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Decrystallization of Lignocellulosic Biomass using Ionic Liquids

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Declaration (Erklæring)

I hereby declare that the work performed in this master thesis has been done idependently and in accordance with the rules and regulations which regulate the master program at the Norwegian University of Science and Technology, NTNU.

(Jeg erklærer herved at jeg har utført arbeidet i forbindelse med denne masteroppgaven selvstendig og i henhold til foreskrift om krav til mastergrad ved NTNU.)

Trondheim 17.06.2012

Jenny Kristin Håseth

Preface

The work presented here has been carried out at the Department of Chemical Engineering, Norwegian University of Science and Technology (NTNU) and the Norwegian Pulp and Paper Research Institute (PFI) under supervision of Associate Professor Størker Moe (NTNU) and Karin Øyaas (Research manager, PFI). The work was carried out between 22.01.2012 and 17.06.2012 in fulfilment of the requirement for a M.Sc at NTNU.

Through PFI, this project is linked to the Sustainable Biofuel program of the Top-level Research Initiative (TRI) under the sub-program Innovations in Bioethanol Production Technologies. TRI is a major Nordic venture, founded by Nordic Energy Research, which focuses on climate, energy and the environment. The goal of the initiative is making a contribution towards solving the global climate crisis by involving and joining the best agencies and institutions in the Nordic region[1].

Acknowledgment

Thanks to NTNU, PFI and Nordic Energy Research for founding this work and to Størker Moe (associate professor, NTNU) and Karin Øyaas (Research manager, PFI) for help and guidance during the semester. The author also wishes to thank Ingebjørg Leirset and Mirjana Filipovic at PFI for helping with the experimental work.

Abstract

This thesis is written in fulfilment of the requirements for a Master in Science at the Norwegian University of Science and Technology (NTNU), Department of Chemical Engineering. The work investigates the effectiveness of pretreatment of norway spruce and sugarcane bagasse with the ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]). The effect of pretreatment temperature and reaction time was evaluated. Enzymatic hydrolysis yield was used as the main evaluation parameter.

Norway spruce was pretreated at 80, 100, and 120 °C for 3, 6, 12, and 24 hours. The sugarcane bagasse raw material was pretreated at the same temperatures for 1 and 3 hours. UV-Vis spectrophotometric analysis was used to determine the amount of lignin removed during the pretreatment. The regenerated solids from the pretreatment was hydrolysed enzymaticly and the digestibility was determined using High-Performance Liquid Chromatography (HPLC).

The pretreatment caused an increase in the enzymatic digestibility for both spruce and bagasse. This effect is believed to arise from a decrease in the crystallinity of the cellulose and an increase in the accessible surface area caused by the increased porosity of the pretreated material.

The digestibility results for spruce shows that, at shorter pretreatment times, higher temperatures are favourable. However, at longer reaction times, too high temperatures can give a reduction in the digestibility. The optimal reaction condition for spruce was in this work found to be 100 °C for 12 hours, giving a digestibility close to 90 wt% of the added glucan. For sugarcane bagasse the optimum was not found, and experiments using harsher conditions was proposed. When comparing the results for pretreatment of spruce with that of bagasse it appear that spruce needs harsher conditions to achieve the same glucan yield as bagasse.

The results of the analysis of the enzymatic digestibility of hemicelluloses (mannan for spruce and zylan for bagasse) concurs very well with the results for glucan presented above.

Regarding the removal of lignin from the biomass, it was found that the degree of delignification in these pretreatment experiments was so low it could be neglected. The low degree of lignin removal was also evident in the darkening of the regenerated biomass from pretreatments using relatively harsh reaction conditions. This darkening was put down to the lignin undergoing condensation reactions.

Suggestions for further work on this area include a thorough investigation into the thermal stability of different ionic liquids at prolonged reaction times and high temperatures, as well as an investigation of the delignification effect of different ionic liquids. As mentioned earlier, pretreatment experiments with bagasse using harsher conditions can also be useful.

Sammendrag

Denne oppgaven er skrevet i henhold til foreskrifter om krav til mastergrad ved NTNU, Institutt for kjemisk prosessteknologi. Oppgaven ser på effekten av forbehandling av gran og bagasse med den ioniske væsken 1-etyl-3-metylimidazolium acetat ([EMIM] [OAc]). Effekten av reaksjonstemperatur og -tid ble evaluert. Enzymatisk hydrolyseutbytte ble brukt som evaluerings parameter.

Gran ble forbehandlet ved 80, 100 og 120 °C i 3, 6, 12 og 24 timer. Bagasse ble forbehandlet ved samme temperaturer i 1 og 3 timer. UV-vis spektrofotometrisk analyse ble brukt til å bestemmes mengden utløst lignin fra forbehandling. Den regenererte biomassen fra forbehandlingen ble hydrolysert enzymatisk og utbyttet ble bestemt ved hjelp HPLC analyse.

Forbehandlingen ga en økning i det enzymatiske hydrolyseutbyttet til både gran og bagasse. Denne effekten antas å være et resultat av en nedgang i krystalliniteten til cellulosen samt en økning i porøsiteten til det forbehandlede materialet.

Resultatene for gran viser at ved kortere forbehandlings tider, er høyere temperaturer gunstige. Ved lengre reaksjonstider kan for høye temperaturer gi en reduksjon i hydrolyseutbyttet. Optimale reaksjonbetingelse for gran ble i dette arbeidet funnet til å være 100 °C og 12 timer, noe som gir et hydrolyseutbytte på nesten 90 vekt% av tilsatt glukan. For bagasse ble ikke optimum funnet, og eksperimenter med tøffere reaksjonsbetingelser er anbefalt. En sammenligning mellom resultatene for hydrolyse av forbehandlet gran og bagasse tyder på at grana trenger tøffere reaksjonsbetingelser enn bagasse for å oppnå samme glukan utbytte.

Resultatene fra analysen av enzymatisk utbytte av hemicelluloser (mannan for gran og zylan for bagasse) stemmer som forventet meget godt overens med resultatene for glukan presentert ovenfor.

Når det gjelder delignifisering av biomassen ble det funnet at delignifiserinsgraden i forsøkene presentert her var så lav at den kan neglisjeres. Den lave graden av ligninfjærning kom også frem av den mørkere farge på den regenererte biomassen fra forbehandlinger ved relativt tøffe reaksjonsbetingelser. Fargeendringen ble betraktet som en indikasjon på at ligninet gjennomgikk en kondensasjonsreaksjon.

Forslag til videre arbeid på dette området omfatter en undersøkelse av den termiske stabiliteten til ulike ioniske væsker ved lengre reaksjonstider, samt en undersøkelse av delignifiseringeffekten av ulike ioniske væskene. I tillegg er det foreslått å utføre forbehandlinger av bagasse ved tøffere reaksjonsbetingelser en de brukt i dette arbeidet.

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List of abbreviations and symbols

AFEX	Ammonia fibre explosion
AIL	Acid insoluble lignin
[AMIM][Cl]	1-alyll-3-methylimidazolium clorid
ASL	Acid soluble lignin
ATMP	Advanced thermo-mechanical pulp
[BMIM][Cl]	1-butyl-3-methylimidazolium clorid
[BnMIM][Cl]	1-benzyl-3-methylimidazolium clorid
DM	Dry matter content
DP	Degree of polymerisation
[EMIM][Cl]	1-ethyl-3-methylimidazolium clorid
[EMIM][OAc]	1-ethyl-3-methylimidazolium acetat
HCl	Hydrochloric acid
HMF	5-hydroxymethylfurfural
HPLC	High-performance liquid chromatography
H_2SO_4	Sulfuric acid
IL	Ionic liquids
LC	Lignocellulosic
LHW	Liquid hot water
T	Temperature (°C)
t	Time (h)
TMP	Thermo-mechanical pulp
UV-Vis	Spectrophotometric analysis in the ultra violet to the visible light area
W	Weight
wt%	Weight percent

1 Introduction

1.1 Motivation

1.1.1 Background

Today the petroleum based industry faces tough challenges in the form of dwindling reserves and increasing energy price, as well as problems connected to the environment and climate[2]. As a result the interest for finding renewable resources capable of replacing the fossil based raw material in production of fuel, energy and materials are increasing. There are several possible renewable sources for energy production such as wind, solar, hydroelectric, wave, and tidal power, but for production of carbon-containing fuels and materials none of these are suitable. In this area biomass is the most promising alternative on account of being the most abundant, carbon containing, renewable resource, with approximately 10^{12} tons available worldwide[3].

Lignocellulosic materials (LC), has a high potential for increased use for production of fuels, commodity chemicals and biodegradable materials[4] without affecting the food production capacity significantly. Wood raw materials are a particularly promising alternative, it being in plentiful supply at a relatively low cost. Today wood is manly used as fuel and building and manufacturing materials as well as being the primary source of cellulose for pulp and paper production[5]. Extensive research are being done on converting lignocellulosic biomass, including agricultural residues, forestry wastes, and energy crops, to valuable products through hydrolysis to monosaccharides and further (usually through fermentation) to a myriad of bio-based products[6]. Pretreatment of biomass is a crucial step in this conversion. In this context ionic liquid pretreatment of biomass has received much attention lately. The work presented her investigates the effect of pretreatment of chosen lignocellulosic materials with ionic liquids to increase the enzymatic degradation into monosaccharides.

1.1.2 Scope of this thesis

This thesis is written in fulfilment of the requirements for a M.Sc at the Norwegian University of Science and Technology. The aim of the master project is to elucidate the solubilising effect of the chosen ionic liquid on one or more lignocellulosic raw materials. Focus of the work is studying the effect of the reaction conditions, manly temperature and reaction time, on the efficiency of pretreatment of norway spruce with 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]). Enzymatic hydrolysis yields will be used as the main evaluation criterion. If time permits, pretreatment of sugarcane bagasse should be studied and other reaction conditions, such as wood:liquid ratio, might also be included.

The procedure for the ionic liquid pretreatment was developed as a part of specialisation project leading up to this master thesis.

1.2 Lignocellulosic materials

Lignocellulosic materials (LC materials) include wood, grass, forest waste, and agricultural waste[2], and consist mainly of cellulose, hemicelluloses, and lignin with the addition of some extractives and ash. Depending on the species, up to 80% of the lignocellulosics can be polysaccharides (cellulose and hemicelluloses) which may theoretically be converted to e.g. fuel alcohols by fermentation. The lignin fraction of the LC material is today mostly burned for process energy, but it has the potential to be utilised for the production of valuable chemicals and materials. Figure 1 gives a representation of some of the carbohydrates found in lignocellulosic biomass. Glucomannan is the most commune hemicellulose in softwoods, like norway spruce, and zylan is the most command in sugarcane bagasse. A representation of the structure of native wood lignin are given in Figure 2.

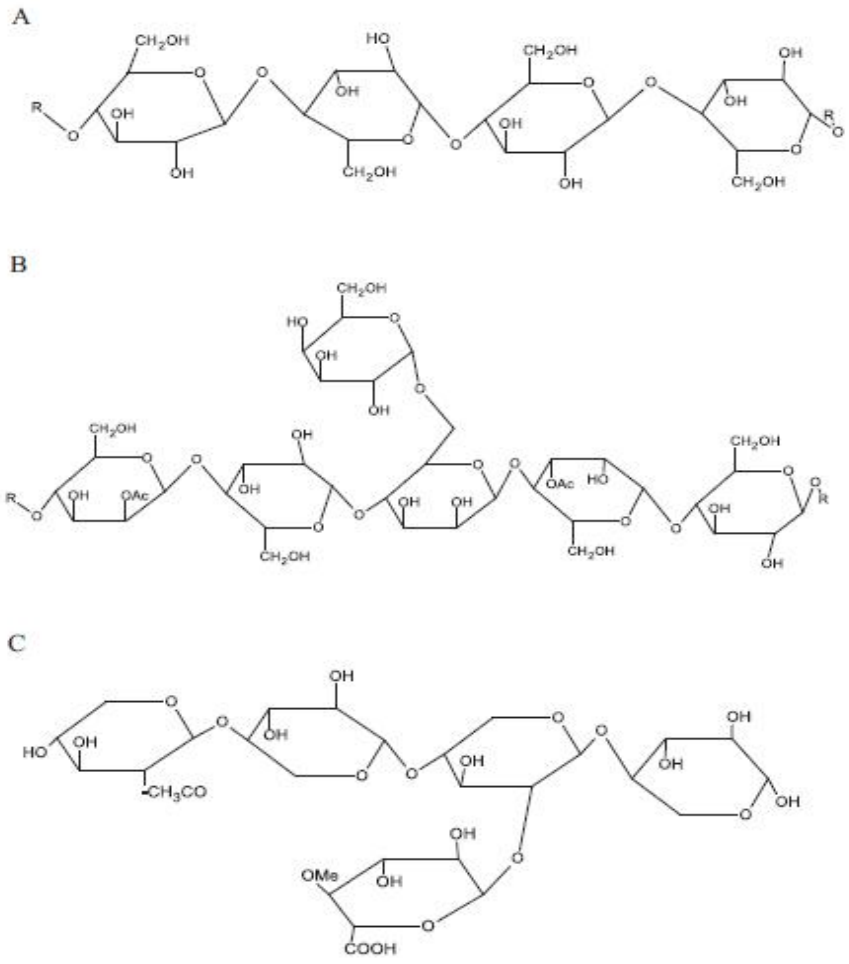

Figure 1: The structure of (A) cellulose and hemicelluloses, (B)glucomannan and (C) xylan[7].

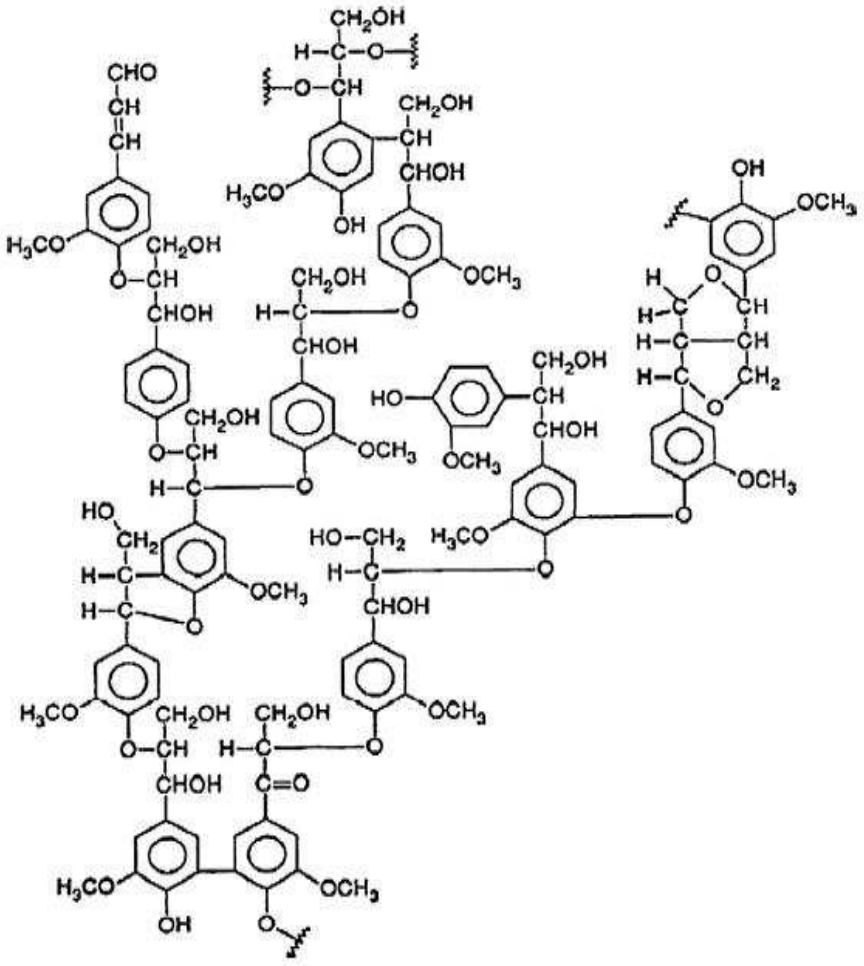

Figure 2: The structure of wood lignin[7].

Native cellulose is a polymeric material of cellubiose, which is made up of two 1,4-β linked glucose units. The degree of polymerisation (DP) can vary considerably between cellulose from different sources. Laboratory synthesised cellulose can have a DP as low as 20 while bacterial cellulose can reach a DP i excess of 10 000[8]. The cellulose structure is reinforced by intra- and intermolecular hydrogen bonds as shown in Figure 3. Native cellulose also has a semicrystalline structure with both crystalline and amorphous regions. Highly crystalline cellulose are more difficult to break down during enzymatic hydrolysis.

Like cellulose, hemicelluloses are carbohydrates, but while cellulose consist of polymers exclusively made of glucose, hemicelluloses are built up of branched, amorphous polymers of several different pentose and hexose sugars. These sugars include glucose, mannose, galactose, xylose, and arabinose[9]. The combinations and contents of these sugars vary considerably in different lignocellulosic materials. The chemical and thermal stability of hemicelluloses are generally lower than that of cellulose. This can be of importance when considering the fractionation of LC materials.

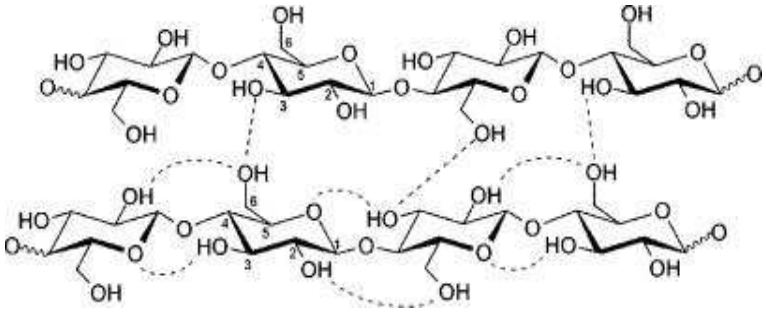

Figure 3: Intra- and intermolecular hydrogen bonds in cellulose[8]

The chemical structure of lignin differs from that of the other main constituents of LC materials in that it is very irregular. Lignin is a network polymer, made from polymerization of phenylpropanoid units. The main function of lignin is to bind with the carbohydrates and form a compact structure[10]. This compact structure causes problems in the fractionation and therefore the utilisation of the LC material.

All the major constituents of LC material can be utilized for the production of fuel and chemicals, but this require the material to be fractionated into its different components[7]. The three-dimensional cross-linked lignin network and the strong hydrogen bonds in the polymeric matrix causes lignocellulosic materials to be highly recalcitrant to chemical and microbial attacks[11]. This is one of the key factors limiting its use as a renewable raw material for production of fuel and chemicals[12]. Figure 4 shows a model of the ultrastructural organization of the wood cell, proposed by Fengel (as stated by Hon[14]), where cellulose-containing fibrils are cemented together by hemicelluloses and lignin. To make the different components available for further utilization this structure needs to be opened. Several different pretreatment methods have been and are being developed to deal with this problem. The different pretreatment methods are discussed below.

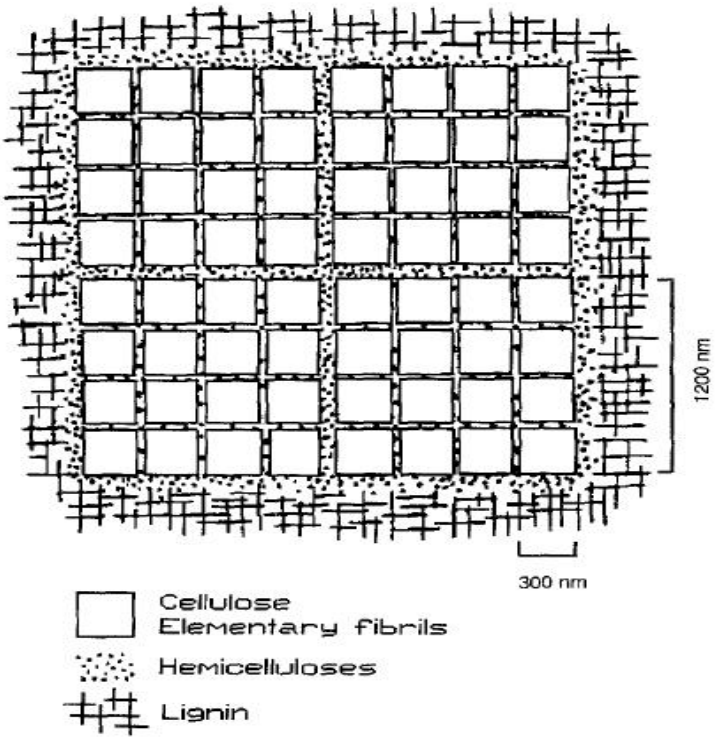

Figure 4: Model of the ultrastructural organization of the wood cell[14]

1.3 Pretreatment technologies

Different pretreatment technologies have different strategies for handling the highly recalcitrant biomass structure. The objective is to alter the structure of the biomass to make it more susceptible to degradation by enzymatic hydrolysis, manly by breaking the lignin seal and disrupting the crystalline structure of the cellulose[2]. Figure 5 shows a simple representation of the main goal of pretreatment of lignocellulosic materials. However, since the physico-chemical characteristics vary considerably between the different lignocellulosic materials, it is necessary to adopt suitable pretreatment technologies based on the properties of each raw material[13].

