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CATALYTIC PRETREATMENT AND HYDROLYSIS OF FIBRE SLUDGE INTO REDUCING SUGARS
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Academic dissertation to be presented with the assent of the Doctoral Training Committee of Technology and Natural Sciences of the University of Oulu for public defence in the Campus auditorium, Kokkola University Consortium Chydenius (Talonpojankatu 2, Kokkola), on 29 November 2013, at 12 noon
Abstract

Decreasing oil reserves, the need to reduce CO₂ emissions and increasing energy demand are issues that are forcing scientists to search for new opportunities in the field of energy. As a result, biofuels have been considered as one possible solution to solve part of these challenges. This research is one small part of that effort.

For both human and economic reasons the use of edible raw materials for biofuel production is not sustainable. This study aims to convert forest industry waste, namely fibre sludge, into reducing sugars (glucose). This platform chemical can then be converted to value-added products, biofuels such as ethanol or butanol for example.

Depolymerisation of fibre sludge (cellulose) to glucose monomers was performed firstly by pretreatment with ionic liquids [BMIM]Cl and [AMIM]Cl and secondly hydrolysed by acids (dilute maleic and sulphuric acids) and enzymes. To go further with the research the two pretreatment steps, dissolution and hydrolysis were combined into a one-step reaction by using a task-specific ionic liquid [SBMIM]Cl.

With the ionic liquid [AMIM]Cl used for pretreatment in this study, we were able to recover 85% of sugars relative to the initial dry mass of the fibre sludge. Corresponding yield was about 30% without pretreatment. The task-specific ionic liquid [SBMIM]Cl was able to dissolve and hydrolyse fibre sludge in a one-step reaction. This ionic liquid was also able to dissolve wet fibre sludge with a moisture content of up to 50%. Enzymatic hydrolysis of [AMIM]Cl pretreated fibre sludge showed also very promising yields of reducing sugars.

Keywords: acid hydrolysis, enzymatic hydrolysis, fibre sludge, glucose, ionic liquid, pretreatment, reducing sugars
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Tiivistelmä

Biotaloudessa keskeisiä globaaleja haasteita ovat kasvava energiantarve, vähenevät fossiilisen öljyvarannot sekä tarve vähentää energiantuotannon ja liikenteen hiilidioksidipäästöjä, mikä on lisännyt viime vuosina aktiivisuutta biopolttoainetutkimuksen saralla. Biopolttoainet voidaan nähdä eräänä mahdollisuutena lisätä uusiutuvien luonnonvarojen käyttöä sekä edistää vähähiilistä taloutta. Uusien kestävän kehityksen periaatteita noudattavien energiantuotantoneutelmiä kehitämisessä on suosittava biomassa, jotka eivät kilpaile ruoantuotannon kanssa samoina raaka-aineista. Tässä suhteessa erityisen keskeisessä asemassa ovat mm. teollisuuden sivutuotteet, joita myös tässä työssä on tutkittu.


Asiasanat: entsymaattinen hydrolyysi, esikäsittely, glukoosi, happohydrolyysi, ioninen liuotin, pelkistävä sokeri
To my Dad
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List of original publications

This thesis is based on the following scientific publications, which are referred to in the text as roman numerals from I to IV:


Jana Holm has been the main author of all four publications. She has performed all the experiments and most of the analyses except NMR analyses and the preparation of ionic liquids. She has also written all the publications.
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1 Introduction

Abatement of CO₂ emissions, decreasing oil reserves and increasing energy prices are challenges that the fossil fuel based economy has to face today (Pachauri & Reisinger 2007). To try and solve some of these challenges biofuels have been considered as one possible solution. Today, bioethanol is produced from corn (in USA), sugar cane (in Brazil and Asia) and sugar beet (in Europe) (Balat et al. 2008). For both humane and economic reasons, the use of edible raw materials for biofuel production is not sustainable. Lignocellulosic biomass offers many potential advantages in comparison with sugary or starchy biomasses because it is very widely available and does not compete with food and feed production (Alvira et al. 2010). Lignocellulosic biomass consists of polymeric carbohydrates (cellulose and hemicellulose) and lignin (Zhang & Zhao 2010). These building blocks are organised in the plant cell wall in a way that is naturally recalcitrant to biological degradation (da Costa Sousa et al. 2009).

Nowadays, material efficiency is one of the key issues when promoting the sustainable use of natural resources, waste materials and industrial by-products. Based on the concept of material efficiency, the following categories of waste hierarchy can be recognised; (1) waste prevention, (2) waste minimisation, (3) waste reuse, (4) recycling, (5) energy recovery, and (6) final disposal (Sligo County Council 2013). In this thesis, waste materials from a pulp mill were used. Samples provided were bark sludge, bio-sludge and fibre sludge that all are secondary products and/or waste products in the forest industry. Based on our preliminary studies of these three sample fractions (Holm et al. 2009), only fibre sludge turned out to be a potential fraction due to its high cellulose content. Therefore, the work within this study was carried out with fibre sludge only. The reuse and composition of fibre sludge differs depending on the pulp mill and the chemical pulping process. Currently, fibre sludge is dried (pressed to a higher solid concentration) at the pulp mill and/or combusted with bark residues in the bark recovery boiler or disposed at landfill sites depending on the pulp mill. For disposal at landfills companies have to pay taxes of 50 €/t from the beginning of 2013 (Waste Tax Act 2010). For example, if 300 000 tonnes of fibre sludge waste is produced, the taxes are around 15 million euros. The chemical pulping process used at the pulp mill is a continuous Kraft process (sulphate process) where Nordic softwoods (pine and spruce) and hardwood (birch) are used as raw materials. The annual amount of fibre sludge waste in the Finnish pulping
industry is approximately 314 000 tonnes (corresponding to 2% of the production capacity as dry solid material) (Ojanen 2001, Pulp production in Finland 2013).

The first aim of this research was to catalytically convert cellulose-rich pulp mill waste to total reducing sugars (TRS) that could be used as platform chemicals in the production of biofuels (ethanol, butanol) or other value added chemicals, for example. The primary constituents of wood pulp are polysaccharides, i.e. cellulose and different types of hemicelluloses. Depolymerisation of these polysaccharides into reducing sugars is mainly carried out under strong acidic or alkaline conditions. Some imidazolium based ionic liquids (ILs), i.e. 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) and 1-allyl-3-methylimidazolium chloride ([AMIM]Cl) possesses the necessary properties for biomass (fibre sludge) pretreatment (Fort et al. 2007, Mosier et al. 2005, Wu et al. 2004) prior to acidic or enzymatic hydrolysis.

