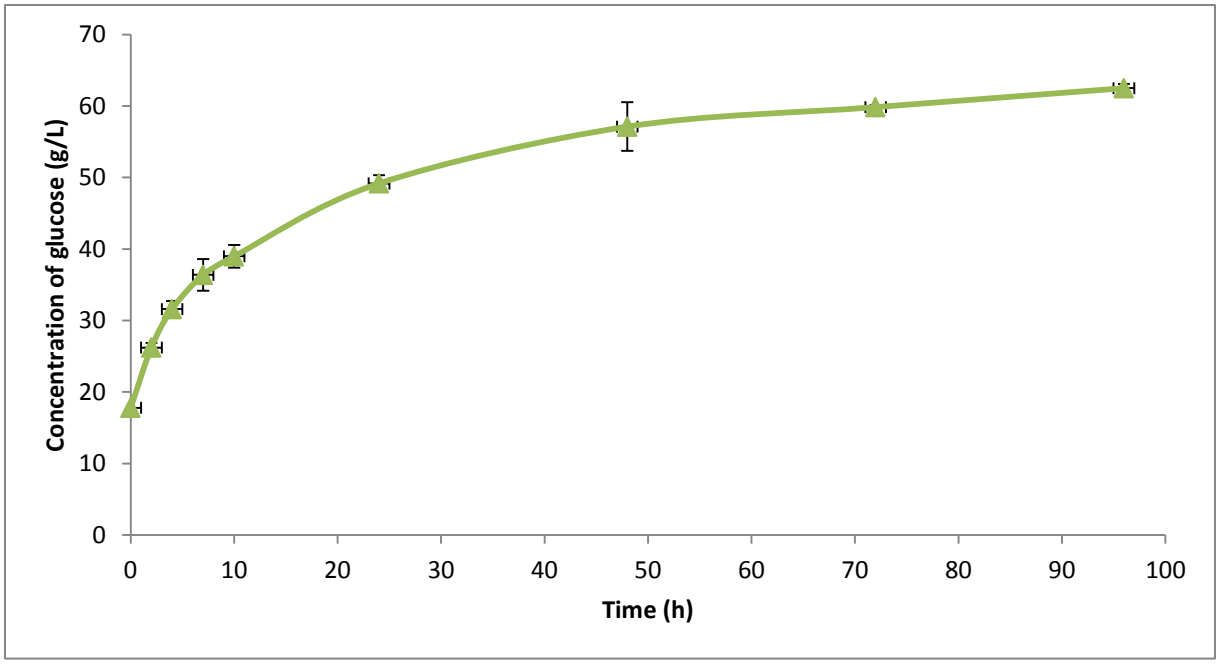

Figure 30. *The concentration profiles of glucose (g/L) for P50 (50% spruce and 50% poplar) during the enzymatic hydrolysis for condition 3. The enzymatic hydrolysis has been run for 96 hours.*

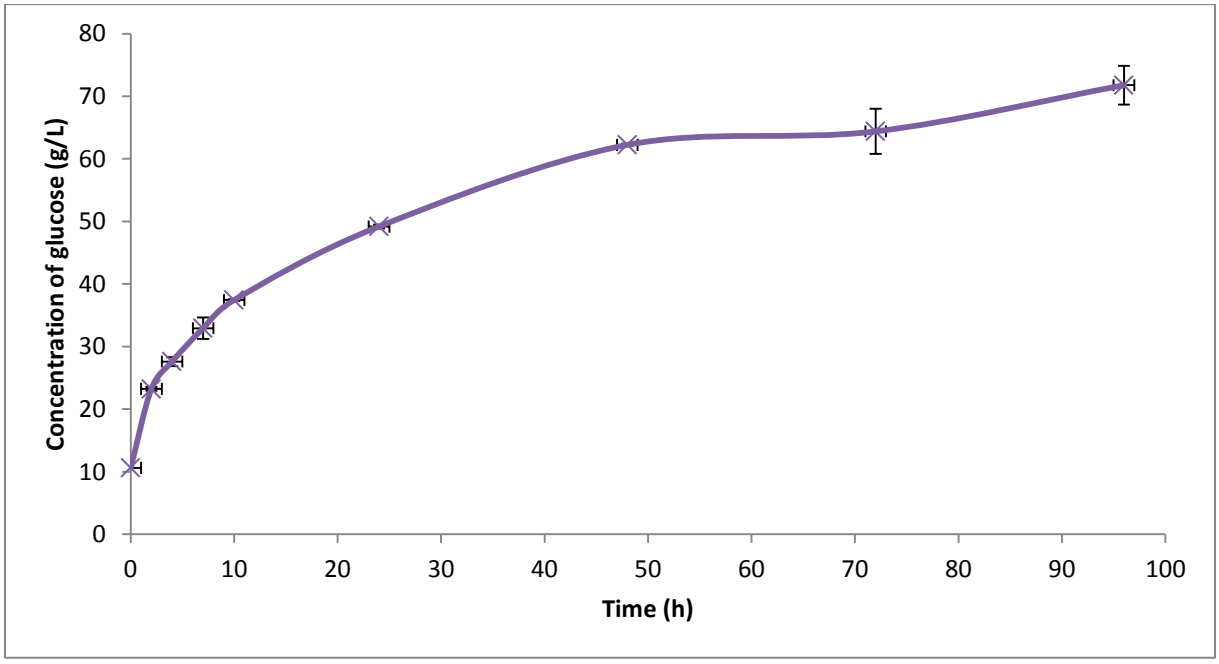

Figure 31. *The concentration profiles of glucose (g/L) for P100 (100% poplar) during the enzymatic hydrolysis for condition 3. The enzymatic hydrolysis has been run for 96 hours.*