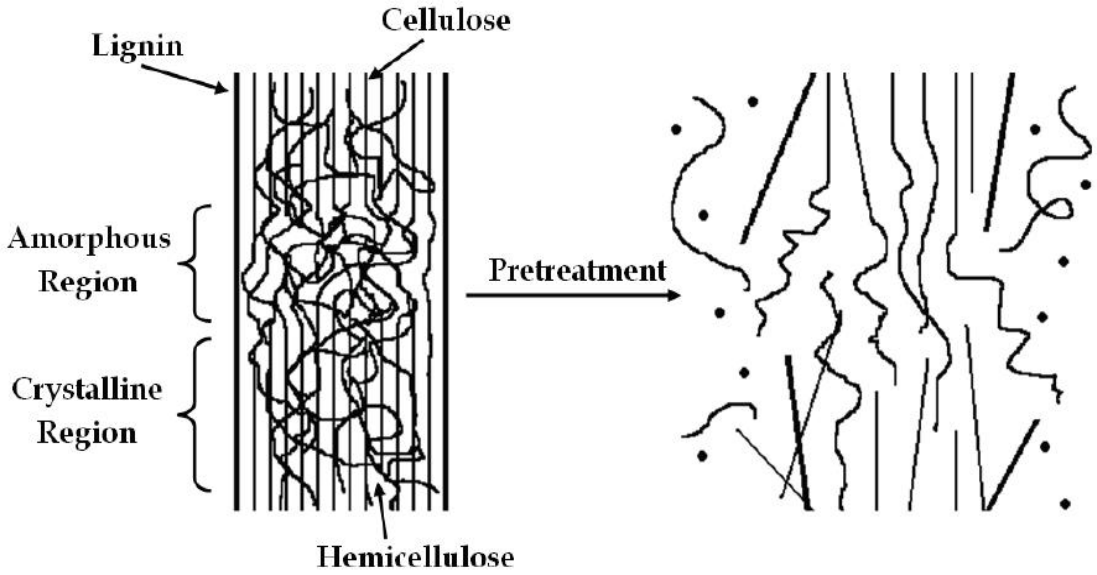

Figure 5: The goal of pretreatment of LC biomass[2]

Pretreatment methods are usually classified into physical, chemical, physico-chemical and biological pretreatments. A description of the different pretreatment methods follows below. Frequent problems with the available pretreatment technologies include the use and production of toxic or harmful substances, sugar degradation into bi-products that may act as inhibitors in the subsequent hydrolysis- and fermentation processes, the need for severe operating conditions like high pressures and/or temperatures and slow reaction rates. This can make the pretreatments environmentally detrimental and expensive[15]. More efficient and environmentally friendly pretreatments, using milder conditions and a "green" and recyclable solvent, are needed. Ionic liquids appears as a promising alternative[16].

1.3.1 Physical pretreatments

Mechanical comminution
Size reduction of the biomass by a combination of chipping, milling and grinding will increase the available surface area and decrease the degree of polymerization and crystallinity of the biomass. This will in turn increase the yield of fermentable sugars from the enzymatic hydrolysis. A drawback of this method is the relatively high power requirements[17].

Extrusion
This method consists of subjecting the biomass to heating, mixing and shearing in an extruder. The result is defibrillation and shortening of the fibres which gives increased accessibility of the cellulose to enzymatic attack[13]. This process could be of particular interest because it can be combined with other pretreatment processes for instance by adding chemicals.

1.3.2 Chemical pretreatments

Alkali pretreatments
Alkali pretreatments can be performed at room temperature, but higher temperatures are often used[18].The pretreatment dissolves lignin while having a limited effect on both hemicelluloses and cellulose, making the biomass more porous and the carbohydrates more easily available to the enzymes. There seems to be less sugar degradation than with acid pretreatment although some inhibitory compounds from degradation of lignin can be formed. The effectiveness of the pretreatment depends heavily on the lignin content in the biomass, and it has proven to be more efficient on agricultural residues than on wood materials[13]. The efficiency can be increased by adding an oxidation agent[18].

Acid pretreatment
Acid pretreatment can be done using either concentrated or diluted acid, but the concentrated acid process is not very attractive due to high production of inhibiting components like furfural and 5-hydroxymethylfurfural (HMF) as well as corrosion concerns. In pretreatment with diluted acid, the material is soaked in a diluted acid and heated to between 140 and 200 °C for up to an hour[17]. The main effect of the pretreatment is to hydrolyse the hemicellulose fraction of the biomass, opening the structure and making the cellulose more accessible.

Sulphuric acid is normally used for this process, it being both inexpensive and efficient. Drawbacks to this pretreatment method includes corrosion concerns, harsh conditions, formation of inhibitors and the need for acid recovery which adds to the overall cost. A positive side to the method is that, while a lot of the other pretreatment methods have proven inefficient for wood raw materials, diluted acid pretreatment can successfully utilize most lignocellulosic materials[18].

Ozonolysis
The ozonolysis pretreatment aim at using ozone as an oxidising agent to facilitate lignin removal. The pretreatment can be performed at very mild conditions and does not lead to the formation of fermentation inhibitors[13]. The pretreatment is suitable for use on agricultural residues. An important drawback is the cost of the large amount of ozone needed.

Organosolv pretreatment
In the organosolv pretreatment lignin is extracted using organic solvents. A solution of the organic solvent and water is added to the biomass and heated to a temperature of 100 to 250 °C[18]. Common solvents are methanol, ethanol, ethylen glycol, acetone, glycerol, tetrahydrofurfuryl alcohol, triethylene glycol and phenol. An acid catalyst, often H_2SO_4 or HCL, can be added[17]. The solvent may act as an inhibitor to the fermentation microorganisms and a high commercial price on some of the solvents makes solvent recycling necessary, adding to the total cost of the pretreatment. For this reason relatively cheap, low molecular weight alcohols are the most popular solvents. One of the major advantages of the organosolv pretreatment is the wide array of LC materials it can utilize and the fact that lignin removal minimises the problem of enzymes being absorbed onto the lignin, resulting in a lowering of the necessary enzyme load in the subsequent hydrolysis. A drawback of the method is the significant amount of inhibitors formed in the process.

Ionic liquids pretreatment
The ionic liquid pretreatment, being the main focus of this work, will be discussed in further detail later.

1.3.3 Physico-chemical pretreatments

Steam explosion
Steam explosion is one of the most widely used methods for pretreating LC material for bioethanol production[18]. The biomass is subjected to pressurised steam for a short period of time followed by explosive decompression. The treatment combines mechanical forces with autohydrolysis caused by the formation of acetic acid from the acetyl groups on the hemicelluloses[13]. This leads to partial hydrolysis of the hemicelluloses and a redistribution and to some extent removal of the lignin.

There are several positive sides to the steam explosion technology such as low environmental impact and lower capital cost as well as higher energy efficiency and the possibility for using a non-hazardous reaction medium[18]. The pretreatment is suitable both for agricultural residue and for hardwoods, but for softwood raw materials addition of an acid catalyst is needed. The main drawbacks of the process are hemicellulose degradation causing the formation of compounds that work as inhibitors for the following hydrolysis and fermentation steps.

Liquid hot water (LHW) pretreatment

In liquid hot water pretreatment the biomass is treated with water at elevated temperatures. This method is similar to steam explosion, but uses lower temperature and lower dry matter content. Despite the decreased temperature, the increase in water content means that the power consumption is higher for the liquid hot water pretreatment than for steam explosion[17]. LHW process solubilises most of the hemicelluloses while leaving the cellulose and lignin mostly unaffected. Some of the advantages of this pretreatment are that no catalyst is needed and the production of inhibitors are low.

Ammonia fibre explosion (AFEX)

The AFEX pretreatment involves treating biomass with liquid anhydrous ammonia at temperatures between 60 and 100 °C at high pressures. The pressure is then released causing the ammonia to vaporise. The effect of the pretreatment is physical disruption and swelling of the fibres, partial decrystallisation of cellulose, and breakdown of the lignin-carbohydrate linkage[18]. The pretreatment is more efficient on agricultural residues than on wood and other high-lignin feedstocks.

Wet oxidation

Wet oxidation employ oxygen or air as an oxidation catalyst along with water at elevated temperatures. The pretreatment causes solubilisation of hemicelluloses and lignin, however the hemicelluloses are not further hydrolysed to fermentable sugars. The formation of inhibitors in this process is very low compared to steam explosion and LHW pretreatment[18].

CO_2 explosion

This pretreatment involves using CO_2 as a supercritical fluid to remove lignin and open the biomass structure. This is done by sudden release of CO_2 pressure and the cellulose and hemicelluloses structures are disrupted giving an increase in the accessible surface area. Addition of co-solvents like ethanol can improve dissolution. Low reaction temperatures reduces the sugar degradation, but the sugar yield is low compared to steam and ammonia explosion[13].

1.3.4 Biological pretreatment

Biological pretreatment are based on the use of brown, white, and soft rot fungi to remove lignin for the biomass. Since it is performed at low temperatures and without the use of chemicals the method is considered environmentally friendly[17]. Other advantages include low capital cost, low energy demand, and no formation of inhibitors. The major drawback of the process is the low reaction rate, making a reaction time of weeks and even months necessary.

1.4 Ionic Liquids

Ionic liquids (ILs) are generally defined as salts that melt at temperatures below 100 °C[8], and are typically composed of large organic cations and small inorganic anions[19]. An overview of commonly used ions for ionic liquids are given i Figure 6. The definition of ILs mentioned above, span such a broad class of substances, defined by a melting point and having salt-like characteristics, that it is next to impossible to finding a set of properties that holds true for all or most ILs[20]. This is an important aspect to consider when describing these substances. On the other hand, the sheer amount of available combinations of cations and anions also gives rise to one of the most important advantages of ionic liquids, the possibility for fine-tuning the properties to fit different uses[2]. Properties that to some extent can be tunes in this way include melting point, thermal stability, refractive index, acid-base character, hydrophilicity, polarity, density, and viscosity[8].

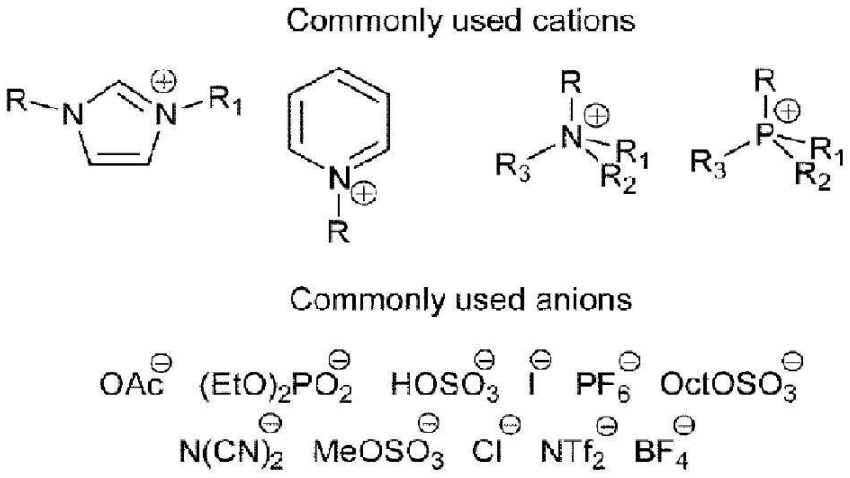

Figure 6: Cations and anions commonly used in ionic liquids[21]

Despite the large variations, some properties that applies to many off the common ionic liquids have been suggested include high chemical and thermal stability, low vapour pressure, low flammability, and high recyclability. Because of these properties ionic liquids have been considered to be "green" solvents[22]. Due to their low vapour pressures, the use of ILs can reduce the chances of air pollution, but many ILs are soluble in water and can be released into the environment through aqueous waste streams[23]. Another advantage is the low melting points, making many ILs liquids at room temperature and therefore easy to work with.

The melting point and viscosity are crucial parameters for the practical use of ionic liquids. These parameters are closely related to the choice of anion and cation. Low symmetry and higher alkyl chain length in the cation decreases the melting point. The absence or presence of strong hydrogen bonds also has an effect on the melting point of the IL. The viscosity of ILs are higher than that of water, and in the general area similar to oils[8]. This is a major drawback because it restrict the diffusion of the liquids into the biomass, lowering the efficiency of the pretreatment[22]. The viscosity can be lowered by applying higher pretreatment temperatures. The high viscosity, lack of basic physico-chemical and toxicological data and the relative high cost of ILs are mayor barriers to the commercial application of these substances as solvent for lignocellulosic materials[19].

The capability of ionic liquids to dissolve cellulose and lignocellulosic materials has received much attention in the last few decades. Several ILs with this particular quality has been found, however, the mechanism for how the biomass is dissolved is not yet well established[24]. A generally excepted suggestion is that the splitting of carbohydrate hydrogen bounds caused by the anion of the IL binding to the cellulose hydroxyl groups play an important part[20]. Liebert[24] reports that 1-ethyl-3-methylimidazolium (EMIM) ion in [EMIM][OAc] might also react with the reducing end groups of cellulose as shown in Figure 7. A dissolution mechanism involving both the anion and the cation of the ionic liquid, suggested by Feng and Chen as sited by Pinkert et al.[8], is shown in Figure 8 for cellulose in [BMIM][Cl].

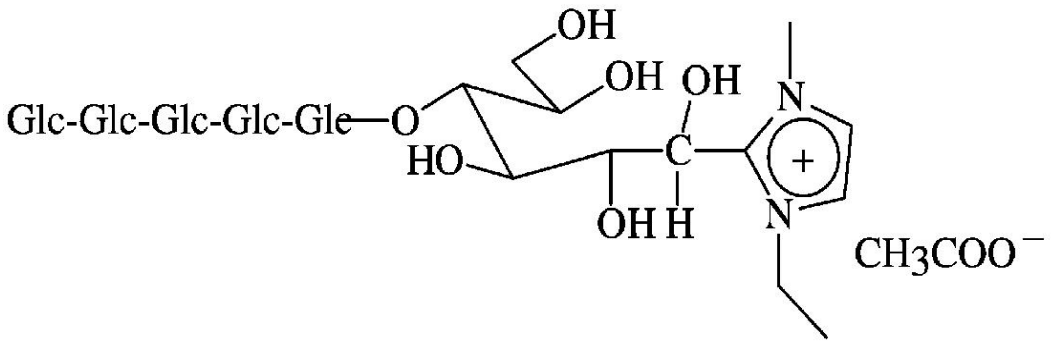

Figure 7: Proposal for covalent binding between [EMIM][OAc] and cellulose[24]

The dissolved biomass can be regenerated by precipitation with a suitable non-solvent such as water, methanol, acetone, or acetonitrile[5] after which the ionic liquid can be regained by evaporation of the non-solvent[10]. The precipitated material have a decreased content of crystalline cellulose[25] and a greatly improved porosity. This facilitates enzymatic hydrolysis and increase the production of fermentable sugars.

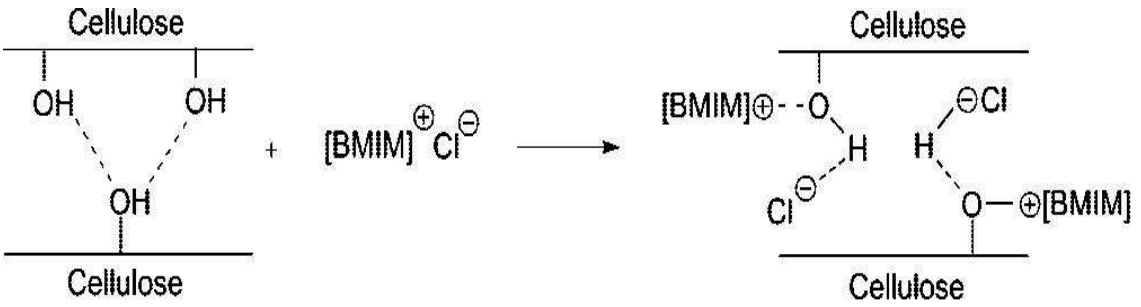

Figure 8: Proposed dissolution mechanism of cellulose in [BMIM][Cl][8]

Many previous studies have reported delignification off the biomass after pretreatment with ILs ([12][26][5]), but others state that lignin is not removed in the pretreatment process ([25][27][15]). It is possible that the delignification effect vary considerably between different ionic liquids. If lignin is not removed in the pretreatment this can be a liability for the following enzymatic hydrolysis since the enzymes are known to be absorbed onto the lignin making a higher enzyme load necessary. However, the opening of the biomass structure and the reduction of the cellulose crystallinity might counter this effect, making IL pretreatment able to compete with pretreatments with a higher degree of delignification.

Zavrel et al[16] have carried out a high-throughput screening to find the most suitable ionic liquid for dissolving lignocellulosics. In this study 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) was found to be the most efficient IL for dissolution of pure cellulose, while 1-allyl-3-methylimidazolium chloride ([AMIM][Cl]) was the most efficient IL for dissolution of wood. When considering the practical use of the ionic liquids, [EMIM][OAc] have several advantages over [AMIM][Cl]. Firstly, the acetate anion of [EMIM][OAc] does not lead to the corrosion problems that can be expected from chloride, reducing the equipment cost. [EMIM][OAc] is also considered bio-degradable and relatively non-toxic[15], and acetate has a higher basicity than chloride inducing increased breaking of hydrogen bounds in cellulose. Figure 9 shows the structure of [EMIM][OAc] and [AMIM][Cl].

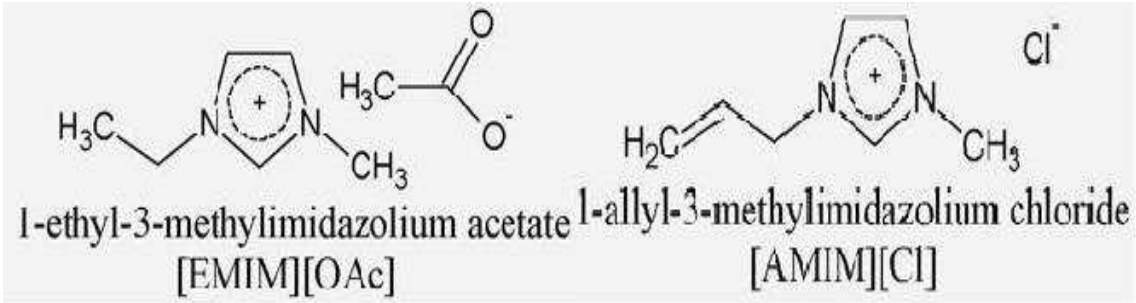

Figure 9: The structure of [EMIM][OAc] and [AMIM][Cl][15]

The thermal stability of ionic liquids is generally considered to be high with a decomposition temperature of 300-400 °C for most ILs. Lately, however, there have been some discussion about whether this holds true when ILs are used at relatively high temperatures for prolonged reaction times[8]. Li et al.[10] suggests that acetate based ILs, like [EMIM][OAc], might exhibit particularly low stability in wood dissolution because wood contains naturally occurring acids with a pK_a value close to that of acetic acid. This might cause the acetate ion to become protonated and form acetic acid. Mäki-Arvela et al.[15] recommend using shorter reaction times when working with [EMIM][OAc]. It might also be worth mentioning that Amarasekara and Owereh[28] have reported excessive charring of the biomass sample at elevated temperatures and prolonged reaction times. This would contribute to a lowered sugar yield from the enzymatic hydrolysis even if the ionic liquids have not deteriorated.

Much work has been done on the area of ionic liquid pretreatment during the last few decades. Many different ionic liquids and raw materials have been tested. Table 1 summarise some of the pretreatment conditions used in previous works on this area. The overview also shows the ILs and biomass raw material used. The data is taken from the literature.

Table 1: Previous work (from the literature).

		Pretreatment conditions		
Ionic liquid	Raw material	T ($^\circ C$)	t (*hours*)	Reference
[AMIM][Cl]	Norway spruce sawdust	90	24	Kilpelainen[29]
[AMIM][Cl]	Norway spruce TMP	130	8	Kilpelainen[29]
[AMIM][Cl]	α-cellulose	90	12	Zavrel[16]
[AMIM][Cl]	Spruce, fir, beech, chestnut	90	12	Zavrel[16]
[AMIM][Cl]	Norway spruce sawdust	80	24	Kilpelainen[29]
[AMIM][Cl]	Norway spruce TMP	120	5	Li, B.[10]
[AMIM][Cl]	Norway spruce TMP	130	4-6	Xie[4]
[BMIM][Cl]	Norway spruce TMP	130	8	Kilpelainen[29]
[BMIM][Cl]	Norway spruce TMP	130	4-6	Xie[4]
[BnMIM][Cl]	Norway spruce TMP	130	4-6	Xie[4]
[BnMIM][Cl]	Norway spruce TMP	130	8	Kilpelainen[29]
[EMIM][OAc]	Indulin AT (kraft lignin)	90	24	Lee[12]
[EMIM][OAc]	α-cellulose	90	12	Zavrel[16]
[EMIM][OAc]	Spruce, beech, chestnut	90	12	Zavrel[16]
[EMIM][OAc]	Maple wood flour	80	24	Lee[12]
[EMIM][OAc]	Triticale straw	150	1.5	Fu[26]
[EMIM][OAc]	Switchgrass	160	3	Li, C.[30]
[EMIM][OAc]	Poplar, switchgrass, corn stover	50	12-14	Samayam[25]
[EMIM][OAc]	Poplar, switchgrass, corn stover	120	1	Samayam[25]
[EMIM][OAc]	Sugarcane bagasse	60-120	2	Silva[31]
[EMIM][OAc]	Avicel	70-140	1-44	Shill[32]

2 Materials and methods

The lignocellulosic biomass used in this work was ATMP refined Norway Spruce from Stora Enso mill in Hylte in Sweden and sugarcane bagasse procured from Borregaard Industries Ltd, Sarpsborg, Norway. The biomass was hammer milled to a particle size between 0.7-1.7 mm, and the extractives removed using a Soxlhet type extraction with a 9:1 cyclohexane:aceton solution for 6 hours. An overview of the chemicals used in this work can be found in Table 2. In addition the enzymes for the hydrolysis (Celluclast and Novozyme 188) was procured from Novozyme.

Table 2: Chemicals used in this work.

Chemical	Formula	Supplier	Purity *[%]*
Acetone	$(CH_3)_2CO$	Romil Ltd	> 99.7
Cyclohexane	C_6H_{12}	Romil Ltd	> 99.5
[EMIM][OAc]	$C_8H_{14}N_2O_2$	Sigma-Aldrich	> 90.0
Sulphuric acid	H_2SO_4	Romil Ltd	$95 - 85$

In short, the biomass was pretreated with ionic liquids at different temperatures for a varying length of time before the dissolved materials was precipitated, filtered out and washed. Two parallels were carried out simultaneously for each experiment, and these were treated separately for all steps in the procedure. The effectiveness of the pretreatment was determined based on enzymatic digestibility of the pretreated biomass. The lignin content in the liquid-fraction from the pretreatment was also analysed. Figure 10 gives a simple representation of the process. The pretreatment and analysis used are further described below.