The secondary aim of this research was to combine the dissolution and hydrolysis steps of pretreatment by using a task-specific ionic liquid (TSIL). In this work, task-specific ionic liquid is determined as a solvent of dual function, i.e. the capability to dissolve and hydrolyse fibre sludge. 1-(4-sulfobutyl)-3-methylimidazolium chloride, [SBMIM]Cl, belongs to the latest generation of ionic liquids which can be used to combine pretreatment steps, dissolution and hydrolysis, into a single one-step process. [SBMIM]Cl is a task-specific ionic liquid that uses the Brønsted-acid function.

The main research questions were:
- Can ionic liquids promote the dissolution of fibre sludge?
- Can dissolution and hydrolysis be performed in a one-step process?
- Can wet raw material also be used?

This thesis concentrates on the pretreatment of fibre sludge with three different ionic liquids i.e. [BMIM]Cl, [AMIM]Cl and [SBMIM]Cl. Furthermore, diluted acid and enzymatic hydrolysis are also used and optimised to obtain reducing sugars (mainly glucose) that can be used as a platform chemical. This thesis does not, however, include any fermentation experiments of sugar solutions to bioethanol, biobutanol or acetone, and on ethanol and butanol mixtures.
2 Lignocellulosic Biomass

2.1 What is lignocellulosic biomass?

Lignocellulosic biomass is a plant biomass that is composed of cellulose (30–50 wt%), hemicellulose (15–35 wt%) and lignin (10–30 wt%) of dry weight (Lynd et al. 2002). The composition of lignocellulose depends on its source. The estimated global production of biomass is approximately $1.0 \times 10^{11}$ tons annually, which make it the most abundant renewable organic material on earth (Sánchez & Cardona 2008, Zhang & Zhao 2010).

The building blocks (cellulose, hemicellulose and lignin) are organised in the plant cell wall in a way that is naturally recalcitrant to biological degradation (see figure 1) (da Costa Sousa et al. 2009). This resistance can be reduced by pretreatment but hydrolysis of cellulose requires another chemical or enzymatic step following this. A pretreatment step is necessary to achieve the accessible cellulose. After the appropriate level of physical size reduction of biomass is achieved which increases the accessibility of lignocellulose, hemicellulose can then be chemically hydrolysed during a chemical pretreatment step. With recent advances in enzyme technology and low environmental impacts, enzymatic hydrolysis is becoming the preferred step for cellulose saccharification (Zhu et al. 2008). The presence of lignin in the cell wall complicates the hydrolysis since it has been implicated as an inhibitor of cellulases (Chandra et al. 2007).

Fig. 1. The structure of wood biomass (Paul de Wild 2011, fig. 1.1).
Sustainable lignocellulosic biomasses include forestry residues, agricultural residues and solid waste from pulping processes, because these do not compete with food and feed production. Cellulose is hardly soluble in conventional solvents (such as water and most common organic liquids) due to its many intramolecular hydrogen bonds (see figure 2) (Swatloski et al. 2002), and therefore new types of solvents (that dissolve cellulose), such as ionic liquids are needed.

Conventional methods for converting (dissolution and hydrolysis) lignocellulose to sugars have been acid hydrolysis or the use of high pressures and temperatures. If acid is used, the recirculation of it is necessary while high pressures and temperatures are energy consuming (Tsao et al. 1979). Ionic liquids (ILs) have raised interest as potential “green” replacements for volatile organic solvents due to their unique properties. Ionic liquids can be considered as non-aqueous (Heinze et al. 2005), non-derivatizing solvents for cellulose (Swatloski et al. 2002). The favourable characteristics of ILs make them a more interesting prospect than classical solvents. Low vapour pressure, high polarity, and high chemical and thermal stabilities are important properties of ILs. ILs with imidazolium, ammonium and pyridinium cations are known to dissolve cellulose (El Seoud et al. 2007, Liebert & Heinze 2008).

Fig. 2. The cellulose network (A is the cellulose chain and B shows inter and intra H-bonds present in cellulose) adopted from (Olivier-Bourbigou et al. 2010) (paper I).
2.2 Pretreatment of lignocellulosic biomass

Pretreatment can be considered as a very important tool for practical conversion processes of lignocellulosic biomass. Pretreatment methods are usually categorised into physical, chemical and biological or a combination of these approaches (physiochemical). However, each of these methods have their own specific drawbacks (Chandra et al. 2007). Cellulose is very difficult to dissolve due to the existence of inter and intra molecular hydrogen bonds (see figure 2) and van der Waals interactions between the cellulose fibrils (Dadi et al. 2006). Pretreatment is needed to make cellulose fibrils more accessible to acids or enzymes.

Fig. 3. Goals of pretreatment adopted from (Mosier et al. 2005) (paper I).

The aim of pretreatment should be to fractionate cellulose, hemicellulose and lignin so that cellulases can attack pure cellulose (Chandra et al. 2007). To achieve a sufficiently fast rate of hydrolysis, the lignin seal needs to be broken and the crystalline structure of cellulose needs to be disrupted (see figure 3). Ionic liquids have shown to be efficient solvents for dissolution and pretreatment of cellulose. An effective pretreatment is characterised by criteria, such as: avoiding the need to reduce the biomass particle size, preserving the pentose (hemicellulose) fractions, limit the formation of degradation products, minimise energy demand and limits costs (Mosier et al. 2005).

Cellulose solvents should have most of the features listed below (Olivier-Bourbigou et al. 2010), i.e. they have to:

1. be able to dissolve cellulose at low temperatures,
2. be non-volatile, non-toxic and chemically stable,
3. not decompose cellulose,
4. be easy to regenerate,
5. be recyclable with high efficiency,
6. be cost-effective and easily processed,
7. non-toxic to enzymatic and microbial fermentation

Ionic liquids [BMIM]Cl and [AMIM]Cl used in the pretreatment of fibre sludge fulfill most of these criteria.
3 Ionic liquids as catalytic reaction media

Ionic liquids (ILs) are organic salts that are liquids at relative low temperatures (below 100 °C). Ionic liquids consist of a large organic cation and an inorganic anion. Many ionic liquids (e.g. [BMIM]Cl, [AMIM]Cl in this study) are liquids at room temperature making them ideal solvents to work with.