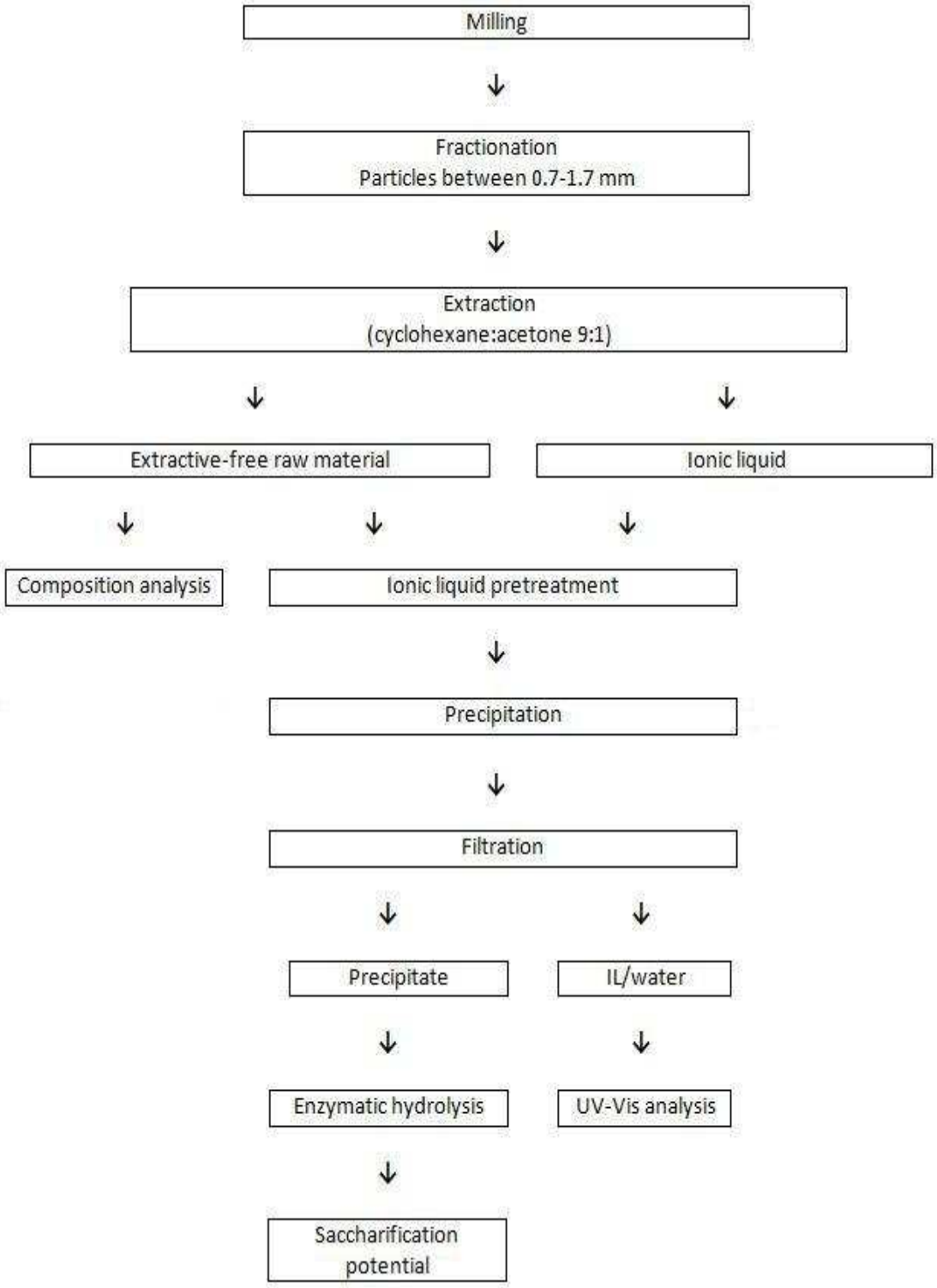

Figure 10: Simple layout of the process

2.1 Ionic liquid pretreatment

[EMIM][OAc] and the wood samples were dried under vacuum at 40 °C for 24 hours before use. Ionic liquid and biomass was transferred to a dry, 100 mL flask at a biomass/IL ratio of 5 wt% under an inert nitrogen atmosphere. In this work 40 mL of ionic liquid was used for each experimental parallel. The reaction solution was stirred at 500 rpm with a magnetic stirrer for a predetermined length of time at the target temperature. The pretreatments of spruce were carried out at 80, 100, and 120 °C for 3, 6, 12, and 24 hours. When using bagasse the same temperatures were employed, but the reaction time was set to 1 and 3 hours. The temperature and stirring was maintained by a Radleys Tech (05-1170) electric heater. Figure 11 shows the experimental setup for the pretreatment.

Figure 11: Experimental setup for the pretreatment

After pretreatment, the dissolved material was recovered by precipitation in water. The reaction solution was vigorously mixed with 400 mL deionised water. The precipitated material was recovered by filtration using a GF/A filter. The filtrate was subjected to UV-Vis analyses to determine the amount of dissolved lignin (Appendix D). The solids were washed with additional water and hydrolysed enzymaticly. A simple flow sheet for the pretreatment process is given in Figure 12. All data from the pretreatment experiments can be found in Appendix A.

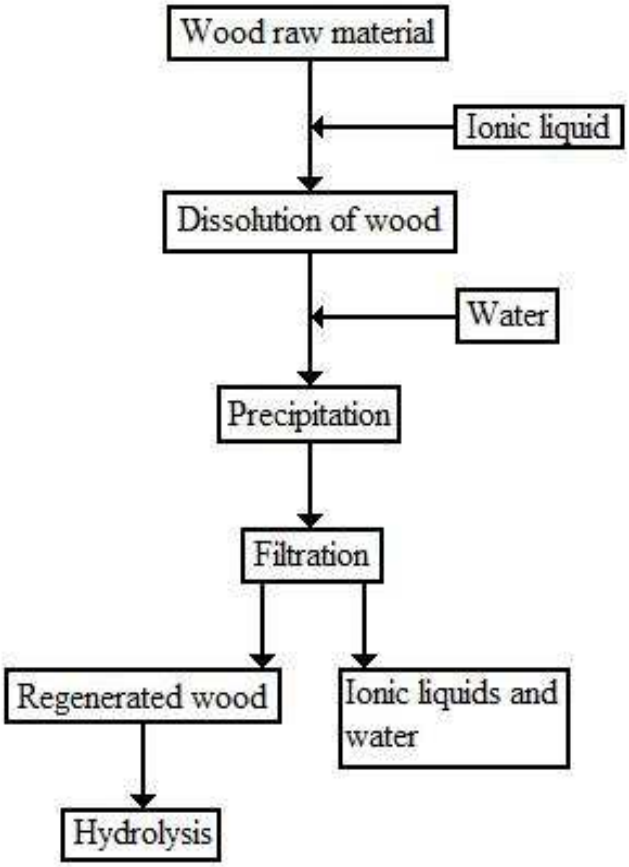

Figure 12: Flow sheet for pretreatment with ionic liquids

2.2 Analysis

2.2.1 Acid hydrolysis

The composition of the biomass was determined by acid hydrolysis followed by lignin- and carbohydrate analysis. The acid hydrolysis was divided into a decrystallisation step and a hydrolysis step. For the decrystallisation 3 mL 72 wt% sulphuric acid was added to 350 mg biomass. It was then kept at 30 °C for 1 hour with constant stirring. For the hydrolysis step the solution was transfered to a 100 mL, plastic capped glass bottle and 84 mL deionised water was added to give an acid concentration of 4 wt%. The hydrolysis solution was kept at 121 °C for 1 hour in an autoclave before being filtered using a preweighed GF/F filter. The acid soluble lignin (ASL) content was determined by UV-Vis analysis of the filtrate at 205 nm using Eq. 1 and an absorption coefficient of 94.5 L/gcm taken from the literature[33]. Absorption measurements at 205 nm was used on the basis that this wavelength has one of the most characteristic absorption maximums for lignin. In addition, the interferences of carbohydrate degradation products are small at this wavelength[33].

$$ASL(g/L) = \frac{UV absorption}{(absorption\, coefficient) \cdot (optical\, cell\, width)} \tag{1}$$

The acid insoluble lignin (AIL) content was determined from the weight of the solid residue and of the ash by using Eq. 2.

$$AIL(\%) = \frac{W_{solid\, residue} - W_{ash}}{W_{sample}} \tag{2}$$

The total lignin content of the biomass was calculated as a sum of the acid soluble and acid insoluble lignin.

The carbohydrate concentration was determined by Dionex ICS-5000 High-Performance Liquid Chromatography analysis (HPLC) with a CarboPac Pa1 column. Appendix B gives the data from the composition analysis of the raw materials.

2.2.2 Enzyme hydrolysis

The procedure for the enzymatic hydrolysis was based on the NREL/TP-510-42629 (March 2008) procedure and was optimised for organosolv pretreatment. A biomass sample equal to the equivalent of 0.1 g of cellulose was weighed out and added to a 20 mL glass vile. 5 mL 0.1 M, pH 4.8 sodium citrate buffer was added to each sample along with 100 μL sodium azide antibiotic to prevent growth of organisms during the hydrolysis. The amount of water added were calculated to give a total volume in each vile of 10 mL (all solutions and the biomass were assumed to have a specific gravity of 1.000 g/mL, see Appendix C). The content of each vile was heated to 50 °C before adding 40 μL Celluclast (74 FPU[1]/mL) and 14 μL Novozyme 188 (226 pNPGU[2]/mL). The samples were subsequently incubated at 50 °C for 48 hours.

After ended incubation time the samples was filtered using a GF/A filter and the amounts of dissolved carbohydrates were determined by HPLC.

2.2.3 Digestibility

The digestibility of the sample was determined from the glucose content in the liquid phase from the enzymatic hydrolysis found by HPLC analysis. The digestibility was calculated on a oven dry weight basis from eq. 3 and 4. The mannan and xylan yield were determined in a similar fashion (see Appendix E).

$$Glucan\,digested\,(g) = glucose\,(g/L) \cdot sample\,volume\,(L) \cdot \frac{162}{180} \tag{3}$$

$$Digestibility\,(\%) = \frac{Glucan\,digested\,(g)}{Glucan\,added\,(g)} \cdot 100\% \tag{4}$$

An overview of quantities of chemicals used and examples of calculations as well as all raw data from the analysis are attached.

[1]FPU: Filter paper units
[2]pNPGU: p-nitrophenyl-glucoside units

3 Results and discussion

3.1 Experimental choices

Norway spruce and sugarcane bagasse was chosen as god representatives for wood and non-wood raw materials respectively. Figure 13 shows the raw materials used. The particle size of the biomass was due to limitations in the available equipment for milling.

Figure 13: The raw materials used in this work, spruce (above) and bagasse (below).

Even though [AMIM][Cl] was found to be the most efficient IL for dissolution of wood [EMIM][OAc] was chosen in this work based on the reasons stated earlier (see section 1.4). These reasons include [EMIM][OAc] being bio-degradable, non-toxic and far less corrosive than [AMIM][Cl]. Acetate also has a higher basicity than chloride, inducing increased breaking of hydrogen bounds in the carbohydrates. Water was chosen as the non-solvent for the precipitation of the dissolved material because it is cheap, readily available and easy and safe to work with.

The reaction conditions for the pretreatment was chosen based on conditions used in earlier experiment found in the literature (see Table 1 in section 1.4). The operating temperature for pretreatment of both spruce and bagasse from the literature is normally in the region of 80 to 130 °C. Pretreatment temperatures was set to 80, 100, and 120 °C for both raw materials. The reaction times used in the literature varied considerably, but was in general shorter for non-wood than for wood raw materials. For this reason spruce was treated for a period of 3, 6, 12, and 24 hours while pretreatment of bagasse was carried out for 1 and 3 hours. Bagasse pretreatment for 6 hours was considered but time did not permit this to be done. A concentration of 5 wt% biomass in IL was used to ensure sufficient mixing during the pretreatment.

3.2 Ionic liquids pretreatment

The procedure for the ionic liquid pretreatment was developed as a part of a specialisation project leading up to this master thesis and was based on procedures reported in the literature[10]. Data from the pretreatments are given in Appendix A. It should be mentioned that parallel 4A had to be excluded from further use because the heat had not been turned on for the pretreatment of this parallel. This experiment was redone (see parallel 11). Parallel 14A was lost when the glass flask containing this parallel was broken. Due to time concerns another experiment using the same conditions was not performed. One of the reasons for this was that the main focus of this work was on pretreatment of spruce and a repetition of the bagasse experiments was not prioritised.

During the pretreatment the IL-biomass solution appeared to become more viscous, almost to the point where it could be described as a gel like substance. This is believed to be caused by formation of hydrogen bounds between the IL and the dissolving biomass. Figure 14 shows the IL-wood solution after pretreatment at 120 °C and 6 hours.

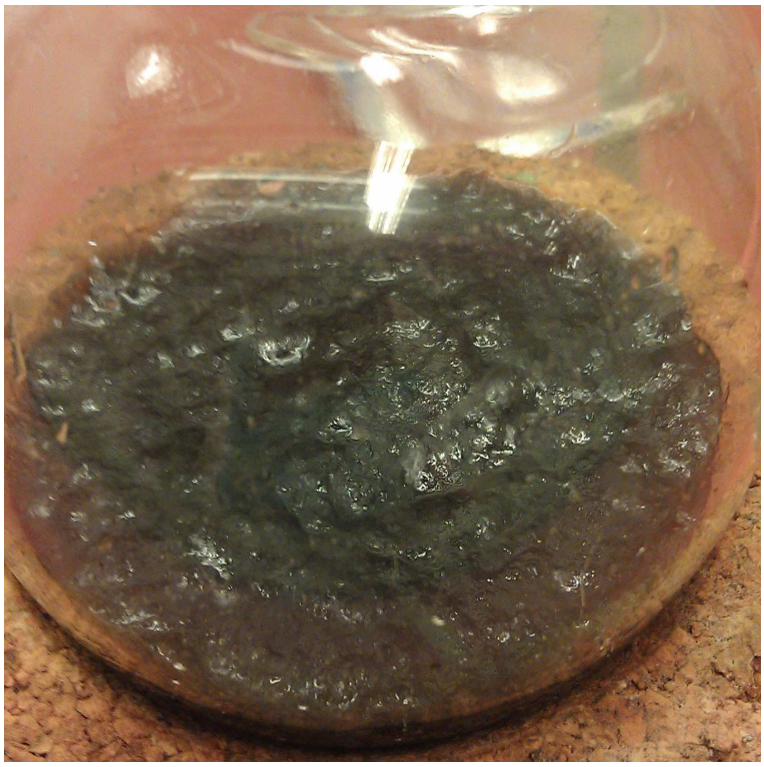

Figure 14: The IL-wood solution after pretreatment

The biomass was recovered by precipitation in water followed by filtration. The solid yield from the pretreatment process was found to be more than 80 wt% for almost all the experiments (see Appendix A). The losses in solids was mainly associated with the filtration process step.

Most of the experiments using spruce as the raw material showed very little visible fibres remaining after pretreatment. For the mildest pretreatment a few intact fibres could be observed, but it was also clear that the porosity had increased dramatically for all pretreated samples. The structure of the recovered samples appeared "fluffy" and more open, which would make it more susceptible to enzymatic degradation. Figure 15 gives a visual representation of the recovered material after precipitation (pretreated at 100 °C for 3 hours). The recovered material from the pretreatment of bagasse showed some visible fibres. This was probably due to the mild pretreatment conditions used for these parallels.

Figure 15: The recovered material was highly porous.

The colour of the recovered material appeared to darken with an increase in the harshness of the pretreatment. Figure 16 gives an indication of this, showing untreated spruce on the left, followed by pretreated spruce at progressively harsher conditions towards the right. The reason for this colour change is most likely condensation of the lignin, making it darker. Amarasekara and Owereh (see section 1.4) reported charring of the sample at high temperatures and long reaction times, however, 120 °C does not seem sufficiently high to cause this. For sugarcane bagasse, no significant darkening of the biomass was observed. This is not surprising considering the mild conditions used in these pretreatments in addition to the darker colour of the original raw material.

Figure 16: Visual comparison of the colour of the different pretreatments. Untreated spruce on the left and progressively harsher pretreatments towards the right.

3.3 Lignin removal

The liquid phase from the filtration after precipitation was analysed using UV-Vis analysis to determine the amount of lignin removed in the pretreatment. Before starting this work, a considerable removal of lignin was expected during the IL pretreatment. However, as mentioned in section 1.4, there are some disagreement in the literature as to whether IL pretreatment removes lignin or not. Figure 17 shows the results of the UV-Vis analysis for spruce and Figure 18 shows the results for the experiments using bagasse.

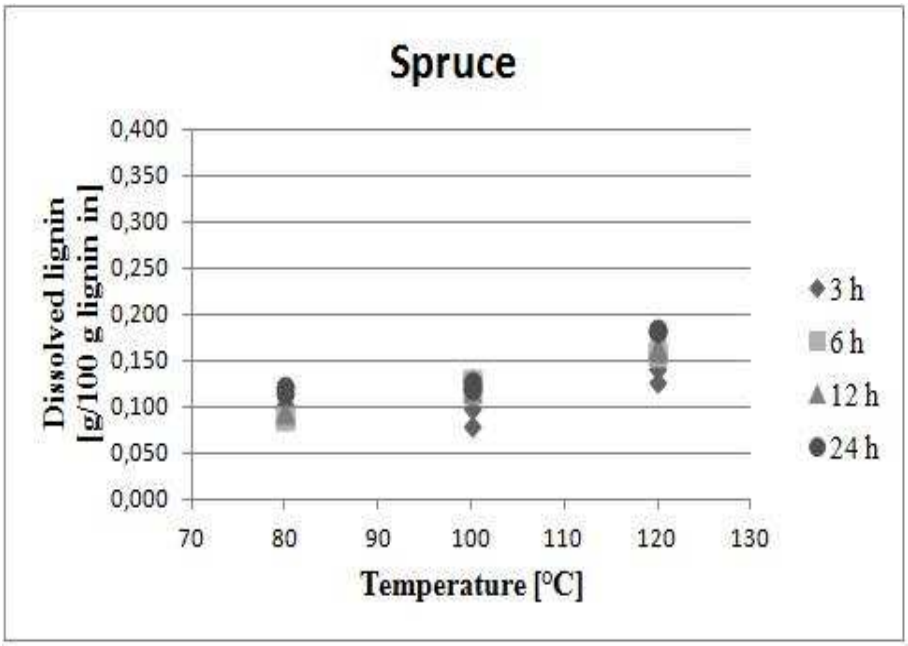

Figure 17: Dissolved lignin in ionic liquid pretreatment of norway spruce.

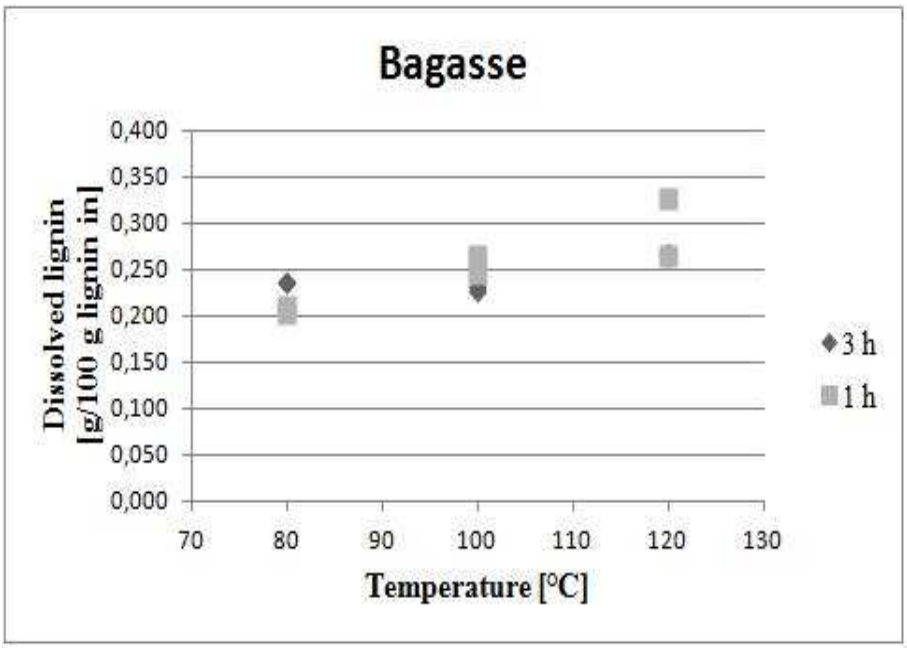

Figure 18: Dissolved lignin in ionic liquid pretreatment of sugarcane bagasse.

The results of the lignin analysis did not comply with the expectations stated above. For spruce it can be seen that that less than 0.2 wt% of the lignin added to the pretreatment was removed from the lignocellulosic material. The lignin removal was higher for sugarcane bagasse than for norway spruce, but it still did not exceed 0.4 wt%. The degrees of delignification in these pretreatment experiments were so low they can be considered neglectable. This agrees with some of the available literature as stated in section 1.4, but not all. More research is needed to settle this.

3.4 Enzymatic hydrolysis

The applied procedure for enzymatic hydrolysis was optimized for the organosolv pretreatment which removes most of the lignin from the biomass. As stated earlier a much higher degree of lignin removal was expected in the ionic liquid pretreatment than what actually occurred. This might cause problems in the subsequent enzymatic hydrolysis. The enzymes can be absorbed onto the remaining lignin, making them inefficient at dissolving carbohydrates.