Ionic liquids as efficient/good reaction media for cellulose dissolution media has been reported as early as 1934 (Graenacher 1934). Almost 70 years later it was reported that ionic liquids can be used as non-degrading solvents for cellulose (Swatloski et al. 2002). Both the cation and anion of an IL can form a complex with the hydroxyl groups of cellulose. This will lead to the disruption of the interchain hydrogen bonding network of the cellulose polymer facilitating its dissolution (Vitz et al. 2009).

Many different ionic liquids have been explored attempting to dissolve carbohydrates. It was concluded that high dissolution efficiency of cellulose may be obtained using halide-based ionic liquids (Olivier-Bourbigou et al. 2010). It is well known that the higher the anion concentration, the better the solubility becomes. Furthermore, the strong electronegativity of the chloride anion and its small size are advantages (Dadi et al. 2006). The chloride anion, being a small hydrogen-bond acceptor, is a good selection in cellulose dissolution (Swatloski et al. 2002). However, the chlorine anion is not that attractive from an environmental point-of-view.

Studies have also shown the existence of a weak interaction between the cation of the ionic liquid and cellulose (El Seoud et al. 2007). The cation interacts also in the dissolution process, i.e. its role in the dissolution mechanism should not be neglected (Dadi et al. 2006). An increase in the length of the alkyl chain on the dialkyl imidazolium cation (with chloride anion) leads to a decrease in the cellulose solubility. The introduction of an allyl group on the imidazolium cation has been shown to provide excellent improvements in the dissolving of cellulose (Dadi et al. 2006). [AMIM]Cl has a small cation because it contains only three carbon atoms in the side chain, while the strong polar of the double bond seems to be an essential part of the cation (Feng & Chen 2008). Overall, it can be said that relatively small cations are efficient in dissolving cellulose. Studies show that [AMIM]+ is more powerful in the dissolution of cellulose than [BMIM]+ due to its smaller size (Feng & Chen 2008, Olivier-Bourbigou et al. 2010).
Fig. 4. Dissolution mechanism of cellulose in [BMIM]Cl adopted from (Feng & Chen 2008).

The free chloride (Cl\(^-\)) ions associate with the cellulose hydroxyl proton and the free cations complex with the cellulose hydroxyl oxygen (see figure 4). This leads to the disruption of hydrogen bonds in cellulose and to the dissolution of cellulose (Zhang et al. 2005). Fukaya et al. (2008) also discovered the same mechanism in their studies (Fukaya et al. 2008).

A structural change of cellulose was observed when the dissolved cellulose was precipitated with water (Zavrel et al. 2009). This could potentially enhance subsequent hydrolysis. The cellulose regenerated from ionic liquids was essentially found to be amorphous and porous, which was much more prone to the degradation by cellulases (Dadi et al. 2006). The celluloses regenerated by rapid precipitation of the dissolved cellulose with an anti-solvent (water) have also demonstrated a great improvement on the kinetics of enzymatic hydrolysis (Kuo & Lee 2009).

Compared with traditional solvents, ionic liquids possess interesting properties such as their broad liquid regions, high thermal stabilities and negligible vapour pressures (Brennecke & Maginn 2001). Ionic liquids are also tunable. By substituting cations and anions physical and chemical properties of the ionic liquid (melting point, viscosity, hydrophobicity and hydrolysis stability) can be affected (Huddleston et al. 2001). Therefore, optimal ionic liquids for a special application can be designed. Simply by making changes to the structure of either the anion or the cation, or both, properties such as solubility, density, refractive index and viscosity can be adjusted to meet the requirements of the task. The tuning of properties is possible by varying the length and branching of the alkyl groups that are incorporated to the cation. There is also the potential for task-specific ionic liquids (TSILs) to be produced (e.g. [SBMIM]Cl in this study).
Today, there are several ionic liquids known to dissolve cellulose. [BMIM]Cl is one of these ionic liquids reported to be efficient in cellulose dissolution (Barthel & Heinze 2006, Swatloski et al. 2002). [BMIM]Cl has the ability to dissolve lignin and polysaccharides simultaneously (Fort et al. 2007). [AMIM]Cl is a nonderivatising solvent for cellulose (Zhang et al. 2005). Kilpeläinen et al. have observed that both [BMIM]Cl and [AMIM]Cl were able to dissolve different types of lignin samples (Kilpeläinen et al. 2007). Similar results were reported by Pu et al. (Pu et al. 2007). The dissolved and pretreated cellulose can be regenerated and separated from lignin and hemicelluloses by adding water as an antisolvent (Fort et al. 2007).

The ability of ionic liquids to dissolve cellulose depends on the nature of the native cellulose (its degree of polymerisation (DP) and crystallinity), on the operating conditions (temperature, reaction time, initial concentration of cellulose in the IL) and the presence of impurities (water). The use of a non-dried ionic liquid can affect the solubility of cellulose, so much that a severely dried ionic liquid is needed to achieve an optimal dissolution (Vitz et al. 2009). Cellulose can be solubilised in [BMIM]Cl even in the presence of 2 wt% water (Rinaldi et al. 2010). In this work, ionic liquids [BMIM]Cl and [AMIM]Cl used in the pretreatment step belongs to the ionic liquids that should be free of water. Into the task-specific ionic liquid [SBMIM]Cl there should be water added to get the ionic liquid to work properly.

Ionic liquids may contain several impurities such as water, halides and volatiles which sometimes affect the colour of the ionic liquid. Water is removed from the ionic liquid by drying it at 60–80 °C overnight in a vacuum oven. Volatile impurities originate from the synthesis process of the ionic liquid and can easily be removed by evaporation. Halides can be removed by halide exchange reactions (Wasserscheid & Welton 2008, chap. 2).

The pretreatment step in this study was mostly performed with [AMIM]Cl. The reason why [AMIM]Cl was chosen over [BMIM]Cl is that [BMIM]Cl is corrosive, extremely hygroscopic and shows some toxicity (Liebert & Heinze 2008, Zhao et al. 2009). Studies show that [AMIM]+ is more powerful in the dissolution of cellulose than [BMIM]+ due to its smaller size (Feng & Chen 2008, Olivier-Bourbigou et al. 2010).

The recyclability of ionic liquids can be achieved by evaporating the antisolvent (water). It should be pointed out that the recycled ionic liquid ([BMIM]Cl) does not need to be anhydrous to be reused, as some water is actually required as a reactant for the hydrolysis of the glycosidic bond (Rinaldi et
It has been found that the IL can be reused up to 4–5 times without affecting sugar yields (Li et al. 2009, Vancov et al. 2012). However, in the present thesis, no recyclability/reuse results are presented even though we have made preliminary recyclability experiments on [AMIM]Cl.

Beside the promising properties of ionic liquids, they also show disadvantages such as high hygroscopicity and sometimes the degradation of cellulose (Vitz et al. 2009). Recyclability of [SBMIM]Cl as a task-specific ionic liquid that uses the Brønsted-acid function is demanding due to that both the achieved sugars and the ionic liquid are highly soluble in water (Amarasekara & Wiredu 2012).
4 Materials and methods

4.1 Samples

Samples used in this research, namely bark sludge, bio-sludge and fibre sludge are provided by a pulp mill in Finland (see figure 6). Figure 5 shows the process flow sheet at the pulp mill and also from which parts of the process these three samples are taken.

The chemical pulping process used at the pulp mill is a continuous Kraft process. This process converts wood chips into pulp (cellulose). Wood chips (celluloses and lignin) are cooked with NaOH + Na₂S (white liquor) to separate lignin from the celluloses due to bond breaking on the bonds that link lignin to cellulose. In
Finland the annual amount of sludge from the pulping process is approximately 500 000 tonnes in the form of dry substance. From this 90 000 tonnes is bio-sludge, 300 000 tonnes is fibre sludge and 120 000 tonnes is deinking sludge (Ojanen 2001).

Fibre sludge (also known as primary sludge) consists of waste water from the sulphate mill. The fibre sludge is a cellulose-rich and lignin poor fraction due to the cooking process. Bark sludge is partly the bark-rich circulation water from wood processing and partly waste water from sludge processing. Bio-sludge is (living) biomass from the active sludge station, which is a part of the waste water treatment process. In this thesis, only fibre sludge is considered in detail.

The solid matter content of fibre sludge when provided from the pulp mill before the roll press was 2 wt% (see Figure 5). The fibre sludge was dried at the pulp mill with a roll press (see figure 7) to a higher solid concentration (moisture content of 40–50 wt%).

Based on preliminary characterisation and some dissolution experiments of fibre sludge, bio-sludge and bark sludge, only fibre sludge showed the potential for further study. Fibre sludge is a cellulose-rich and lignin low waste fraction due to the cooking process, and therefore, only fibre sludge is considered in this thesis (see figure 7).
4.1.1 Fractionation of fibre sludge pretreatment and hydrolysis

In the present study, the first fractionation scheme (see figure 8) involved separate steps for pretreatment. Dissolution was performed in ILs ([BMIM]Cl and [AMIM]Cl) followed by hydrolysis (acids or enzyme catalyzed). In the later part of this study these two steps were combined. This was possible by using a task-specific ionic liquid (TSIL) 1-(4-sulfonylbutyl)-3-methylimidazolium chloride [SBMIM]Cl. In this thesis, TSIL is determined as an ionic liquid that is able to both pretreat and hydrolyse the sample (fibre sludge) in a one-step reaction.
A redeeming feature of the two-step dissolution and hydrolysis reaction is that the ionic liquid can be recycled. In the one-step dissolution and hydrolysis method (see figure 9), a complete recovery for reuse of the ionic liquid is challenging since both the resulting sugars and the ionic liquid are highly soluble in water (Amarasekara & Wiredu 2012). Ionic liquids with the Bronsted-acid function possess a dual effect, i.e. they can act both as a solvent and as a catalyst. Additionally, no neutralisation and separation of the acid catalyst is required, and there is no waste in the acid, as the acid is in the solvent itself (Amarasekara & Owereh 2009).
4.2 Preparation and characterisation of ionic liquids

All ionic liquids, i.e. [BMIM]Cl, [AMIM]Cl and [SBMIM]Cl, used in this research for dissolution and/or simultaneous dissolution and hydrolysis of fibre sludge worked well. For experiments in paper II [BMIM]Cl and [AMIM]Cl were synthetised at the University of Oulu (see figure 11) whilst [AMIM]Cl and [SBMIM]Cl for experiments in papers III and IV were synthetised at Kokkola University Consortium Chydenius.

From figure 10 it can be seen how well fibre sludge dissolves in the ionic liquid and how the colour of the IL changes after the dissolution process.
Fig. 10. Dissolution of dry fibre sludge in the ionic liquid [BMIM]Cl (paper II).

[BMIM]Cl (Oulu University, used in paper II) was prepared from butyl chloride (159.5 g, 1.72 mol) and N-methylimidazole (103.0 g, 1.25 mol) in a 500 ml flask by mixing and refluxing until all methylimidazole had reacted (24–48 h). The crude product was then recrystallised from an ethyl acetate-acetonitrile mixture (55:45). The yield of white [BMIM]Cl was 174.8 g (80%). $^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 0.96 (3H, t, $J_{HH} = 7.3$ Hz), 1.41 (2H, m), 1.89 (2H, m), 4.13 (3H, s), 4.34 (2H, t, $J_{HH} = 7.3$ Hz), 7.47 (1H, t, $J_{HH} = 1.8$ Hz), 7.62 (1H, t, $J_{HH} = 1.8$ Hz), 10.67 (1H, s). MS(ESI$^+$) [m/z (rel. int. (%))]: 139 (100, [BMIM]). MS(ESI$^-$) [m/z (rel. int. (%))]: 210 (100, Cl[BMIM]Cl) (Holm et al. 2009).