HPLC analysis of the liquid phase from the enzymatic hydrolysis was done to determine the amount of dissolved sugars in the sample. The analysis focused on glucose, this being the monomer for cellulose. In addition the mannose content was analysed for the pretreatments using spruce on account of this being one of the monomers in glucomannan, the most common hemicellulose in softwood. The most common hemicellulose in bagasse is zylan, so an analysis of the zylose content was done for the parallels using this raw material. The results of the HPLC analysis of the dissolved sugars after enzymatic hydrolysis are given below (see Appendix E for raw data). It is important to note that the parallels are completely separate experiments, not just different measurements on the same experiment. This, along with the fact that a substantial amount of time past between the analysis of some of the parallels, can help explain the somewhat large deviation in the measured data between some of the experimental parallels using the same pretreatment conditions.

3.4.1 Glucan yield

The main goal of the pretreatment is to increase the digestibility of the biomass. The glucan yield from the enzymatic hydrolysis of the samples was chosen to represent the digestibility. Enzymatic hydrolysis of the untreated raw material gave a very low glucan yield of 0.04 and 0.03 wt% for untreated spruce and bagasse respectively. The digestibility of the pretreated spruce samples are reported in Figure 19 to 22.

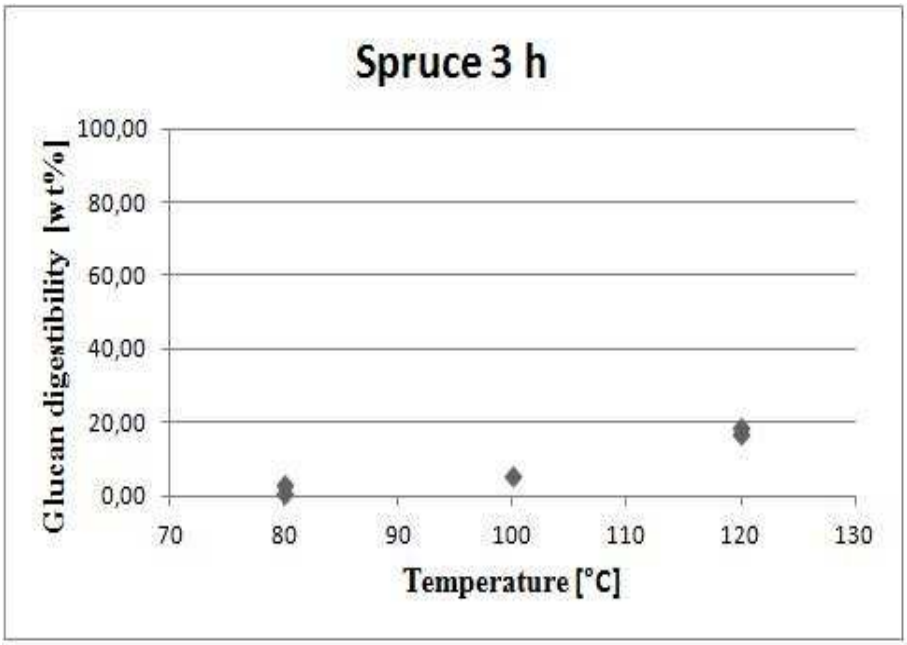

Figure 19: Digestibility of spruce pretreated for 3 hours.

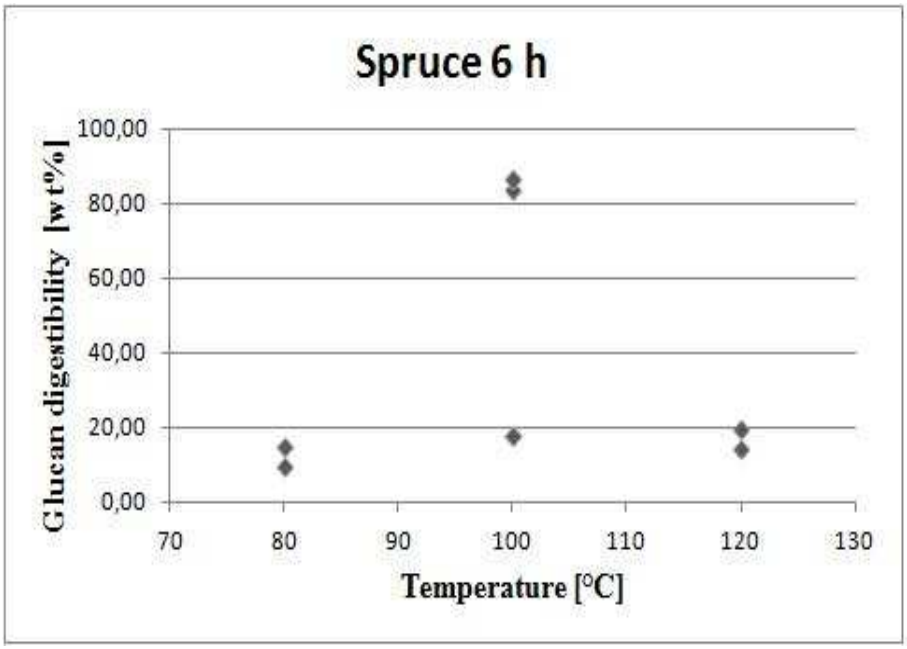

Figure 20: Digestibility of spruce pretreated for 6 hours.

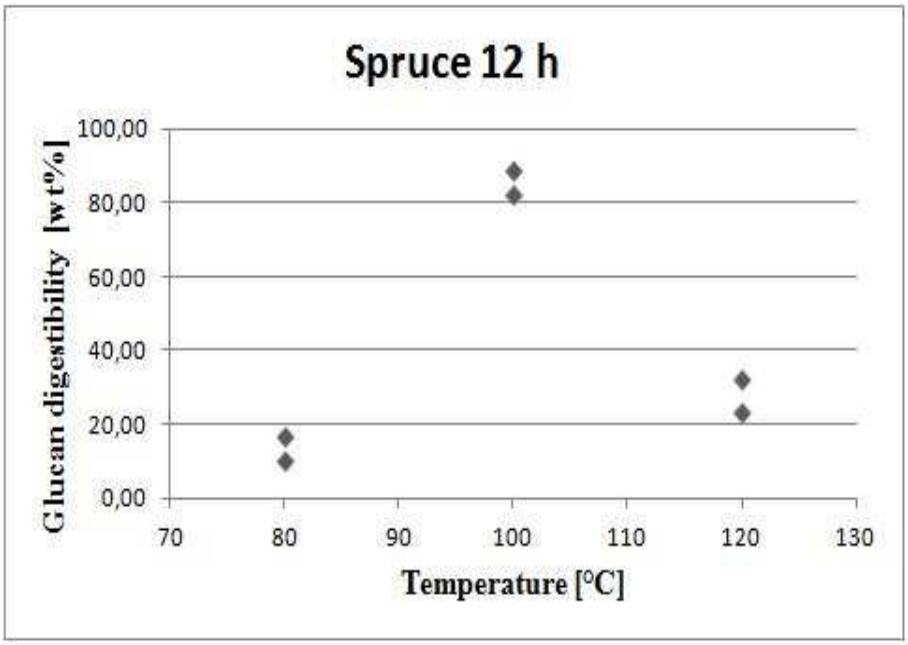

Figure 21: Digestibility of spruce pretreated for 12 hours.

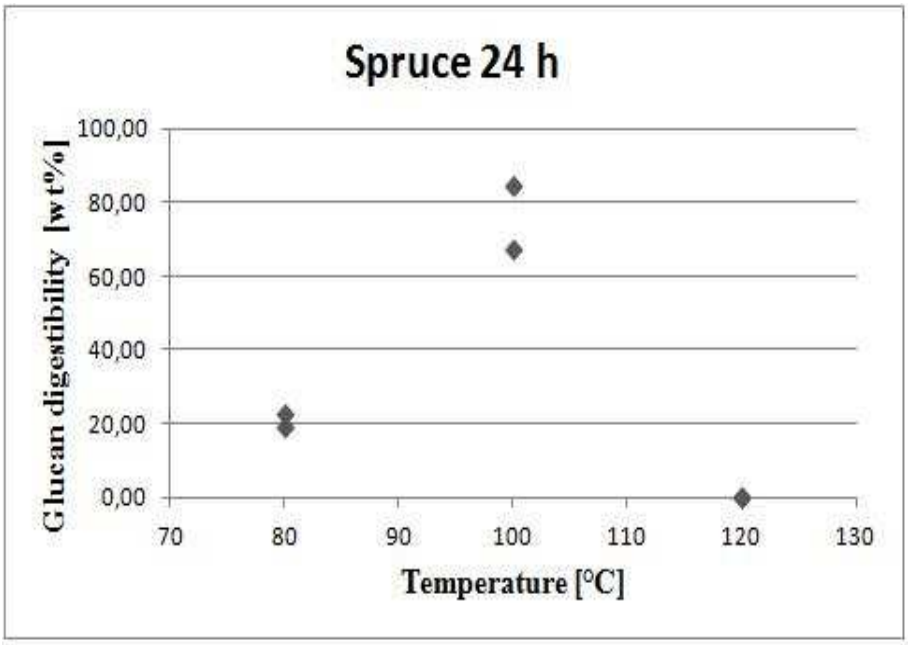

Figure 22: Digestibility of spruce pretreated for 24 hours.

All the pretreated parallels show an increased enzymatic digestibility compared to the untreated samples. This is believed to arise from a decrease in the crystallinity of the cellulose and an increase in the accessible surface area caused by the higher porosity of the pretreated material. For the experiments using a pretreatment time of 3 hours there appears to be an increase in the glucan yield with increasing temperatures. This implies that for such a short pretreatment time the system can tolerate even higher temperatures than 120 °C. However, the sugar yields do not exceed 20 wt% (or 20 g dissolved glucan per 100 g of glucan in the sample). This is relatively low compared to the maximum yields obtained during this work.

When considering the results of the experiments using 6 hours, the data from the pretreatments at 100 °C needs to be mentioned. The different data points are from separate experiments using the same conditions, but the deviation between the parallel with high yield and the ones with lower yields are almost 400 %. It is assumed that the experiments with the lower yields are the correct results on account of there being two parallel experiments with very little deviations at this point. The two values for the high yield parallel are different measurement of the same experiment. The explanation for this deviation was not found, but it is believed to be a simple error in execution of the pretreatment, despite that no special observations was noted when performing neither of the experiments.

After removing the parallel with the highest yield the results shows a trend not very different for the 3 hour parallels. The yield appear to be relatively stable at 10-20 wt%, with a slight increase from 80 °C to 100 and 120 °C. Compared with the 3 hour parallels the yield does not seem to have increased much when doubling the pretreatment time. On the other hand, if the parallel with the highest yield at 100 °C was considered to be correct, the data for the 6 hour pretreatment share a striking resemblance to the pretreatments using a reaction time of 12 and 24 hours. The best approach would be to redo the experiment, but the inconsistency was discovered so late in the work that this was not possible.

For the experiments using a pretreatment time of 12 and 24 hours there are a clear optimum of the hydrolysis yield at 100 °C. The highest yield found in this work was almost 90 wt% of the added glucan and corresponded to pretreatment at 100 °C for 12 hours. The considerable decrease of the glucan yield at 120 °C for longer reaction times could point to a decomposition of the IL at these conditions, as mentioned in the literature (section 1.4). It is also possible that the harsh operating conditions could lead to charring of the biomass which would affect the yield, but, as mentioned earlier, it is not believed that the temperature in these experiments is sufficiently high to cause this kind of reaction.

Figure 23 gives the glucan yield from the parallels using bagasse as raw material. It shows that the digestibility of bagasse increased with increasing temperature as well as reaction time. There is no apparent decrease in the glucan yield at high temperatures using these shorter reaction times. This indicates that the optimal conditions have not been employed in this work and harsher pretreatment conditions can be used. When comparing the 3 hour parallels for the different raw materials, it appears that spruce needs harsher conditions to achieve the same glucan yield as bagasse.

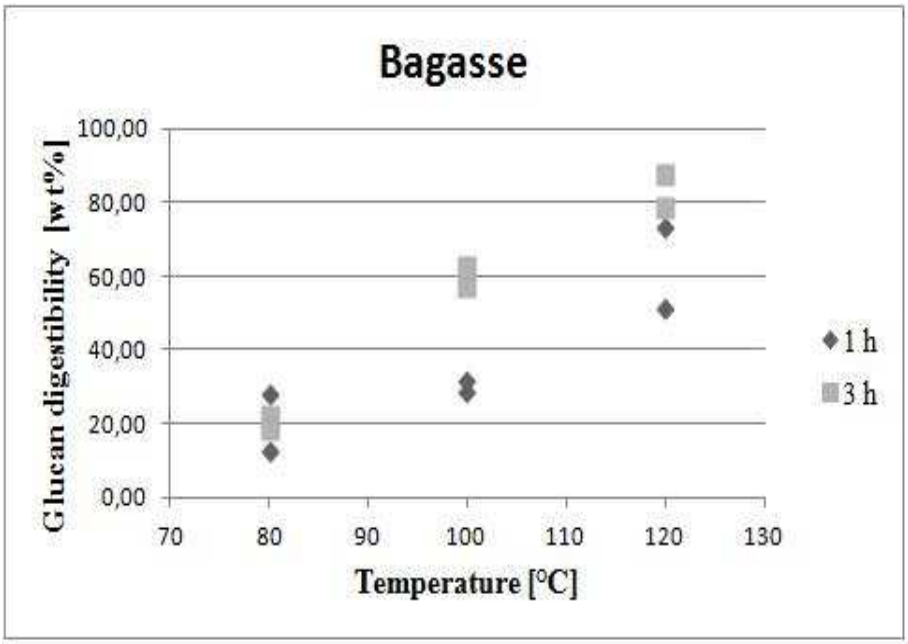

Figure 23: Digestibility of pretreated sugarcane bagasse.

3.4.2 Mannan yield

As mentioned before, the mannose content was analysed for the experiments using spruce, to give an indication of the dissolution of the hemicelluloses. The results of these analysis are given in Figure 24 to 27.

As expected, the results for hemicellulose dissolution are consistent with the results for cellulose dissolution (see discussion for glucan yield). When assuming that the parallel with the highest yield at 100°and 6 hours was not correct(see discussion above), the highest mannan yield was found for the same parallel as the optimum for glucan (100 °and 12 hours) and reached about 70 wt% of the mannan added in the sample.

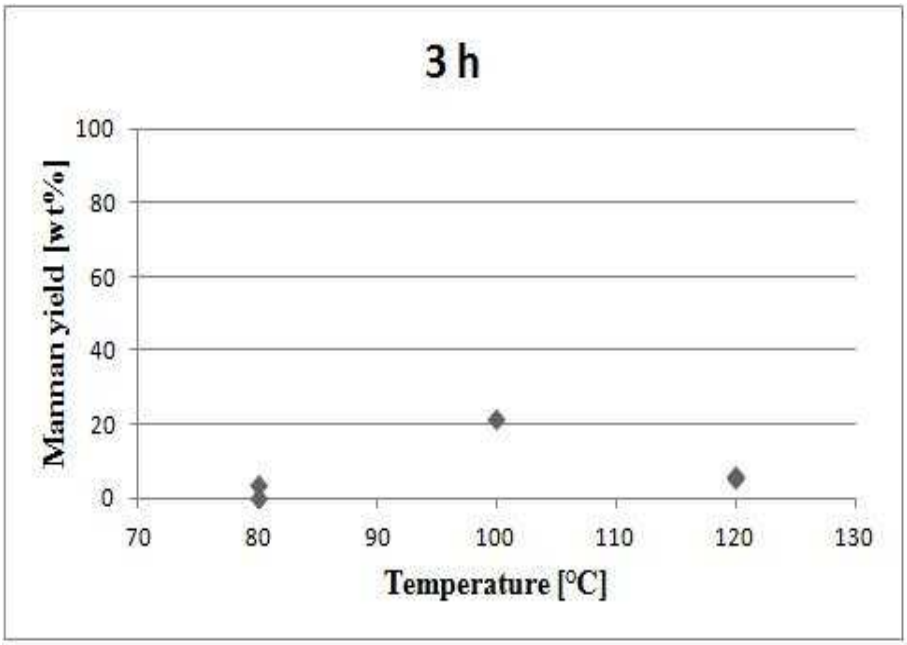

Figure 24: Mannan yield of spruce pretreated for 3 hours.

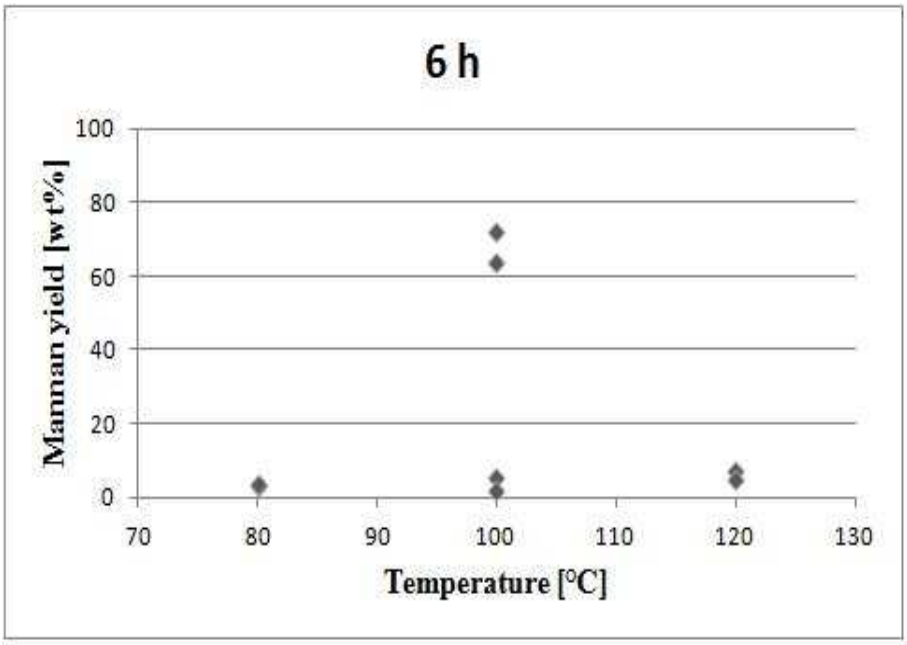

Figure 25: Mannan yield of spruce pretreated for 6 hours.

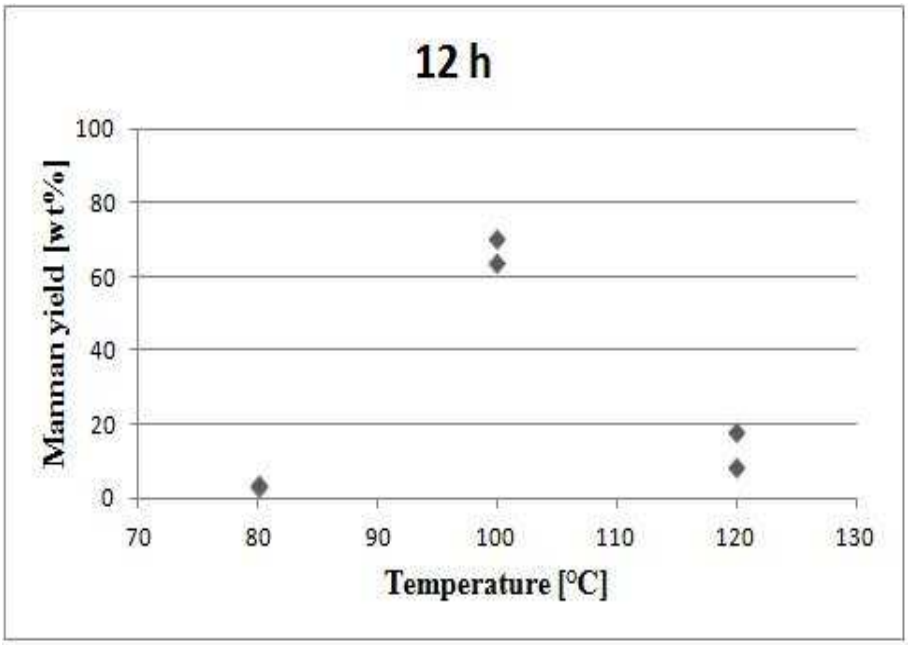

Figure 26: Mannan yield of spruce pretreated for 12 hours.

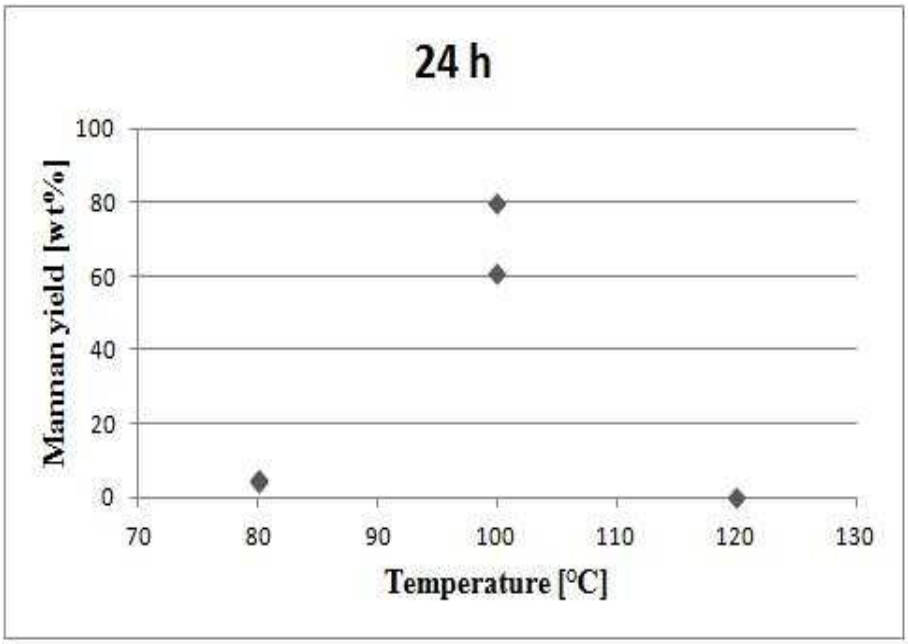

Figure 27: Mannan yield of spruce pretreated for 24 hours.