Preparation of [AMIM]Cl (Oulu University, used in paper II) was carried out by placing N-methylimidazole (125.0 g, 1.53 mol) in a 500 ml round-bottom flask and cooling the reaction vessel in an ice bath. Allyl chloride (155.0 g, 2.03 mol) was added slowly to the cooled flask. The reaction was then mixed for a short period at 0 °C and allowed to warm up to room temperature. Next, the reaction mixture was gently heated up with an oil bath and refluxed for 24 h until all N-methylimidazole had reacted. The resulting crude product was washed with ethyl acetate (3 × 40 ml) and dried overnight in a high vacuum at 50 °C. The yield was 236.1 g (98%). $^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 4.09 (3H, s), 4.99 (2H, d, $J_{HH} = 6.2$ Hz), 5.44 (2H, m), 5.99 (1H, m), 7.39 (1H, t, $J_{HH} = 1.8$ Hz), 7.59 (1H, t, $J_{HH} = 1.8$ Hz), 10.72 (1H, s). MS(ESI$^+$) [m/z (rel. int. (%))]: 123 (100, [AMIM]). MS(ESI$^-$) [m/z (rel. int. (%))]: 193 (100, Cl[AMIM]Cl) (Holm et al. 2012).
Fig. 11. Ionic liquids [BMIM]Cl (left) and [AMIM]Cl (right) were both synthesised at the University of Oulu.

Synthesis of [AMIM]Cl (Kokkola University Consortium Chydenius, used in paper III) was carried out according to the procedure of Zhang et al. with some small modifications (Zhang et al. 2005). Using a ratio of 1:1.25, allylchloride was poured as droplets into 1-methylimidazole in a round-bottom flask. The solution was stirred for 18 hours at 55°C. The ionic liquid was then washed several times with ethyl acetate and cyclohexane. In order to obtain a clean ionic liquid, activated charcoal and methanol were added and stirred together for 90 minutes and then filtered through Celite® (Srivastava, Nitin et al. 2010). A clean light yellowish solution (see figure 12) was obtained which was then dried under a vacuum line. Residual water content of [AMIM]Cl was determined by a Karl Fisher Coulometer, giving a water content of <0.1%. 1H NMR spectra of [AMIM]Cl (Kokkola University Consortium Chydenius, used in article 3) was recorded with a Bruker DPX 200 spectrometer (200.13 MHz) with the following peaks: 1H NMR (200 MHz, CDCl₃) d: 4.13 (3H, s), 5.04 (2H, d, J_{HH} = 6.3 Hz), 5.45 (2H, m), 6.04 (1H, m), 7.58 (1H, t, J_{HH} = 1.8 Hz), 7.81 (1H, t, J_{HH} = 1.8 Hz), 10.39 (1H, s). The methanol residue peak from the cleaning steps is assigned to a singlet at 3.38 ppm. The corresponding peak of H₂O in CDCl₃ at around 1.56 ppm is absent (Holm et al. 2013).
The synthesis of [SBMIM]Cl (Kokkola University Consortium Chydenius, used in paper IV) was done according to Gui et al. with some small modifications (Gui et al. 2004) by the following protocol. 1,4-butane sultone (0.2 mol) was added dropwise to 1-methylimidazole (0.2 mol) whilst being stirred in a 250 mL round-bottom flask. The solution was heated to 70 °C for 1 hour and the resulting solid was then cooled down, crushed and washed several times with toluene and cyclohexane. The zwitterion obtained was dried in a vacuum oven for 12 hours (yield higher than 98%) followed by adding droplets of hydrochloric acid (37%) to the zwitterion in stoichiometric proportions. The solution was stirred and heated at 70 °C for 2 hours. The resulting mixture was washed with toluene and cyclohexane before being cleaned with activated charcoal in dry methanol to obtain a clear solution. The solvents were then evaporated with a rotary evaporator and a yellowish ionic liquid was formed in the inner layer of the pear-bottom flask. The ionic liquid was dried again in a vacuum oven for 12 hours at 70 °C. 1H NMR spectra of ILs were recorded with a Bruker DPX 200 spectrometer (200.13 MHz). Spectroscopic data of [AMIM]Cl was identical to previous literature: 1H NMR (200 MHz, CDCl₃) δ : 4.13 (3H, s), 5.04 (2H, d, J_HH = 6.3 Hz), 5.45 (2H, m), 6.04 (1H, m), 7.58 (1H, t, J_HH = 1.8 Hz), 7.81 (1H, t, J_HH = 1.8 Hz), 10.39 (1H, s). However, a singlet at 3.38 ppm corresponds to methanol residues from the cleaning steps. No peak was observed around 1.56 ppm that corresponds to the H₂O peak in CDCl₃ (Holm et al. 2013).

Spectroscopic data of [SBMIM]Cl followed values published in existing literature: 1H NMR (200 MHz, D₂O) δ : 1.72 (2H, m), 1.98 (2H, m), 2.91 (2H, t, J_HH = 7.6 Hz), 3.86 (3H, s), 4.22 (2H, t, J_HH = 7.0 Hz) 7.41 (1H, t, J_HH = 1.8 Hz), 7.47 (1H, t, J_HH = 1.8 Hz), 8.72 (1H, s).
4.3 Pretreatment of fibre sludge

Fibre sludge pretreatment was performed by adding 50 mg (100 mg) of dried grinded fibre sludge and 2.5 g (5.0 g) of ionic liquid ([BMIM]Cl, [AMIM]Cl) into a 50 ml round-bottom flask. The solution was then heated in an oil bath at 100 °C for 30 minutes and stirred at a rate of 150 rpm. 10 ml (20 ml) of hot distilled water was then added to the hot solution of fibre sludge and ionic liquid to act as an antisolvent for the regeneration of cellulose. The solution was left to set for 15 minutes during which a solid precipitate (pretreated cellulose from fibre sludge) was formed. The supernatant containing the ionic liquid was removed by filtration with a Büchner funnel and the precipitated cellulose was washed three times with distilled water.

4.4 Procedure for regeneration of fibre sludge (cellulose) from the solution

After the dissolution of fibre sludge in the ionic liquid the pretreated fibre sludge (cellulose) needs to be recovered from the ionic liquid medium. The regeneration of fibre sludge (cellulose) is achieved by adding hot distilled water to the solution of ionic liquid and dissolved pretreated fibre sludge. The antisolvent (distilled water) needs to be hot otherwise gelling of the ionic liquid can occur if the distilled water is at ambient temperature (see figure 13). If gelling of the ionic liquid does occur it is very challenging to recover the pretreated fibre sludge. Furthermore, the recovery of the ionic liquid for reuse will become more difficult. Regeneration of cellulose from [BMIM]Cl was difficult since cellulose and [BMIM]Cl formed a fused gel-like substance (Bose et al. 2012).

Compared with the initial cellulose, the regenerated cellulose has the same degree of polymerisation but its macro and micro structure (especially the degree of crystallinity) can be manipulated (Zhu 2008). The cellulose regenerated from ILs was found essentially as amorphous and porous, and was much more responsive to degradation by cellulases (Dadi et al. 2006, Zhao et al. 2009).
4.5 Task-specific ionic liquid [SBMIM]Cl

Combination of a two-step reaction to a one-step reaction was one aim of this thesis. To modify the pretreatment step with the hydrolysis step of fibre sludge was achieved by using a task-specific ionic liquid (TSIL). 1-(4-sulfobutyl)-3-methylimidazolium chloride, [SBMIM]Cl, belongs to the latest generation of ionic liquids which are used to combine pretreatment steps, dissolution and hydrolysis, into a one-step process. [SBMIM]Cl is a task-specific ionic liquid that uses the Brønsted-acid function in which a chloride anion participates in the pretreatment step (dissolution) (Remsing et al. 2006) whilst –SO₃H (sulfonic acid group) enables the hydrolysis or depolymerisation of cellulose.
From the image in figure 14, it can clearly be seen that the task-specific ionic liquid, [SBMIM]Cl, had a very high viscosity. Therefore, it was impossible to dissolve fibre sludge into [SBMIM]Cl without adding a mass fraction of 15% distilled water of the initial ionic liquid mass (2.5 g) to the TSIL. After adding the water, the viscosity of [SBMIM]Cl decreased. The distilled water was added in order to dissociate the sulfonic acid function.

Acid in an ionic liquid as a system for hydrolysis of lignocellulose has previously been studied by Li et al. Their results showed maleic acid as an organic acid which effectively accelerates the hydrolysis reaction in [BMIM]Cl (Li et al. 2008).

4.6 Hydrolysis of lignocellulosic samples

Three major hydrolysis processes are used for the depolymerisation of lignocellulosic materials, namely dilute acid hydrolysis, concentrated acid hydrolysis and enzymatic hydrolysis. Usually mineral acids or enzymes are used to catalyse the hydrolysis of cellulose to glucose and other platform chemicals. Enzymatic hydrolysis is preferred over mineral acid hydrolysis for the reasons that acid hydrolysis typically leads to the formation of undesirable degradation products of glucose. Degradation products such as furfural from pentoses and 5-HMF from hexoses decrease glucose yields and inhibit subsequent fermentation. In the presence of water, 5-HMF produces levulinic acid and formic acid (Mosier et al. 2005). Acids are also corrosive and need to be re-circulated.
4.6.1 Acid hydrolysis

Diluted sulphuric acid has been primarily used for pretreatment of lignocellulosic materials before enzymatic hydrolysis of cellulose. The traditionally preferred diluted acid (0.75 – 5%), used for hydrolysis, requires high temperatures (160 – 200 °C) and pressures (around 1 MPa). However, the hydrolysis rate is still slow and sugar degradation is high (Li et al. 2008, Sánchez & Cardona 2008). Sulphuric acid degrades glucose achieved in the hydrolysis process (Mosier et al. 2005) whilst acid hydrolysis of cellulose is predominantly limited to some mineral acids.

Organic acids (fumaric acid and maleic acid) are becoming popular alternatives to enhance cellulose hydrolysis for ethanol production. These organic acids can be used for pretreatment with high efficiency. Maleic acid has been shown to be more effective than fumaric acid (Alvira et al. 2010). In the present study maleic acid was used to hydrolyse fibre sludge. It caught our attention because fibre sludge is a lignin poor raw material, and the main objective of acid pretreatment is to dissolve the hemicellulosic fraction of the biomass and to make the cellulose more accessible to enzymes (Alvira et al. 2010).

Acid hydrolysis in this study was carried out with sulphuric acid and maleic acid at different concentrations. Based on preliminary experiments optimal acid concentrations for fibre sludge hydrolysis were 2–3 M. Acid hydrolysis was also tested with different reaction times (30 min-180 min) and different temperatures (23–100 °C). Optimal hydrolysis conditions were shown to be 90 minutes and 100 °C.

4.6.2 Enzymatic hydrolysis

Enzymes are bond specific since a combination of selective enzymes is required to ensure a maximum yield of total reducing sugars during the enzymatic hydrolysis. Enzymes that hydrolyse cellulose are called cellulases.

Cellulases include a large number of endo- and exoglucanases which hydrolyse -1,4-glucosidic bonds of the cellulose chains. Exoglucanases remove cellobiose from the chain ends of cellulose molecules whereas endoglucanases cuts in the middle of the cellulose chain causing a decrease in the degree of polymerisation of cellulose. β-glucosidase hydrolyses cellobiose and oligosaccharides to glucose (see figure 15). Operating conditions such as temperature, pH and reaction time affects the enzyme performance while the
enzyme dosage depends on the specific biomass feedstock composition (i.e. cellulose, hemicellulose and lignin).

In this study two different enzyme mixtures were used, namely an ACCELLERASE® 1500 enzyme complex and a mixture of a cellulose complex + β-glucosidase provided by Novozymes. ACCELLERASE® 1500 is a mixture used to catalyse the breakdown of lignocellulosic material, mainly by exo- and endoglucanase, hemi-cellulase and β-glucosidase. The cellulose complex provided by Novozymes catalyses the breakdown of cellulosic material into glucose, cellobiose and higher glucose polymers. The main reaction products are cellobiose and glucose. To maximise the TRS yield (synergism) β-glucosidase (known as cellobiase) was also added as this hydrolyses cellobiose to glucose. ACCELLERASE® 1500 enzyme complex had its best operational stability at temperatures between 50–65 °C and for a pH between 4.0–5.0. For the Novozymes cellulose complex and β-glucosidase, their optimal hydrolysis temperatures and pHs were 45–50 °C for pH 5.0–5.5 and 45–70 °C for pH 2.5–6.5 respectively.