3.4.3 Xylan yield

To give an indication of the dissolution of the hemicelluloses from bagasse, the xylose content in the liquid phase from the enzymatic hydrolysis was analysed. These results are given in Figure 28.

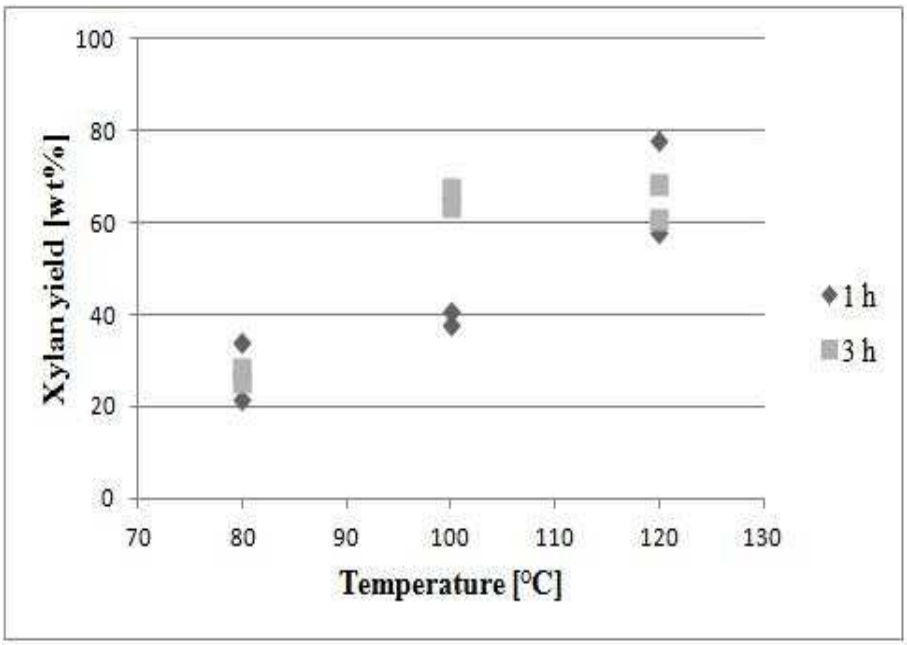

Figure 28: Xylan yield from enzymatic hydrolysis of pretreated sugarcane bagasse.

As for spruce, the results of the dissolution of the hemicellulose in bagasse are consistent with the results for cellulose dissolution in the same experiments (see discussion for glucan yield). The highest xylan yield registered was in the range of 60-70 wt% for the upper end of the temperature range, but it is believed that this can be improved if harsher pretreatment conditions are applied.

3.5 Further work

As mentioned earlier (section 1.4), there are some disagreement in the literature as to whether ionic liquid pretreatment removes lignin from the biomass or not. In the work presented here removal of lignin was not observed, but this is still a subject that needs to be investigated closer. The results presented here shows a dramatic reduction in the enzymatic hydrolysis yield of pretreated spruce if the pretreatment are performed at high temperatures for a prolonged period of time. Among other things, this was attributed to the ionic liquid used ([EMIM][OAc]) and an investigation to see if other ILs show the same tendencies could be of interest. On the same subject, making a more detailed study of the mass balances of the process to determined which stage the loss in fermentable sugar yield are related to could be useful. It is also evident from the results that the conditions used for pretreatment of bagasse could be harsher without giving a decrease in sugar yields. Therefore, pretreatment experiments with bagasse applying longer reaction times (maybe as much as 12 hours) and perhaps higher temperatures as well are proposed as possible continuations of this work.

4 Conclusion

The pretreatment of Norway spruce and sugarcane bagasse with 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) caused an increase in the enzymatic digestibility of the biomass. This is believed to arise from a decrease in the crystallinity of the cellulose and an increase in the accessible surface area caused by increased porosity of the pretreated material.

The digestibility results for spruce shows that, at shorter pretreatment times, higher temperatures are favourable. However, at longer reaction times, too high temperatures can give a reduction in the digestibility. The optimal reaction condition for spruce was in this work found to be 100 °C for 12 hours, giving a digestibility close to 90 wt% of the added glucan. For sugarcane bagasse the optimum was not found, and experiments using harsher conditions was proposed. When comparing the results for pretreatment of spruce with that of bagasse it appear that spruce needs harsher conditions to achieve the same glucan yield as bagasse.

The results of the analysis of the enzymatic digestibility of hemicelluloses (mannan for spruce and zylan for bagasse) concurs very well with the results for glucan presented above.

Regarding the removal of lignin from the biomass, it was found that the degree of delignification in these pretreatment experiments was so low it could be neglected. The low degree of lignin removal was also evident in the darkening of the regenerated biomass from pretreatments using relatively harsh reaction conditions. This darkening was put down to the lignin undergoing condensation reactions.

Suggestions for further work on this area include a thorough investigation into the thermal stability of different ionic liquids at prolonged reaction times and high temperatures, as well as an investigation of the delignification effect of different ionic liquids. As mentioned earlier, pretreatment experiments with bagasse using harsher conditions can also be useful.

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Appendices

A Pretreatment

This is an overview of the reaction parameters and quantities of [EMIM][OAc] and wood fibres used in the different experiments. The required amount of lignocellulosic fibres were calculates from the weighed [EMIM][OAc], the target biomass/IL ratio and the predetermined dry matter content (DM) of the biomass using Eq. 6. The computation example is based on experiment 5A.

$$Biomass\,(g) = \frac{[EMIM][OAc]\,(g) \cdot biomass/IL\,ratio}{DM_{biomass}} \quad (5)$$

$$= \frac{40.5040\ g \cdot 0.05}{0.9583} = 2.1144\ g \quad (6)$$

The solid yield of the pretreatment was calculated by Eq. 8.

$$Solid\,yield\,(wt\%) = \frac{Solid\,product\,(g) \cdot DM_{product}}{Biomass\,in\,(g) \cdot DM_{biomass}} \cdot 100\ \% \quad (7)$$

$$= \frac{9.9081\ g \cdot 0.1918}{2.1144\ g \cdot 0.9583} \cdot 100\ \% = 93.8\ wt\% \quad (8)$$

Table 1 gives an overview of the reaction conditions, chemical loading and the calculated solid yield of different experimental parallels. For parallel 4A the heat was not turned on so this experiment was excluded from later calculations. Experiment 1 to 13 used spruce as the raw material while the remaining experiments (14-19) used sugar cane bagasse.

Table 1: Parameters, quantities and yields of the pretreatment parallels.

Parallel	Temperature *(°C)*	Time *(hours)*	[EMIM][OAc] *(g)*	$Biomass_{in}$ *(g)*	$Solids_{out}$ *(g)*	Solid yield *(wt %)*
1A	100	3	37.5988	1.8866	1.5954	84.6
1B	100	3	40.9878	2.0509	1.8217	88.8
2A	100	24	41.5027	2.0780	1.8320	88.2
2B	100	24	37.8858	1.8929	1.6591	87.7
3A	100	12	42.4897	2.1274	2.1164	99.6
3B	100	12	41.4734	2.0751	2.2274	107.3
4A	100	6	40.5855	2.0123	1.9464	96.7
4B	100	6	38.5289	1.9105	1.7636	92.3
5A	80	3	40.5040	2.0262	1.9004	93.8
5B	80	3	42.6196	2.1313	2.0400	95.7
6A	120	24	42.5384	2.1282	1.8819	88.4
6B	120	24	41.2012	2.0618	1.9734	95.7
7A	120	12	41.7111	2.0865	1.8594	89.1
7B	120	12	43.3545	2.1703	1.8663	86.0
8A	80	6	40.5898	2.0305	1.8036	88.8
8B	80	6	39.7026	1.9867	1.8625	93.8
9A	80	12	39.9664	1.9963	1.6281	81.6
9B	80	12	40.8286	2.0440	1.8073	88.4
10A	120	6	38.5012	1.9270	1.5915	82.6
10B	120	6	40.3692	2.0198	1.8295	90.6
11A	100	6	40.8249	2.0410	1.6376	80 2
11B	100	6	41.3963	2.0713	1.7569	84.8
12A	80	24	41.0054	2.0516	1.7861	87.1
12B	80	24	41.6273	2.0814	1.8536	89.1
13A	120	3	41.1052	2.0568	1.8446	89.7
13B	120	3	40.5454	2.0271	1.8635	91.9
14A	80	3	38.0197	1.9010	1.7584	92.5
14B	80	3	38.9914	1.9501	1.6533	84.8
15A	100	3	40.8841	2.0440	1.7268	84.5
15B	100	3	40.1096	2.0055	1.5676	78.2
16A	120	3	40.2892	2.0157	1.6202	80.4
16B	120	3	41.1086	2.0562	1.6317	79.4
17A	80	1	40.6666	2.0325	1.7284	85.0
17B	80	1	40.7880	2.0300	1.9850	97.8
18A	100	1	40.7605	2.0392	1.8629	91.4
18B	100	1	39.4477	1.9736	1.6212	82.1
19A	120	1	41.2836	2.0640	1.7531	84.9
19B	120	1	39.8876	1.9954	1.6844	84.4

B Chemical composition of the raw materials III

As described under Materials and methods in the report, the composition of the raw material was found by the use of acid hydrolysis followed by HPLC and lignin analysis. The lignin content was found as a combination of acid soluble (ASL) and acid insoluble (AIL) lignin. The ASL content was determined by UV-Vis analysis at 205 nm using Eq. 10 and an absorption coefficient of 94,5 L/gcm. Calculation example for spruce follows.

$$ASL\,(\%) = \frac{UV absorption}{(abs.\,coefficient)\cdot(optical\,cell\,width)} \cdot \frac{liquid\,phase}{W_{sample}} \cdot 100 \quad (9)$$

$$= \frac{3.406}{94.5\,L/gcm\cdot 1\,cm} \cdot \frac{0.140\,L}{0.351\,g} \cdot 100\,\% = 1.44\,\% \quad (10)$$

ASL for the raw materials can be found in Table 2.

Table 2: Acid soluble lignin content

Raw material	Absorption 205 nm	Liquid phase *(L)*	Biomass in *(g)*	ASL *(wt%)*
spruce	3.353	0.140	0.351	1.42
spruce	3.406	0.140	0.351	1.44
bagasse	1.197	0.140	0.350	0.51
bagasse	1.147	0.140	0.350	0.49

The acid insoluble lignin (AIL) content was determined from the weight of the solid residue and of the ash by using Eq. 11.

$$AIL\,(\%) = \frac{W_{solid\,residue} - W_{ash}}{W_{sample}} \cdot 100 = \frac{0.0979\,g - 0.0006\,g}{0.351\,g} \cdot 100\,\% = 27.71\,\% \quad (11)$$

The total lignin content on a weight basis was calculated from Eq. 12.

$$Lignin\,content\,(\%) = ASL + AIL = 1.44\,\% + 27.71\% = 29.15\,\% \quad (12)$$

AIL for the different raw materials is given in Table 3.

Table 3: Acid insoluble lignin and total lignin content.

Raw material	Solid residue *(g)*	Ash *(g)*	Biomass in *(g)*	ASL *(wt%)*	Total lignin *(wt%)*	
spruce	0.0964	0.0002	0.351	27.54	28.96	29.05
spruce	0.0979	0.0006	0.351	27.71	29.15	
bagasse	0.0757	0.0023	0.350	20.96	21.47	21.48
bagasse	0.0760	0.0026	0.350	21.00	21.48	

The glucan content was found by HPLC analysis and calculated by eq. 14. The mannan and xylane content of spruce and bagasse respectively was found in the same fashion as the glucan content (using a correction factor of 132/150 for xylan).

$$Glucan\,content\,(\%) = glucose \cdot \frac{162}{180} \cdot \frac{liquid\,phase}{W_{sample}} \cdot 100 \quad (13)$$

$$= 1.1685\ g/L \cdot \frac{162}{180} \cdot \frac{0.140\ L}{0.351\ g} \cdot 100\,\% = 41.98\,\% \quad (14)$$

Table 4 gives an overview of the raw data from the HPLC analysis and the glucan, mannan and xylan content of the raw materials. The liquid phase was 0.140 L for all parallels.

Table 4:Carbohydrate content.

Raw material	Glucose *(g/L)*	Mannose *(g/L)*	Xylose *(g/L)*	Biomass in *(g)*	Glucan *(wt%)*	Mannan *(wt%)*	Xylan *(wt%)*
spruce	1.1685	0.2207	-	0.351	41.98	7.93	-
spruce	1.1682	0.2227	-	0.351	41.92	7.99	-
bagasse	1.3163	-	0.6526	0.350	47.37	-	22.96
bagasse	1.3429	-	0.6639	0.350	48.41	-	23.40

C Enzyme hydrolysis

For the enzymatic hydrolysis an amount of biomass equivalent to 100 mg of cellulose was needed. The biomass needed was calculated from Eq. 15. The calculation example is based on untreated spruce.

$$Biomass\,(g) = \frac{cellulose\,wanted}{Cellulose\,content \cdot DM} = \frac{0.100\,g}{0.4195 \cdot 0.2910} = 0.8192 \tag{15}$$

The water added was calculated to give a total volume of 10 mL by Eq. 18.

$$\begin{aligned} Water\,(mL) &= Total\,V - buffer - antibiotics - enzyme - biomass && (16)\\ &= 10\,mL - 5\,mL - 0.1\,mL - 0.054\,mL - 0.8185\,mL && (17)\\ &= 4.0275\,mL && (18) \end{aligned}$$

Table 5 gives an overview of the volumes used in the different enzymatic hydrolysis parallels. The amount of buffer and antibiotics are not listed since these are 5 and 0.1 mL respectively for all parallels.

Table 5: Quantities for the enzymatic hydrolysis.

Parallel	Biomass *(mL)*	Enzyme *(mL)*	Water *(mL)*
Spruce	0.8185	0.054	4.0275
Bagasse A	0.9785	0.054	3.8675
Bagasse B	0.9788	0.054	3.8672
1A	1.2005	0.054	3.6455
1B	1.8706	0.054	2.9754
2A	2.1464	0.054	2.6996
2B	1.6618	0.054	3.1842
3A	1.5086	0.054	3.3374
3B	1.4650	0.054	3.3810
4B	1.1066	0.054	3.7394
5A	1.1408	0.054	3.7052
5B	1.0484	0.054	3.7976
6A	1.2939	0.054	3.5521
6B	1.2597	0.054	3.5863
7A	1.1267	0.054	3.7193
7B	1.3005	0.054	3.5455
8A	1.2498	0.054	3.5962
8B	1.0264	0.054	3.8196
9A	1.0276	0.054	3.8184
9B	1.1596	0.054	3.6864
10A	1.3233	0.054	3.5227
10B	1.2637	0.054	3.5823
11A	1.1166	0.054	3.7294
11B	1.1544	0.054	3.6916
12A	1.1508	0.054	3.6952
12B	1.1103	0.054	3.7357
13A	1.1911	0.054	3.6549
13B	1.2883	0.054	3.5577
14B	0.8898	0.054	3.9562
15A	1.3753	0.054	3.5247
15B	1.3506	0.054	3.4954
16A	1.6008	0.054	3.2452
16B	1.6762	0.054	3.1698
17A	1.2302	0.054	3.6158
17B	1.4394	0.054	3.6158
18A	1.1557	0.054	3.6903
18B	1.1474	0.054	3.6986
19A	1.3541	0.054	3.4919
19B	1.5998	0.054	3.2462

D UV-Vis analysis

The lignin content in the liquid phase from the precipitation after pretreatment were calculated from the absorption measurements at 205 nm using an absorption coefficient (Abs.) of 94.5 L/gcm, the optical cell length (l) of 1 cm and Beer's law as stated in Eq. 20. The numeric examples are based on experiment 5A.

$$Dissolved\,lignin\,(g/L) = \frac{absorption}{abs.\,(L/gcm)\cdot l\,(cm)} \tag{19}$$

$$= \frac{0.124}{94.5\;L/gcm\cdot 1\;cm} = 0.0013\;g/L \tag{20}$$

The original concentration of lignin was calculated from Eq. 22 by using the predetermined lignin content of the biomass.

$$Lignin_{in}\,(g/L) = \frac{Biomass\,(g)\cdot lignin\,fraction}{liquid\,phase\,(L)} \tag{21}$$

$$= \frac{2.0262\;g\cdot 0.2905}{0.440\;L} = 1.2728\;g/L \tag{22}$$

Dissolved lignin as a fraction of the total amount of lignin in the system was found from Eq. 24.

$$Dissolved\,lignin\,(g/g\;lignin_{in}) = \frac{Dissolved\,lignin\,(g/L)}{Lignin_{in}\,(g/L)} \tag{23}$$

$$= \frac{0.0013\;g/L}{1.2728\;g/L}\% = 0.00103\;(g/g\;lignin_{in}) \tag{24}$$

Table 5 gives the data from the UV-Vis analysis. The liquid phase is excluded from the table on account of it being 0.44 L for all the parallels.

Table 5: Data from UV-Vis analysis.

Parallel	Absorption			Lignin content	$Biomass_{in}$	$Lignin_{in}$	Dissolved lignin
	280 nm	*220 nm*	*205 nm*	*(g/L)*	*(g)*	*(g/L)*	*(g/g $lignin_{in}$)*
1A	0.429	0.007	0.094	0.0010	1.8866	1.1852	0.00084
1B	1.231	0.052	0.124	0.0013	2.0509	1.2883	0.00102
2A	1.864	1.131	0.164	0.0017	2.0708	1.3054	0.00133
2B	1.479	0.080	0.141	0.0015	1.8929	1.1891	0.00125
3A	1.682	0.105	0.155	0.0016	2.1247	1.3347	0.00123
3B	1.918	0.144	0.168	0.0018	2.0751	1.3035	0.00136
4A	-0.096	0.000	0.085	0.0009	2.0123	1.2641	0.00071
4B	1.492	0.091	0.152	0.0016	1.9105	1.2001	0.00134
5A	0.651	0.042	0.124	0.0013	2.0262	1.2728	0.00103
5B	0.747	0.052	0.136	0.0014	2.1313	1.3388	0.00107
6A	1.968	0.213	0.242	0.0026	2.1282	1.3369	0.00192
6B	2.025	0.212	0.235	0.0025	2.0618	1.2952	0.00192
7A	2.013	0.186	0.208	0.0022	2.0865	1.3107	0.00168
7B	2.041	0.203	0.222	0.0023	2.1703	1.3633	0.00172
8A	0.820	0.033	0.110	0.0012	2.0305	1.2755	0.00091
8B	0.802	0.031	0.110	0.0012	1.9867	1.2480	0.00093
9A	1.028	0.460	0.118	0.0012	1.9963	1.2541	0.00100
9B	0.906	0.042	0.120	0.0013	2.0440	1.2840	0.00099
10A	1.875	0.157	0.187	0.0020	1.9270	1.2105	0.00163
10B	2.032	0.182	0.201	0.0021	2.0198	1.2688	0.00168
11A	1.658	0.109	0.163	0.0017	2.0410	1.2821	0.00135
11B	1.286	0.077	0.147	0.0016	2.0713	1.3012	0.00120
12A	1.417	0.089	0.155	0.0016	2.0516	1.2888	0.00127
12B	1.300	0.080	0.148	0.0016	2.0814	1.3075	0.00120
13A	1.782	0.121	0.162	0.0017	2.0568	1.2921	0.00133
13B	1.890	0.148	0.178	0.0019	2.0271	1.2734	0.00148
14A	2.036	0.180	0.208	0.0022	1.9010	0.9068	0.00243
14B	2.014	0.185	0.212	0.0022	1.9501	0.9303	0.00241
15A	2.036	0.191	0.218	0.0023	2.0440	0.9751	0.00237
15B	2.028	0.183	0.209	0.0022	2.0055	0.9567	0.00231
16A	2.079	0.225	0.248	0.0026	2.0157	0.9616	0.00273
16B	2.088	0.230	0.251	0.0027	2.0562	0.9809	0.00271
17A	1.678	0.133	0.190	0.0020	2.0325	0.9696	0.00207
17B	1.821	0.151	0.197	0.0021	2.0300	0.9684	0.00215
18A	2.030	0.203	0.230	0.0024	2.0392	0.9728	0.00250
18B	2.041	0.214	0.241	0.0026	1.9736	0.9415	0.00271
19A	2.052	0.224	0.251	0.0027	2.0640	0.9846	0.00270
19B	2.089	0.237	0.300	0.0032	1.9954	0.9519	0.00334

E HPLC analysis

Here follows the data from the HPLC analysis. The dissolved cellulose was calculated from the glucose concentration from the HPLC analysis using eq. 26. Example based on parallel 10A.

$$Glucan\,recovered\,(mg) = glucose\,(mg/L) \cdot liquid\,phase\,(L) \cdot \frac{162}{180} \quad (25)$$

$$= 2163.9531\ mg/L \cdot 0.01\ L \cdot \frac{162}{180} = 19.48\ mg \quad (26)$$

The enzymatic hydrolysis yield was determined from Eq. 28.

$$Enzymatic\,hydrolysis\,yield\,(wt\%) = \frac{Glucan\,recovered\,(mg)}{Glucan\,in\,sample\,(mg)} \cdot 100\ \% \quad (27)$$

$$= \frac{19.48\ mg}{100\ mg} \cdot 100\ \% = 19.48\ wt\% \quad (28)$$

Table 6 shows the data obtained from the HPLC analysis. The liquid phase was 0.01 L and the glucan content into the hydrolysis was 100 mg for all parallels. In addition the mannan content into the hydrolysis was 19 mg for the spruce parallels (parallel 1-13) and the content of xylose was 46 mg for the bagasse parallels (parallel 14-19).

The attachments that follows Table 6 gives the raw data from the HPLC analysis.