Cellulases (enzymes that hydolyse cellulose) have a modular structure consisting of a catalytic domain (CD), carbohydrate-binding module (CBM) and an interdomain linker peptide. The carbohydrate-binding module identifies the substrate and binds on it, the catalytic domain is the active site and the linker coordinates the action of these two (Beckham et al. 2011) (see figure 16).
Figure 16 shows the steps that exoglucanase (celllobiohydrolase CBH) undergoes when hydrolysing cellulose: (a) binding to cellulose through a carbohydrate-binding module, (b) surface diffusion to find a free, reducing chain end, (c) threading the cellulose chain into the tunnel, (d) formation of the active complex, (e) hydrolysis of cellulose to cellobiose and (f) product expulsion (Beckham et al. 2011).

Endoglucanases are commonly characterised by a cleft, into which any part of a linear cellulose chain can fit. The exoglucanases bear tunnel-like active sites, which can only accept a substrate chain via its end (see figure 17).
An endo-acting enzyme can produce new chain ends in the internal part of the cellulose chain. Two newly exposed chains would then be available for exo-acting enzymes, in which two different types of exoglucanases (cellobiohydrolases) can then act on the cellulose chain from opposite ends (i.e., reducing versus the non-reducing end).

As cellulose is hydrolysed, the lignin and hemicellulose that accumulate in the hydrolysis residue can potentially restrict access to cellulases and decrease the hydrolysis rate (Chandra et al. 2007). Therefore pretreatment should aim to produce a readily hydrolysable substrate by increasing the accessibility to cellulases and limiting the negative effects of hemicellulose and lignin on hydrolysis, while maximising the total carbohydrate recovery. IL residues from the regenerated cellulose should be carefully removed because their presence has harmful effects on cellulose activity depending on the amount of IL remaining (Zhao et al. 2009). Hemicellulose degradation products such as furfural and hydroxymethyl furfural inhibit subsequent fermentation (Chandra et al. 2007).

Substrate-related factors that limit enzymatic hydrolysis are crystallinity, degree of polymerisation, available surface area, lignin barrier, hemicellulose content, feedstock particle size and porosity.

### 4.7 Analysis of total reducing sugars and glucose

Total reducing sugars (TRS) which can form some aldehyde or ketone (including disaccharides and monosaccharides) can be measured by the dinitrosalicylic acid method (DNS method). The amount of total reducing sugars (TRS) was measured according to the DNS method by using 1% dinitrosalicylic acid reagent (DNS) (Miller 1959). 3,5-Dinitrosalicylic acid (DNS) reacts with the reducing sugars and other reducing molecules to form 3-amino-5-nitrosalicylic acid, which absorb light strongly at the wavelength of 540 nm.

Yield of total reducing sugars (TRS%) from fibre sludge was calculated as follows:

$$\text{Total reducing sugar yield (TRS\%)} = \frac{\text{Reducing sugars weight}}{\text{Fibre sludge weight}} \times 100\%$$

In the case of pretreated fibre sludge 300 µl of the solution to be analysed was added to 300 µl of DNS reagent and boiled for 5 minutes precisely. Afterwards, the solution was cooled down under tap water to quench the oxidation reaction and 2.4 ml of distilled water was added. Analysis of the solution was then
performed with a Shimadzu UV-Spectrophotometer UV-1800 at a wavelength of 540 nm. The concentration of reducing sugars was determined according to the standardisation performed on glucose.

The DNS method is not quantitative enough and the accuracy of the measurements is estimated to be ± 5% for TRS analysis. However, in this study the DNS method was used to show the reducing sugar levels during different pretreatment steps and the results are comparable.

Glucose (GO) assay kit (Sigma) is an enzymatic analysing method for glucose concentration. Enzymatic methods are specific, sensitive and rapid. In this method D-glucose is oxidised to D-gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen in the presence of peroxide reacts with reduced o-dianisidine (colourless) to form brown oxidised o-dianisidene. Oxidised o-dianisidene reacts further with sulphuric acid to form a more stable coloured product namely pink oxidised o-dianisidine. This compound can be detected by UV-VIS at 540 nm and its concentration is proportional to the original glucose concentration (Sigma-Aldrich 2005). Shimadzu UV-Spectrophotometer UV-1800 was used in this study to detect the colour differences in solutions.
5 Results and discussion

The crystallinity of cellulose, protection of cellulose by lignin, accessible surface area, the heterogeneous character of biomass particles and cellulose sheathing by hemicellulose all contribute to the recalcitrance of lignocellulosic biomass to hydrolysis (Mosier et al. 2005, Zhu et al. 2009). Cellulose can be hydrolysed into glucose or other platform chemicals either chemically by acids or enzymatically by cellulases. Hydrolysis includes the conversion of carbohydrate polymers to monomeric sugars.

Of the three samples provided by the pulp mill, namely bark sludge, bio-sludge and fibre sludge, only fibre sludge dissolved in [BMIM]Cl (Holm et al. 2009). That is why this cellulose-rich waste fraction was chosen for further studies. It is possible to get pure fibre sludge or then a mixture of fibre and bark sludge from the pulp mill.

5.1 Fibre sludge characterisation

There is a strong agreement that lignin removal plays an important role in enhancing biomass digestibility (Draude et al. 2001). Fibre sludge is considered to be a delignified residue after the continuous Kraft process (sulphate pulping process) where wood chips are converted into pulp, namely cellulose. Nordic soft woods (pine and spruce) and hardwood (birch) are used as raw materials in the pulping process. Some of the hemicelluloses are dissolved out during pulping. The fibre length is relatively short and susceptible to hydrolysis because of its high surface area (Mora & Banerjee 2012).

Currently in Finland fibre sludge is mostly used for energy production and/or disposed at landfill sites depending on the pulp mill. Fibre sludge has a high cellulose content (appr. 80% of dry-solid organic material) and also from the material efficiency point-of-view it seems to be too valuable a material to be used for energy production or disposed at landfills.

Organic matter content in fibre sludge was determined to be approximately 80%, of which α-cellulose was 80 wt%, β-cellulose 13 wt% and γ-cellulose 7 wt%. Of these, α-cellulose indicates the presence of a undegraded, higher molecular weight cellulose in pulp, β-cellulose indicates the degraded cellulose and γ-cellulose consists mainly of hemicellulose (Tappi Standard T 203 cm-09 - Alpha-, Beta- and Gamma-Cellulose in Pulp 2009). The chemical composition of fibre sludge changes depending on which wood species and cooking
circumstances are used. It is important to perform the characterisation of fibre sludge before pretreatment (dissolution).