Table 6: Data from HPLC analysis

Parallel	Glucose *(mg/L)*	Mannose *(mg/L)*	Xylose *(mg/L)*	Glucan yield *(wt %)*	Mannan yield *(wt %)*	Xylan yield *(wt %)*
Spruce	4.9268	3.9891	-	0.04	0.19	-
Bagasse	2.7982	-	1.7277	0.03	-	0.03
1A	586.2582	457.1835	-	5.28	21.66	-
2A	7474.4463	1283.6470	-	67.27	60.80	-
2B	9414.6453	1679.7758	-	84.73	79.57	-
3A	9147.0013	1341.9518	-	82.32	63.57	-
3B	9855.6413	1483.5212	-	88.70	70.27	-
4B	9292.5684	1527.8978	-	83.63	72.37	-
4B	9636.4260	1351.5916	-	86.73	64.02	-
5A	330.0826	76.2948	-	2.97	3.61	-
5B	38.7672	4.9531	-	0.35	0.23	-
6A	4.9592	1.4954	-	0.04	0.07	-
7A	2609.3261	177.6342	-	23.48	8.41	-
7B	3557.3747	373.9631	-	32.02	17.71	-
8A	1089.7388	60.8502	-	9.81	2.88	-
8B	1673.1322	73.7736	-	15.06	3.49	-
9A	1150.9445	63.1233	-	10.36	2.99	-
9B	1832.9330	83.1103	-	16.50	3.94	-
10A	2163.9531	148.5625	-	19.48	7.04	-
10B	1598.8014	102.1415	-	14.39	4.84	-
11A	1986.5175	117.2452	-	17.88	5.55	-
11B	1954.4131	34.7030	-	17.59	1.64	-
12A	2145.7493	106.1965	-	19.31	5.03	-
12B	2552.8428	88.1121	-	22.98	4.17	-
13A	1890.8096	123.7883	-	17.02	5.86	-
13B	2065.6407	121.1902	-	18.59	5.74	-
14B	2439.9664	-	1340.4409	21.96	-	25.64
14B	2069.4005	-	1485.5274	18.62	-	28.42
15A	6378.8236	-	3318.4296	57.41	-	63.48
15B	6931.7449	-	3525.2181	62.39	-	67.44
16A	8734.2415	-	3182.6476	78.61	-	60.89
16B	9717.5190	-	3578.9645	87.61	-	68.47
17A	1376.4123	-	1125.0776	12.39	-	21.52
17B	3137.2627	-	1766.1163	28.24	-	33.79
18A	3517.6622	-	1975.9702	31.66	-	37.80
18B	3154.8499	-	2115.0327	28.39	-	40.46
19A	5709.9247	-	3018.0892	51.39	-	57.74
19B	8128.4339	-	4067.3366	73.16	-	77.81

Spruce

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 06.06.12-

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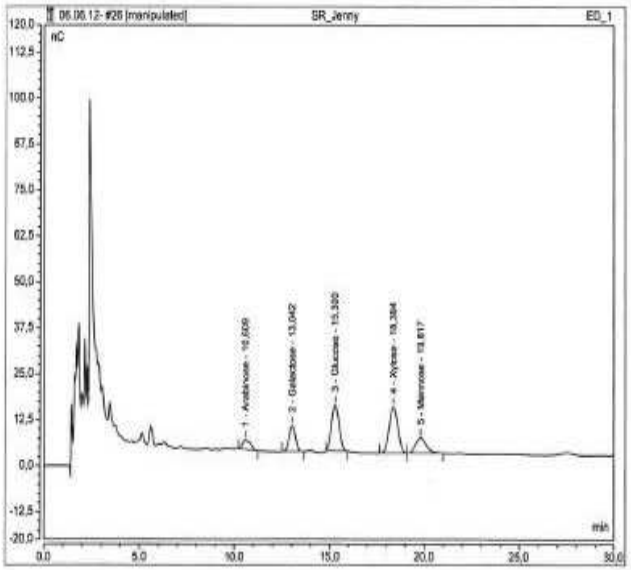
Peak Integration Report

Sample Name:	SR_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	05-jun-2012 / 10:12	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	10,81	Arabinose	BMB*	1,219	2,571	1,7802
2	13,04	Galactose	BMB	2,726	7,015	3,8517
3	15,30	Glucose	BMB*	6,877	12,521	4,9268
4	18,36	Xylose	BM	6,754	12,595	6,0653
5	19,82	Mannose	MB*	2,686	4,126	3,9891
		TOTAL:		19,27	38,83	20,09



Cation/Integration

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Bagasse

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Instrument: PFI-LABBA_1
Sequence: 06.06.12-

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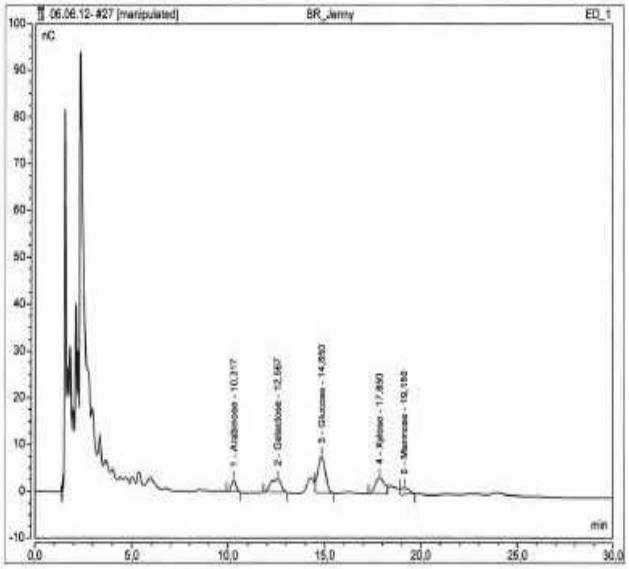
Peak Integration Report

Sample Name:	BR_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	05-jun-2012 / 11:33	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	10,32	Arabinose	BMB*	0,096	2,475	1,0320
2	12,57	Galactose	BMB*	1,840	2,824	1,9984
3	14,85	Glucose	MB*	3,588	7,723	2,7982
4	17,85	Xylose	BM *	1,814	3,402	1,7277
5	19,15	Mannose	MB*	0,781	1,466	1,0337
		TOTAL:		8,72	17,89	8,59



Cation/Integration

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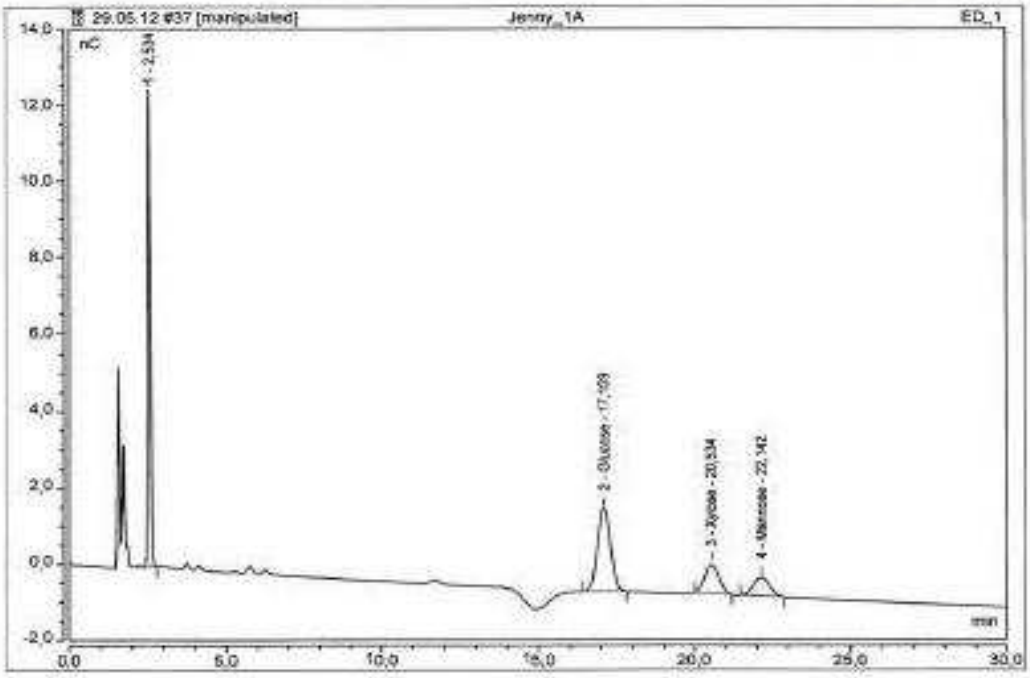
Instrument: PFI-LABBA_1
Sequence: 29.05.12

1A

Peak Integration Report

Sample Name:	Jenny_1A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM naoqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	31-mai-2012 / 18:45	Run Time:	79,36

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	17,11	Glucose	BMB	1,080	2,183	585,2884
3	20,63	Xylose	BMB*	0,403	0,764	307,3479
4	22,14	Mannose	BMB*	0,298	0,499	457,1635
TOTAL:				1,78	3,44	1350,79



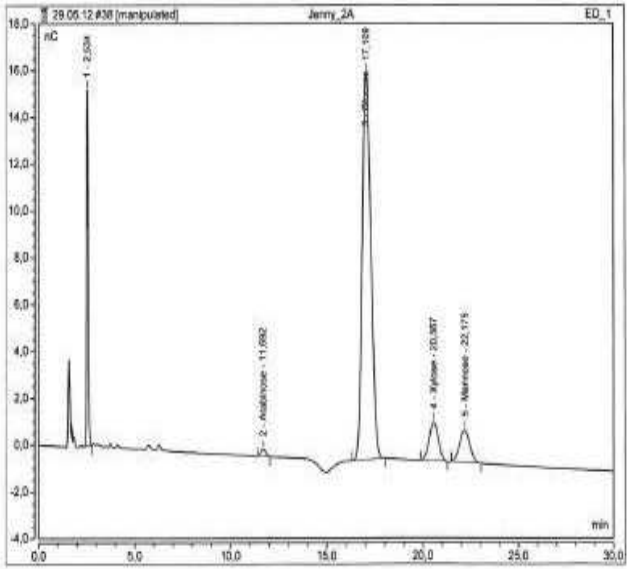
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

2A

Peak Integration Report

Sample Name:	Jenny_2A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	31-mai-2012 [23:05	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,69	Arabinose	BMB*	0,086	0,287	137,5548
3	17,11	Glucose	BMB	8,658	16,536	7474,4463
4	20,57	Xylose	BMB*	0,916	1,651	771,5672
5	22,18	Mannose	BMB*	0,828	1,375	1283,6470
TOTAL:				10,50	19,85	9667,22



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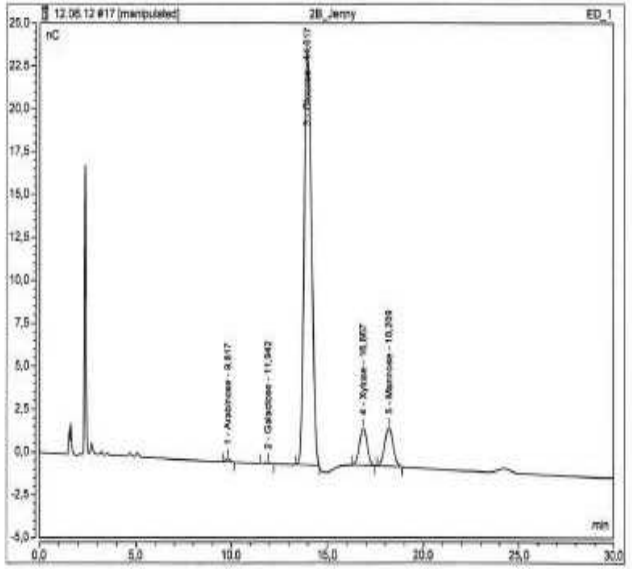
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 12.06.12

2B

Peak Integration Report

Sample Name:	2B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	13-jun-2012 [22:47	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	9,82	Arabinose	BMB*	0,082	0,230	101,2656
2	11,94	Galactose	BMB*	0,029	0,087	48,8061
3	14,02	Glucose	BMB*	10,858	24,103	9414,6453
4	16,87	Xylose	BMB	1,000	2,162	820,9238
5	18,21	Mannose	BMB	1,181	2,266	1679,7759
TOTAL:				12,91	28,86	12065,22



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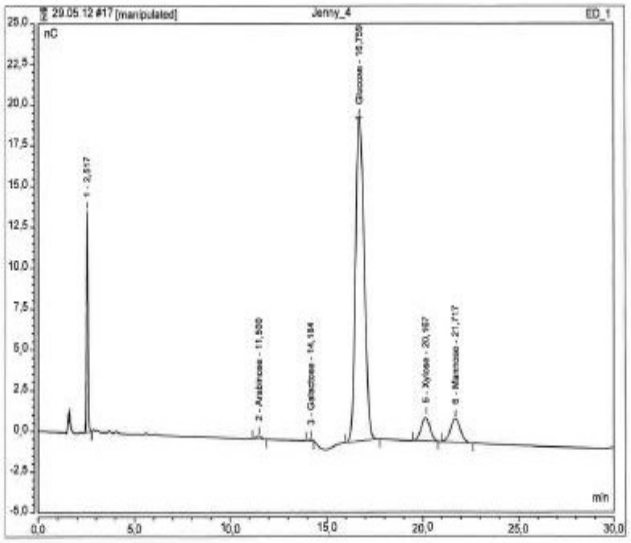
3A

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

Peak Integration Report

Sample Name:	Jenny_4	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	30-mai-2012 / 10:06	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,50	Arabinose	BMB*	0,069	0,223	115,2666
3	14,18	Galactose	BMB*	0,015	0,070	46,6743
4	16,76	Glucose	BMB	10,323	19,760	8147,0013
5	20,17	Xylose	BMB*	0,772	1,431	640,8101
6	21,72	Mannose	BMB*	0,877	1,454	1341,9518
TOTAL:				12,06	22,94	11291,70



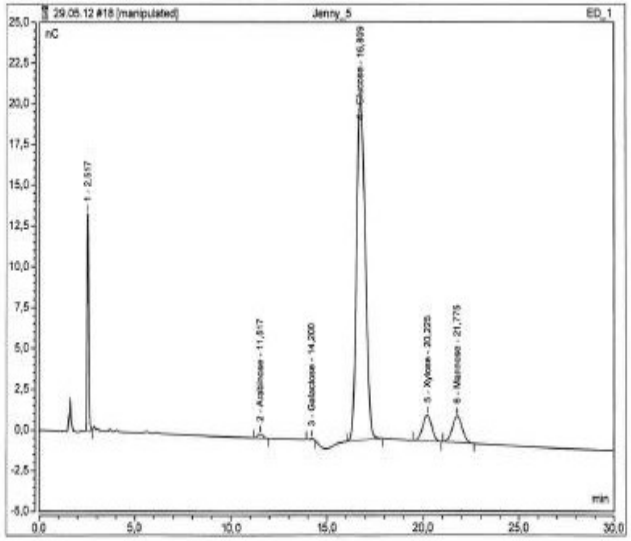
3B

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

Peak Integration Report

Sample Name:	Jenny_5	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	30-mai-2012 / 10:29	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,52	Arabinose	BMB*	0,078	0,248	127,0972
3	14,20	Galactose	BMB*	0,016	0,068	48,4246
4	16,81	Glucose	BMB	11,008	20,854	9855,6413
5	20,23	Xylose	BMB*	0,875	1,592	734,1471
6	21,78	Mannose	BMB*	0,969	1,693	1483,5212
TOTAL:				12,95	24,37	12249,43



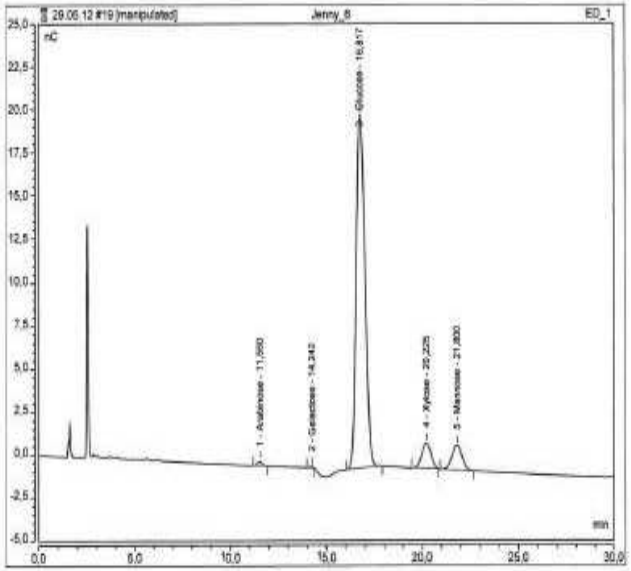
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

4B

Peak Integration Report

Sample Name:	Jenny_6	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	30-mai-2012 / 17:58	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	11,55	Arabinose	BMB*	0,072	0,232	118,6141
2	14,24	Galactose	BMB*	0,013	0,062	44,8760
3	16,82	Glucose	BMB	16,798	20,466	9635,4260
4	20,23	Xylose	BMB*	0,796	1,481	863,9881
5	21,80	Mannose	BMB*	0,883	1,458	1361,5916
TOTAL:				12,56	23,70	11815,51



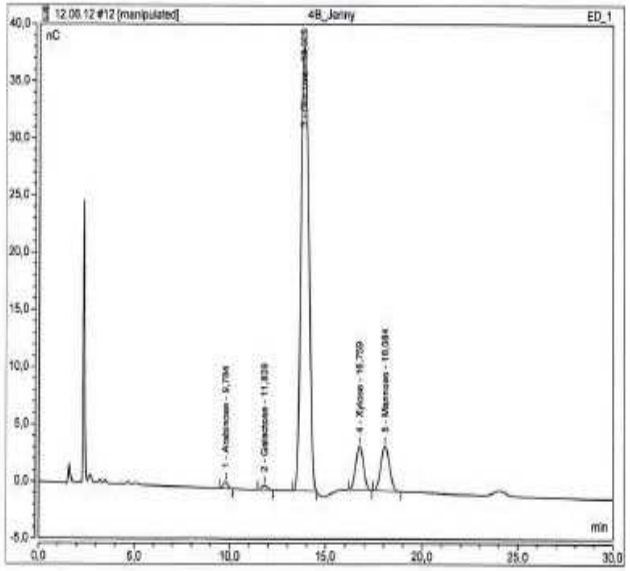
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 12.06.12

4B

Peak Integration Report

Sample Name:	4B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	500,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	13-jun-2012 / 16:02	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	9,78	Arabinose	BMB*	0,162	0,604	114,4964
2	11,81	Galactose	BMB*	0,138	0,410	76,7480
3	13,91	Glucose	BMB*	18,421	38,768	9292,8684
4	16,76	Xylose	BMB*	1,779	3,848	757,1468
5	18,08	Mannose	BMB*	2,095	3,949	1527,8976
TOTAL:				22,53	47,58	11768,86



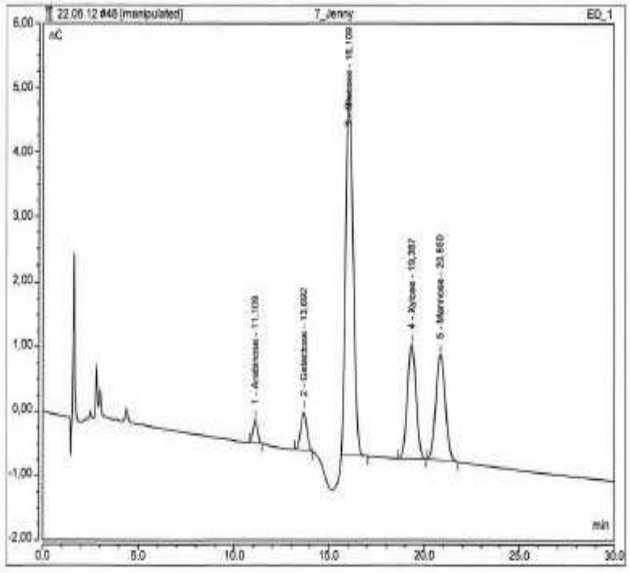
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 22.05.12

5A

Peak Integration Report

Sample Name:	7_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	200,0000
Program:	Gradient 1,5-200mM reagvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	25-mai-2012 / 17:53	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	11,11	Arabinose	BMB*	0,102	0,359	26,9612
2	13,69	Galactose	BMB*	0,230	0,688	44,2266
3	16,11	Glucose	BMB*	2,568	5,636	384,8556
4	19,37	Xylose	BMB*	0,934	1,777	172,3685
5	20,85	Mannose	BMB*	0,950	1,643	318,7493
TOTAL:				4,78	10,01	957,25



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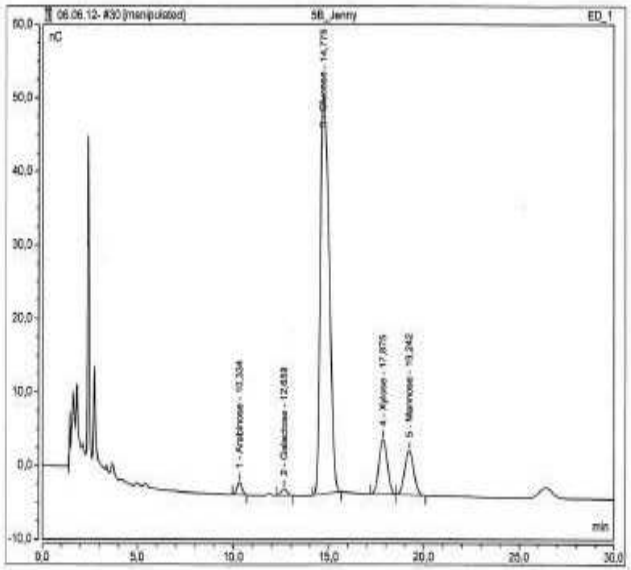
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 06.06.12-

5B

Peak Integration Report

Sample Name:	5B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1,0000
Program:	Gradient 1,5-200mM reagvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	08-jun-2012 / 15:36	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	10,33	Arabinose	BMB*	0,490	1,711	0,7341
2	12,66	Galactose	BMB*	0,311	0,866	0,3838
3	14,78	Glucose	BMB*	28,959	54,345	38,7672
4	17,86	Xylose	BMB*	3,729	7,398	3,5339
5	19,24	Mannose	BMB*	3,308	5,966	4,9631
TOTAL:				36,80	70,29	48,37



Cation/Integration

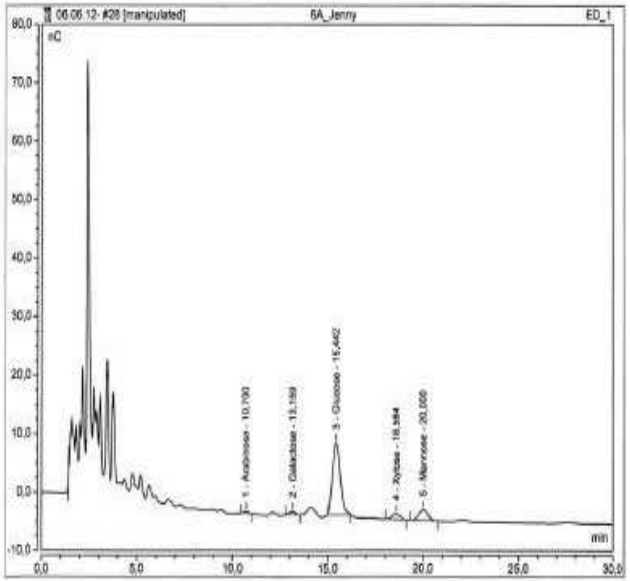
Chromeleon (c) Dionex 1998-2009
Version 7.1.0.888

6A

Peak Integration Report

Sample Name:	6A_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	08-jun-2012 / 12:54	Run Time:	55,99

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	10,70	Arabinose	BMB*	0,102	0,386	0,1535
2	13,16	Galactose	BMB*	0,166	0,582	0,2569
3	15,44	Glucose	M*	5,811	12,321	4,9592
4	18,68	Xylose	BMB*	0,456	0,938	0,4935
5	20,00	Mannose	BMB	1,033	1,827	1,4254
TOTAL:				7,70	16,03	7,29

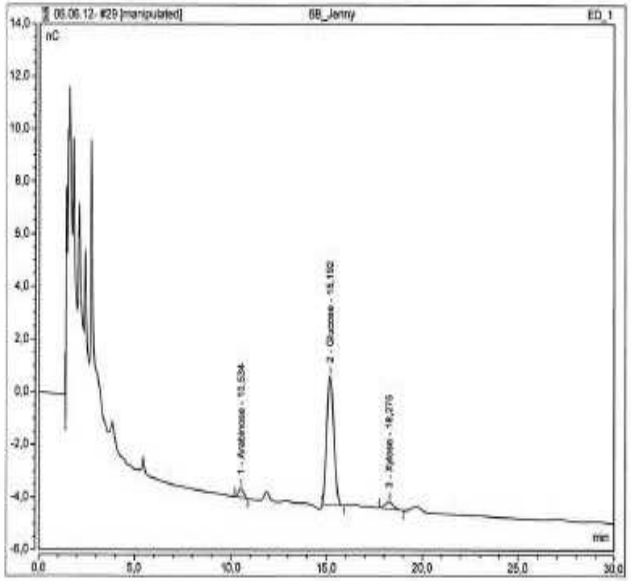


6B

Peak Integration Report

Sample Name:	6B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	08-jun-2012 / 14:15	Run Time:	71,01

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	10,53	Arabinose	BMB*	0,089	0,347	0,1496
2	15,19	Glucose	BMB*	2,103	4,697	1,4639
3	18,29	Xylose	BMB*	0,134	0,279	0,2058
TOTAL:				2,34	5,52	1,82



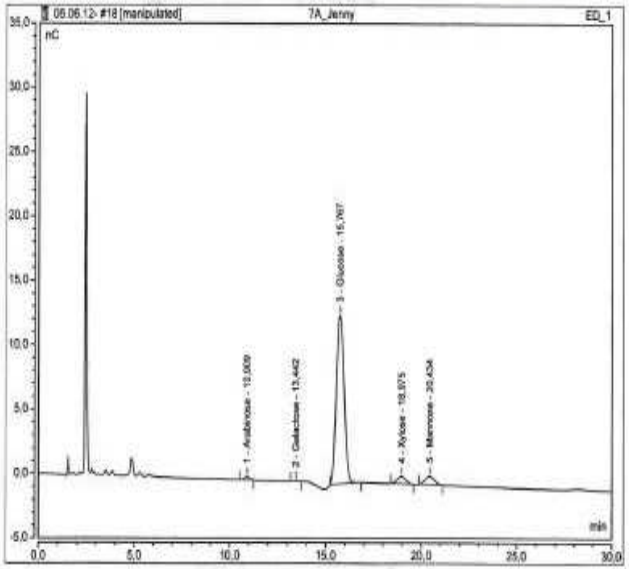
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 06.06.12-

7A

Peak Integration Report

Sample Name:	7A_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	500,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	07-jun-2012 / 11:34	Run Time:	79,96

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	10,81	Arabinose	BMB*	0,063	0,227	46,0657
2	13,44	Galactose	BMB*	0,012	0,042	20,2405
3	15,77	Glucose	BMB*	6,183	13,091	2608,3261
4	18,96	Xylose	BMB*	0,271	0,543	163,7971
5	20,43	Mannose	BMB*	0,344	0,637	177,6342
TOTAL:				6,87	14,54	3017,95



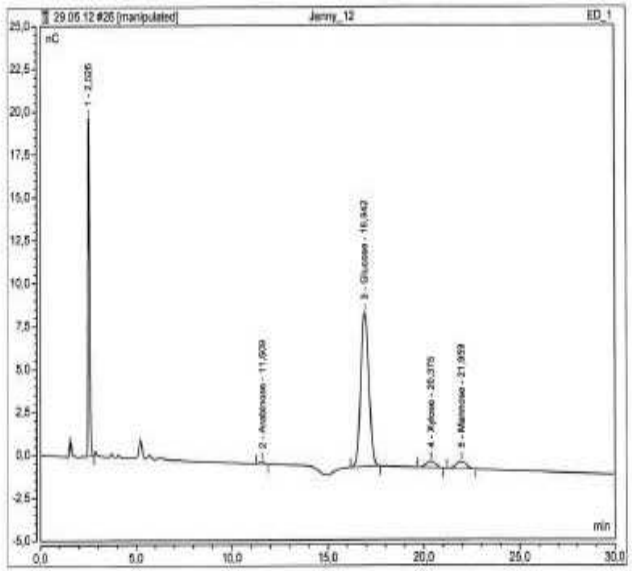
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

7B

Peak Integration Report

Sample Name:	Jenny_12	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	31-mai-2012 / 03:18	Run Time:	79,96

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,61	Arabinose	BMB*	0,038	0,128	74,0788
3	16,94	Glucose	BMB	4,491	8,953	3657,3747
4	20,38	Xylose	BMB*	0,195	0,370	121,3604
5	21,96	Mannose	BMB*	0,744	0,410	373,9631
TOTAL:				4,97	9,86	4126,78



8A

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

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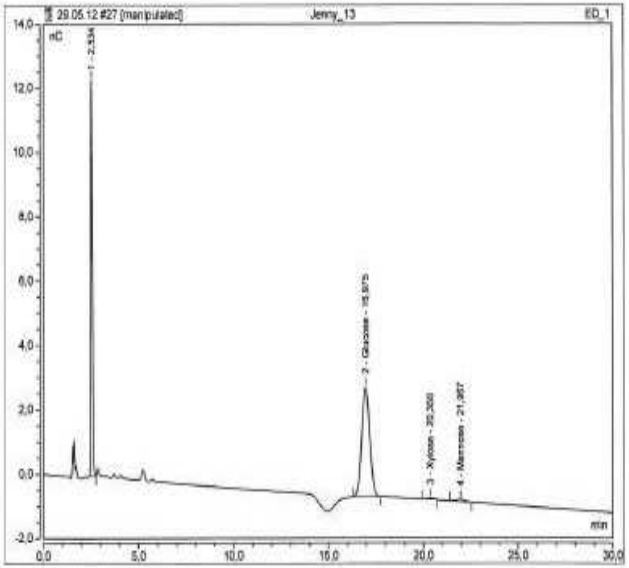
1.6.2012 12:21 PM

Peak Integration Report

Sample Name:	Jenny_13	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	31-mai-2012 / 04:39	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	16,98	Glucose	BMB	1,673	3,411	1069,7388
3	20,38	Xylose	BMB*	0,019	0,044	n.a.
4	21,97	Mannose	BMB*	0,038	0,066	80,8502
TOTAL:				1,73	3,52	1150,89



Cation/Integration

Chromeleon (c) Dionex 1996-2009
Version 7.1.0.898

8B

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

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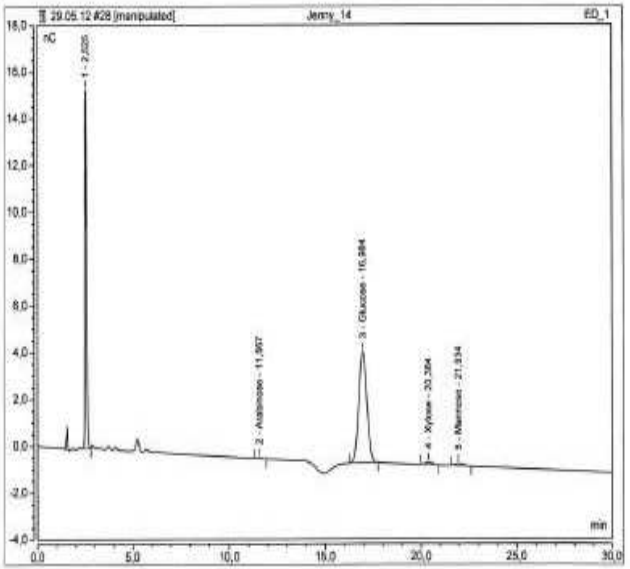
Peak Integration Report

Sample Name:	Jenny_14	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	31-mai-2012 / 09:09	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,57	Arabinose	BMB*	0,050	0,032	36,2668
3	16,98	Glucose	BMB	2,263	4,753	1673,1302
4	20,38	Xylose	BMB*	0,041	0,083	n.a.
5	21,93	Mannose	BMB*	0,046	0,088	73,7736
TOTAL:				2,48	4,97	1785,17



Cation/Integration

Chromeleon (c) Dionex 1996-2009
Version 7.1.0.898

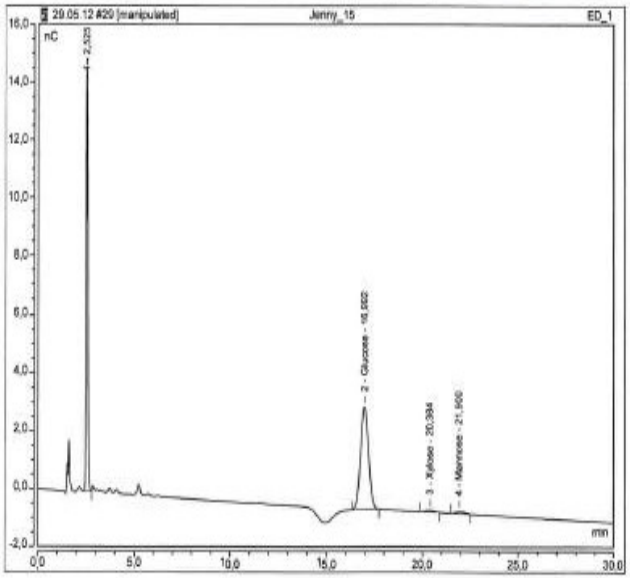
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

9A

Peak Integration Report

Sample Name:	**Jenny_15**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1000,0000**
Program:	**Gradient 1,5-200mM reeqvil 30grC**	*Operator:*	**Labbetuss**
Inj. Date / Time:	**31-mai-2012 / 07:21**	*Run Time:*	**79,98**

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	16,89	Glucose	BMB	1,745	3,550	1150,9445
3	20,38	Xylose	BMB*	0,031	0,063	n.a.
4	21,90	Mannose	BMB*	0,039	0,069	63,1233
TOTAL:				1,82	3,68	1214,07



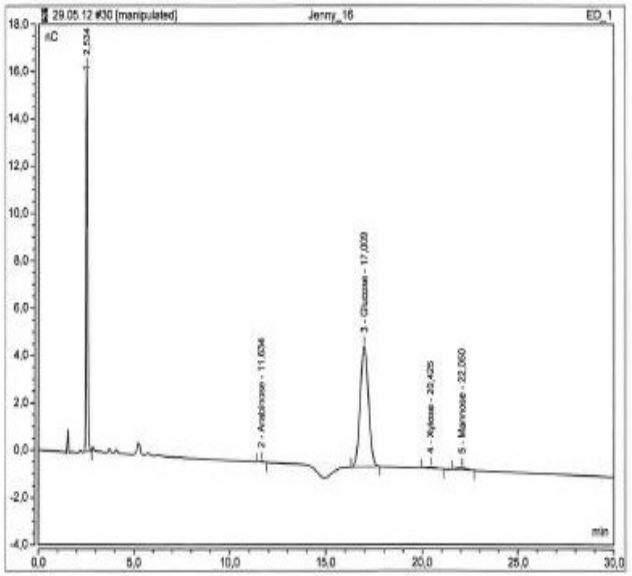
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

9B

Peak Integration Report

Sample Name:	**Jenny_16**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1000,0000**
Program:	**Gradient 1,5-200mM reeqvil 30grC**	*Operator:*	**Labbetuss**
Inj. Date / Time:	**31-mai-2012 / 08:42**	*Run Time:*	**79,98**

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,63	Arabinose	BMB*	0,010	0,036	37,2805
3	17,01	Glucose	BMB	2,538	5,111	1832,9330
4	20,43	Xylose	BMB*	0,095	0,071	n.a.
5	22,05	Mannose	BMB*	0,052	0,097	83,1103
TOTAL:				2,83	5,32	1953,32



XX

10A

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

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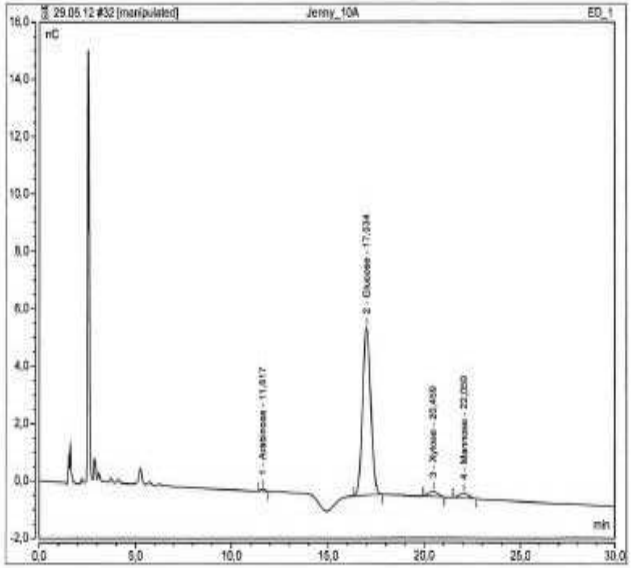
Peak Integration Report

Sample Name:	Jenny_10A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-250mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	31-mai-2012 / 11:39	Run Time:	79,88

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	11,62	Arabinose	BMB*	0,023	0,085	55,3067
2	17,03	Glucose	BMB	2,818	0,856	2163,9531
3	20,46	Xylose	BMB*	0,095	0,181	32,2089
4	22,06	Mannose	BMB*	0,096	0,166	148,5625
TOTAL:				3,13	6,29	2400,03



Cation/Integration

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Version 7.1.0.898

10B

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

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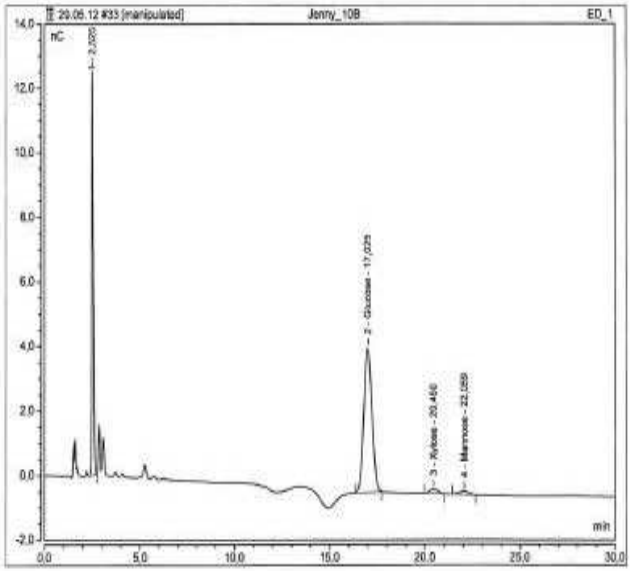
Peak Integration Report

Sample Name:	Jenny_10B	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-250mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	31-mai-2012 / 13:20	Run Time:	79,88

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	17,03	Glucose	BMB	2,220	4,483	1558,8014
3	20,45	Xylose	BMB*	0,069	0,138	9,0525
4	22,06	Mannose	BMB*	0,065	0,112	102,1415
TOTAL:				2,35	4,73	1670,00



Cation/Integration

Chromeleon (c) Dionex 1996-2009
Version 7.1.0.898

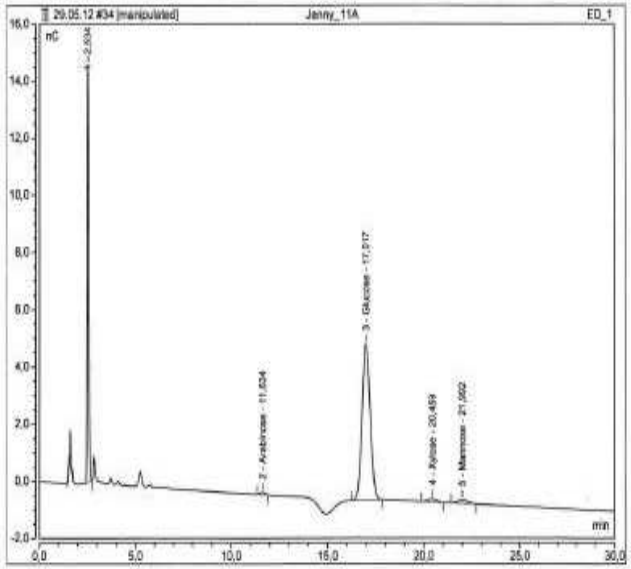
11A

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

Peak Integration Report

Sample Name:	Jenny_11A	*Inj. Vol.:*	25,00
Injection Type:	Unknown	*Dilution Factor:*	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	*Operator:*	Labbetuss
Inj. Date / Time:	31-mai-2012 / 14:41	*Run Time:*	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,83	Arabinose	BMB*	0,024	0,085	36,0297
3	17,02	Glucose	BMB	2,714	5,455	1986,5175
4	20,46	Xylose	BMB*	0,008	0,131	6,2023
5	21,99	Mannose	BMB*	0,075	0,127	117,2462
TOTAL:				2,88	5,79	2165,99



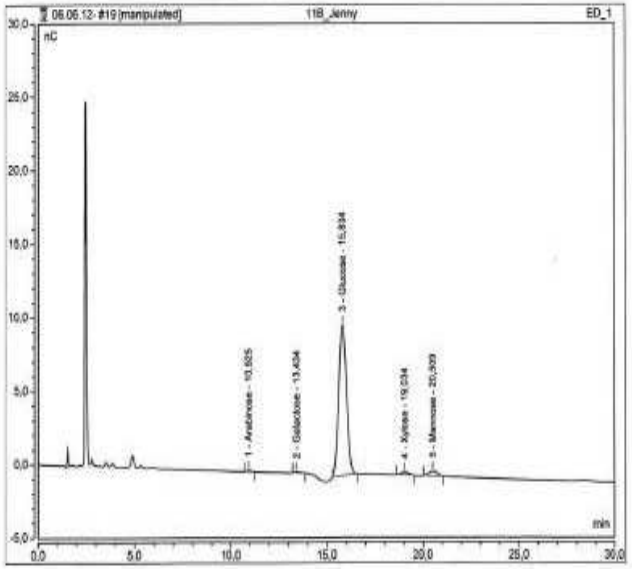
11B

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 06.06.12-

Peak Integration Report

Sample Name:	11B_Jenny	*Inj. Vol.:*	25,00
Injection Type:	Unknown	*Dilution Factor:*	500,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	*Operator:*	Labbetuss
Inj. Date / Time:	07-jun-2012 / 12:55	*Run Time:*	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	10,93	Arabinose	BMB*	0,024	0,092	16,5824
2	13,43	Galactose	BMB*	0,016	0,049	23,0194
3	15,83	Glucose	BMB*	4,796	10,257	1954,4131
4	19,03	Xylose	BMB*	0,082	0,182	79,4457
5	20,61	Mannose	BMB*	0,159	0,307	34,7030
TOTAL:				5,08	10,89	2108,16



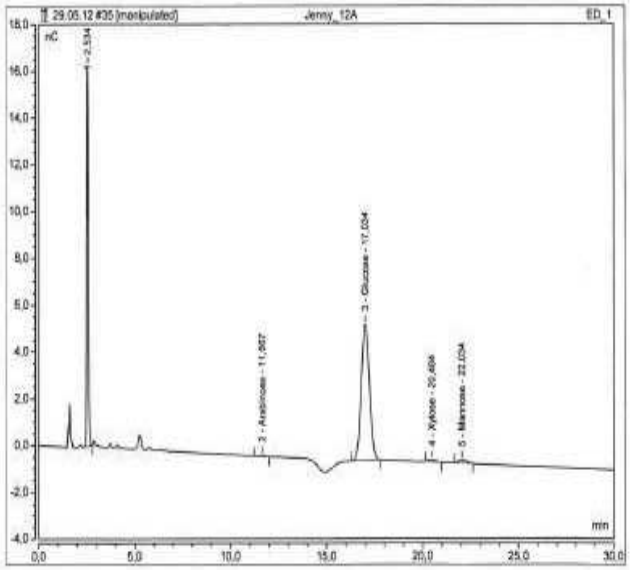
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

12A

Peak Integration Report

Sample Name:	Jenny_12A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	31-mai-2012 / 16:02	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,67	Arabinose	BMB*	0,018	0,053	49,3165
3	17,03	Glucose	BMB	2,897	5,617	2145,7493
4	20,48	Xylose	BMB*	0,037	0,086	n.a.
5	22,03	Mannose	BMB*	0,068	0,128	106,1965
TOTAL:				3,02	6,08	2301,26



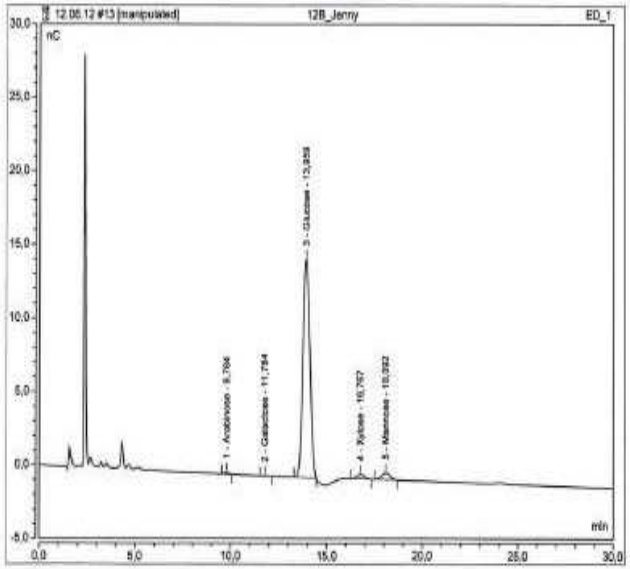
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 12.06.12

12B

Peak Integration Report

Sample Name:	12B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	500,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	13-jun-2012 / 17:23	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	9,78	Arabinose	BMB*	0,094	0,134	32,4755
2	11,78	Galactose	BMB*	0,014	0,041	17,6743
3	13,96	Glucose	BMB*	6,260	14,838	2552,8428
4	16,77	Xylose	BMB*	0,133	0,297	31,5740
5	18,09	Mannose	BMB*	0,218	0,441	86,1121
TOTAL:				6,66	15,75	2722,68



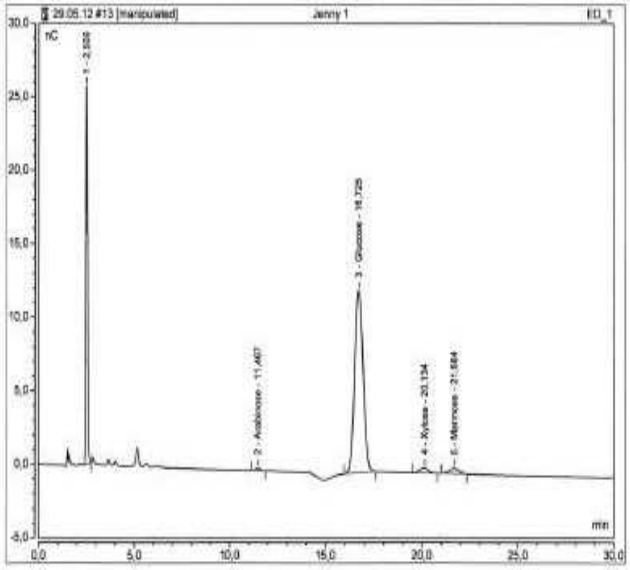
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

13A

Peak Integration Report

Sample Name:	Jenny 1	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	400,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	30-mai-2012 / 09:28	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,47	Arabinose	BMB*	0,047	0,161	34,5135
3	16,73	Glucose	BMB	8,245	12,365	2665,6407
4	20,13	Xylose	BMB*	0,178	0,330	42,9514
5	21,68	Mannose	BMB*	0,201	0,350	123,7883
TOTAL:				8,67	13,21	2866,89



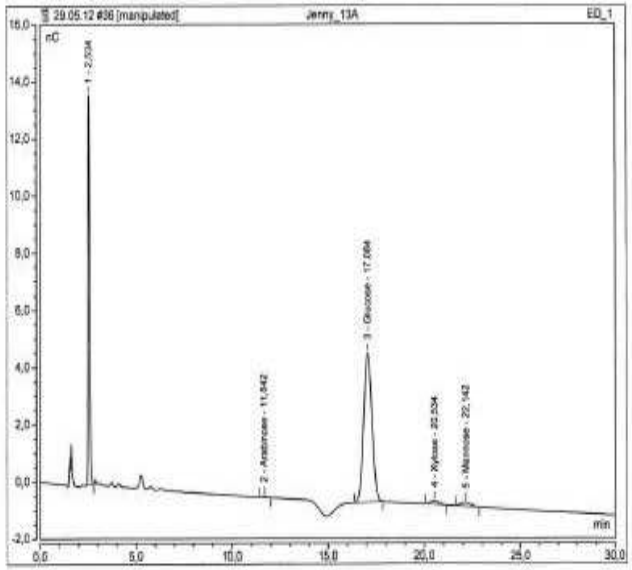
Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

13A

Peak Integration Report

Sample Name:	Jenny_13A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	31-mai-2012 / 17:24	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,64	Arabinose	BMB*	0,016	0,054	46,1126
3	17,08	Glucose	BMB	2,604	5,229	1890,8095
4	20,53	Xylose	BMB*	0,058	0,119	n.a.
5	22,14	Mannose	BMB*	0,078	0,139	121,1902
TOTAL:				2,78	5,54	2058,11



14B

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

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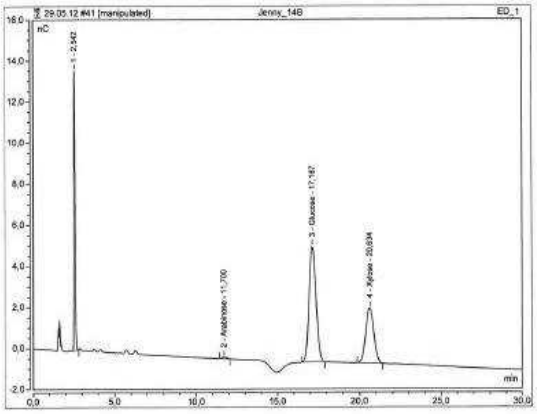
Peak Integration Report

Sample Name:	Jenny_14B	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	01-jun-2012 / 00:09	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,70	Arabinose	BMB*	0,026	0,061	58,3119
3	17,17	Glucose	BMB	2,810	5,606	2069,4005
4	20,63	Xylose	BMB	1,537	2,702	1340,6409
TOTAL:				4,37	8,41	3468,15



14B

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 12.06.12

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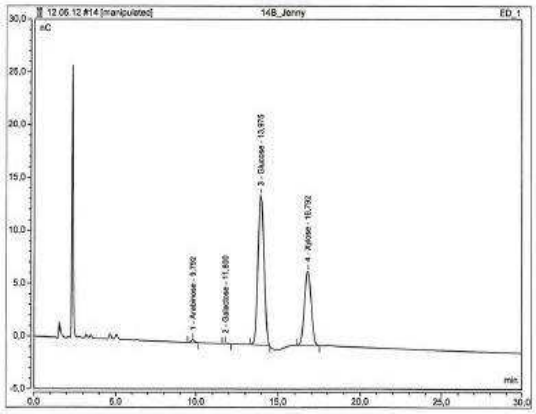
14.6.2012 9:30 AM

Peak Integration Report

Sample Name:	14B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	500,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	13-jun-2012 / 18:44	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	9,79	Arabinose	BMB*	0,071	0,267	56,3269
2	11,80	Galactose	BMB*	0,018	0,058	19,7710
3	13,98	Glucose	BMB*	6,017	14,193	2439,9664
4	16,79	Xylose	BMB	3,370	7,066	1465,5274
TOTAL:				9,48	21,58	4001,59



15A

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

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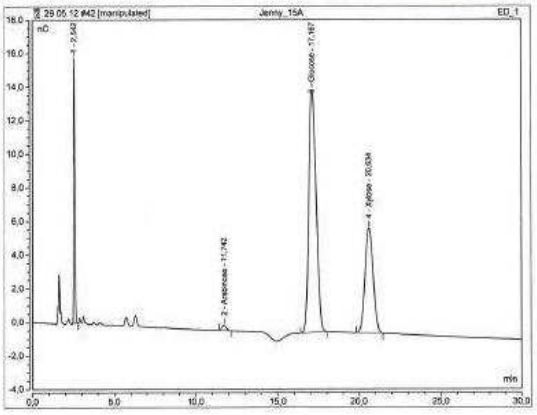
Peak Integration Report

Sample Name:	Jenny_15A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	01-jun-2012 / 01:39	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,74	Arabinose	BMB*	0,068	0,317	152,5223
3	17,17	Glucose	BMB	7,530	14,487	6578,8236
4	20,63	Xylose	BMB	3,625	6,245	3318,4296
TOTAL:				11,25	21,05	9649,78



15B

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 12.06.12

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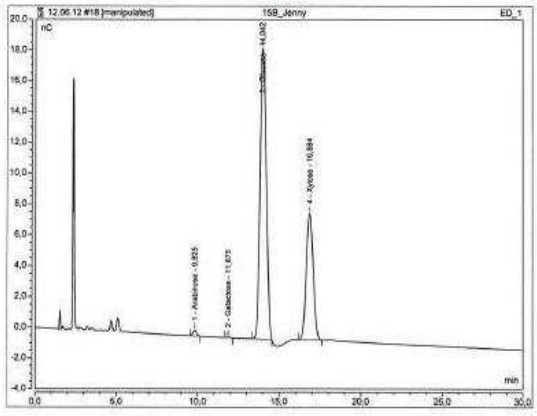
14.6.2012 11:14 AM

Peak Integration Report

Sample Name:	15B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	14-jun-2012 / 00:09	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	9,83	Arabinose	BMB*	0,109	0,382	152,9807
2	11,80	Galactose	BMB*	0,011	0,043	32,3852
3	14,04	Glucose	BMB*	8,181	18,634	6831,7448
4	16,88	Xylose	BMB	3,959	8,209	3525,2181
TOTAL:				12,25	27,57	10542,33



16A

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

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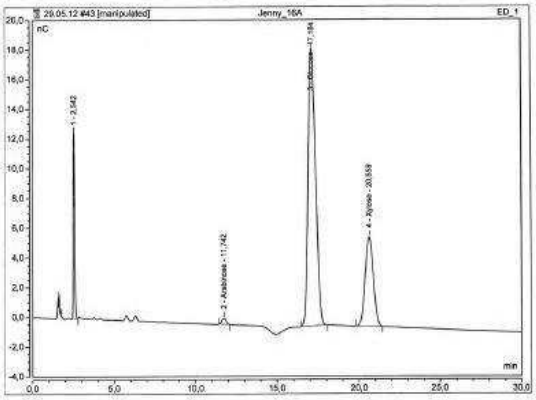
1.6.2012 1:07 PM

Peak Integration Report

Sample Name:	Jenny_16A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	01-jun-2012 / 02:51	Run Time:	79,96

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,74	Arabinose	BMB*	0,122	0,412	184,9403
3	17,18	Glucose	BMB	9,919	18,705	8734,2415
4	20,66	Xylose	BMB	3,485	6,005	3182,6486
TOTAL:				13,59	25,12	12101,83



16B

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 12.06.12

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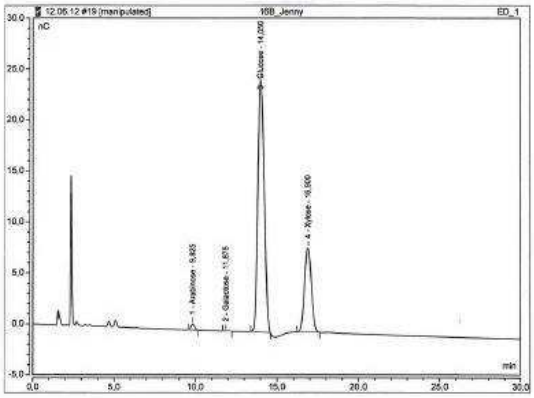
14.6.2012 11:17 AM

Peak Integration Report

Sample Name:	16B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	14-jun-2012 / 01:30	Run Time:	79,97

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	9,83	Arabinose	BMB*	0,159	0,582	224,0803
2	11,86	Galactose	BMB*	0,012	0,042	33,5792
3	14,05	Glucose	BMB*	10,947	24,753	9717,5190
4	16,80	Xylose	BMB	4,016	8,312	3578,9645
TOTAL:				15,13	33,69	13554,15



17A

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

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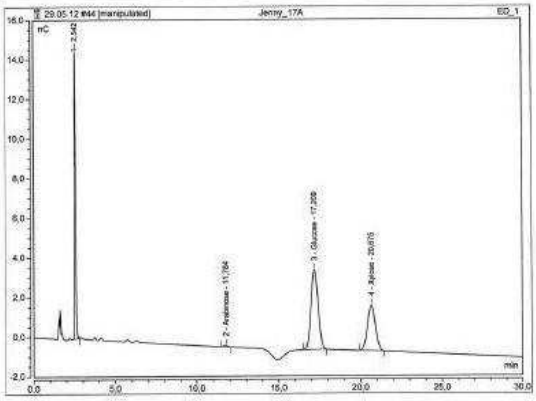
1.6.2012 1:09 PM

Peak Integration Report

Sample Name:	Jenny_17A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	01-jun-2012 / 04:13	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,78	Arabinose	BMB*	0,022	0,073	53,4464
3	17,21	Glucose	BMB	2,008	4,029	1376,4123
4	20,68	Xylose	BMB	1,303	2,302	1126,0278
TOTAL:				3,33	6,40	2554,94



17B

Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 12.06.12

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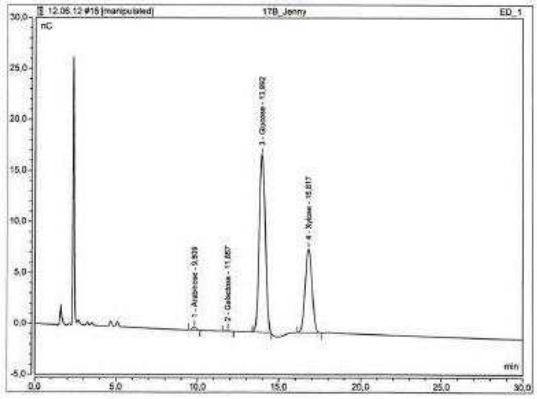
14.6.2012 8:34 AM

Peak Integration Report

Sample Name:	17B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	500,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	13-jun-2012 / 20:05	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	9,81	Arabinose	BMB*	0,067	0,321	66,0807
2	11,87	Galactose	BMB*	0,026	0,079	23,3029
3	13,99	Glucose	BMB*	7,500	17,438	3137,2627
4	16,82	Xylose	BMB*	3,967	8,232	1786,1163
TOTAL:				11,58	26,07	4992,76



Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

18A

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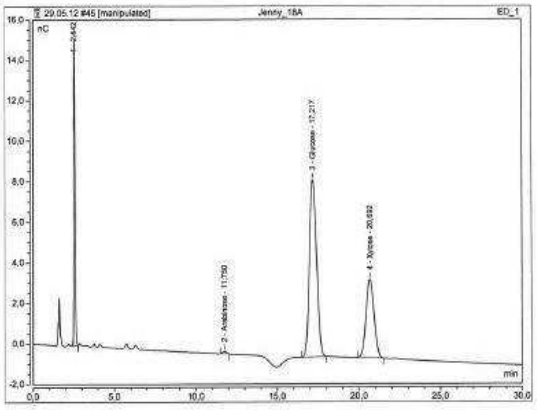
Peak Integration Report

Sample Name:	Jenny_18A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	01-jun-2012 / 05:14	Run Time:	79,97

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
2	11,75	Arabinose	BMB*	0,034	0,120	88,8768
3	17,22	Glucose	BMB	4,447	8,751	3517,9622
4	20,69	Xylose	BMB	2,220	3,873	1875,9702
TOTAL:				6,70	12,74	5502,81



Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 12.06.12

18B

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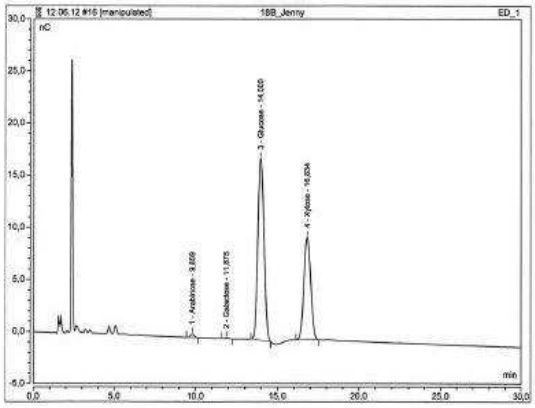
Peak Integration Report

Sample Name:	18B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	500,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	13-jun-2012 / 21:26	Run Time:	79,97

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	9,81	Arabinose	BMB*	0,091	0,334	66,9486
2	11,88	Galactose	BMB*	0,023	0,073	22,0717
3	14,00	Glucose	BMB*	7,537	17,434	3154,8499
4	16,83	Xylose	BMB	4,770	9,852	2151,0927
TOTAL:				12,42	27,67	5396,90



Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 29.05.12

19A

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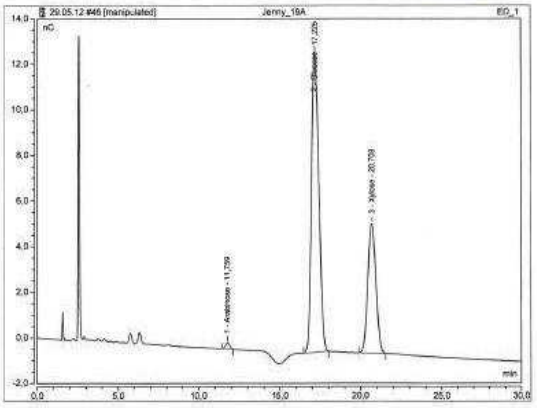
1.6.2012 1:12 PM

Peak Integration Report

Sample Name:	Jenny_19A	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	01-jun-2012 / 05:55	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	11,76	Arabinose	BMB*	0,060	0,261	129,8787
2	17,23	Glucose	BMB	6,827	13,195	5256,9247
3	20,71	Xylose	BMB	3,315	6,709	3018,0692
TOTAL:				10,22	19,16	8657,89



Logged on User: Labbetuss
Instrument: PFI-LABBA_1
Sequence: 12.06.12

19B

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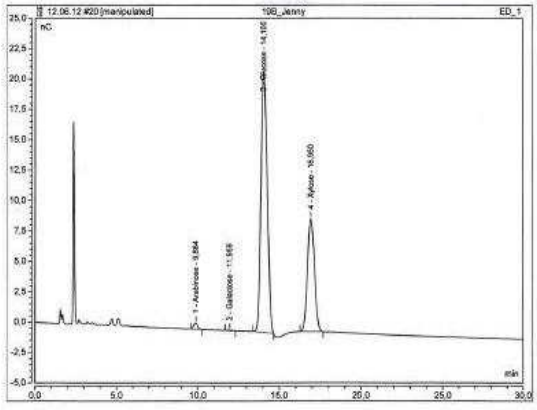
14.6.2012 11:21 AM

Peak Integration Report

Sample Name:	19B_Jenny	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1000,0000
Program:	Gradient 1,5-200mM reeqvil 30grC	Operator:	Labbetuss
Inj. Date / Time:	14-jun-2012 / 02:51	Run Time:	79,98

No.	Time min	Peak Name	Peak Type	Area nC*min	Height nC	Amount mg/l
1	9,85	Arabinose	BMB*	0,146	0,540	208,0383
2	11,96	Galactose	BMB*	0,017	0,055	38,6954
3	14,10	Glucose	BMB*	8,384	21,382	8128,4339
4	16,95	Xylose	BMB	4,827	9,296	4067,3088
TOTAL:				14,08	31,27	12442,60



13.06.2012

NTNU	Risk assessment	Utarbeidet av:	Nummer:	Dato:	
		HMS-avd.	HMSRV2603	04.02.2011	
HMS IKB		Godkjent av:	Side:	Erstatter:	

Unit: *Kjemisk prosessteknologi* **Date:** 22.01.12

Line manager: *Øyvind Gregersen*

Participants in the identification process (including their function): Stærker Moe (advisor), Jenny Kristin Håseth (student)

Signatures: Jenny Kristin Håseth

ID no.	Activity from the identification process form	Potential undesirable incident/strain	Likelihood	Consequence:				Risk value	Comments/status Suggested measures
			Likelihood (1-5)	Human (A-E)	Environment (A-E)	Economy/ material (A-E)	Reputation (A-E)	Human	
1	Handling of wood material	None	1	A	A	A	A	A1	
2	Handling of ionic liquids	Spills on person	2	B	B	A	C	B2	Little data on long-term toxicity found in MSDS.
3	Dissolution of wood in ionic liquid	Spills on person, burns	2	B	B	A	C	B2	Little data on long-term toxicity found in MSDS.
4	Precipitation of dissolved wood	Spills on person, burns	2	B	B	A	C	B2	Little data on long-term toxicity found in MSDS.
5	Filtration of precipitate	Spills on person	2	B	B	A	C	B2	Little data on long-term toxicity found in MSDS.
6	Handling of filtrate	Spills on person	2	B	B	A	C	B2	Little data on long-term toxicity found in MSDS.
7	Acid hydrolysis	Spills on person, Acid burns, burns	2	B	B	A	C	B2	
8	Enzymatic hydrolysis	Spills on person	2	B	B	A	C	B2	
9	Lignin analysis	Spills on person	2	A	A	A	A	A2	
10	Preparation for carbohydrat analysis	Spills on person	2	A	A	A	A	A2	