Prior to ionic liquid pretreatment, dried fibre sludge was ball milled for 75 minutes with 55 bigger balls (Ø 200 mm) and 30 smaller balls (Ø 90 mm). Unfortunately, significant size reduction requires energy.

5.2 Effect of ionic liquid pretreatment

The yield of precipitated fibre sludge, mostly cellulose, after the ionic liquid pretreatment with [BMIM]Cl and [AMIM]Cl (both synthesised at Oulu University) was approximately 50% (calculated from mass balance) of the dry fibre sludge. The precipitated fibre sludge yields improved remarkably when using [AMIM]Cl synthesised at Kokkola University Consortium Chydenius. With this new [AMIM]Cl fibre sludge recovery yields were 85%. The degree of recovery for the pretreated reference sample (Whatman filter paper) was over 95 wt%. This indicates that we have been able to make a good working ionic liquid. The ionic liquids were developed from different modified protocols as previously described in Section 4.2. The impurities (e.g. methanol residues from the cleaning step) in the [AMIM]Cl (see Section 4.2.) might have enhanced the recovery process of the fibre sludge, and this could explain why the recovery yields are remarkably higher. According to literature, the presence of residual methanol in ionic liquids has an influence on the precipitation of dissolved cellulose (López-Pastor et al. 2006).

Based on our unpublished data [AMIM]Cl, synthetised at Kokkola University Consortium Chydenius, was also able to totally dissolve wet fibre sludge (moisture content around 50%) within 10 minutes. The yield of the precipitated wet fibre sludge after ionic liquid pretreatment was 33%, which is remarkably lower than recovery yields (85%) for dry fibre sludge. The data clearly shows that water in the dissolution process affects the dissolution ability. The use of a non-dried ionic liquid can affect the solubility of cellulose, so much that a severely dried ionic liquid is needed to achieve an optimal dissolution (Vitz et al. 2009). Cellulose can be solubilised in [BMIM]Cl even in the presence of 2 wt% water (Rinaldi et al. 2010).

SEM (Scanning Electron Microscope) images of dried fibre sludge and [AMIM]Cl pretreated fibre sludge (see figure 18) clearly shows the effect of the ionic liquid pretreatment. The structure of fibre sludge has been interrupted, which allows a more effective hydrolysis.
5.3 Acid hydrolysis of fibre sludge

Pretreated, regenerated cellulose samples were hydrolysed in sulphuric acid or maleic acid at different acid concentrations (2–3M). Results of acid hydrolysis are presented in Figure 19. Hydrolysis with 3 M maleic acid provided the best TRS yield of 1.4%, with a hydrolysis period of 90 minutes at 100°C, compared to 3 M sulphuric acid which resulted in a TRS yield of 7.4%. There are no significant concentration dependencies using 2 M or 3 M maleic acid and sulphuric acid respectively as shown in figure 19. On the other hand, the use of sulphuric acid results in much higher yields compared with maleic acid. Hemicellulose is readily hydrolysed by dilute acids under moderate conditions. The addition of sulphuric acid has been initially applied to remove hemicellulose (Mosier et al. 2005). Yields in this study for acid hydrolysis were not that high, however the hydrolysis conditions used were not that extreme either (temperature 100°C and time 90 minutes).
5.4 Enzyme-catalysed hydrolysis of fibre sludge

5.4.1 Enzymatic hydrolysis of fibre sludge with ACCELLERASE® 1500 enzyme mixture

ACCELLERASE® 1500 enzyme complex has its best operational stability at temperatures between 50–65 °C and for a pH range 4.0–5.0. In this study pH was adjusted to 5.0 and temperature was set at 50°C.

Enzymatic hydrolysis with ACCELLERASE® 1500 gave a TRS yield of over 30% in 90 minutes at 50°C and a maximum yield of TRS 34.4% after 120 minutes (see figure 20). Based on these results, enzymatic hydrolysis provided higher TRS yields than acid hydrolysis in milder conditions, and so is the most suitable hydrolysis method for fibre sludge samples.
As can be seen in Figure 21, the use of ionic liquid pretreatment gave significantly better results. Compared to the results of enzymatic hydrolysis without any IL pretreatment, a 50% increase in the relative amount of measured sugars was obtained. There were no significant differences between the use of [BMIM]Cl and [AMIM] Cl ILs for the pretreatment of fibre sludge.
5.4.2 Enzymatic hydrolysis of fibre sludge with a Novozymes enzyme mixture

It is known that highly crystalline cellulose is less accessible to cellulose attack than amorphous cellulose. A common method to reduce crystallinity is ball milling which also decreases the particle size and increases the surface area (Alvira et al. 2010, Zhu et al. 2009).

The heterogeneous nature of biomass and the multiplicity of enzymes make it difficult to fully understand the interactions of enzymes and substrates (Zhu et al. 2009).

In this study, enzymatic hydrolysis was performed to [AMIM]Cl pretreated fibre sludge by a mixture of two enzyme types: cellulase complex NS22086 and β-glucosidase NS22118. Both of these two enzymes were provided by Novozymes, Denmark.

For the Novozymes cellulase complex the optimal hydrolysis temperature is 45–50 °C with a pH of 5.0–5.5, compared to β-glucosidase with a temperature of 45–70 °C and a pH of 2.5–6.5. The optimum operating temperature for cellulose degrading enzymes has been proven to be 50°C and therefore, enzymatic hydrolysis is performed at this temperature.

Different pH conditions (5.0, 5.2 and 5.5) were tested in this study. pH slightly affected the yield of TRS during enzymatic hydrolysis. However, this effect was only small in the pH range of 5.0 – 5.5 after 180 minutes of hydrolysis. The maximum TRS yield was achieved in a shorter hydrolysis time (90 min) when the pH was adjusted to 5.2 compared to 5.0. However after 180 min (pH 5) the TRS yields were almost identical. The highest TRS yield was achieved with a pH of 5.5 but in that case the enzyme mix loading was 50 µl instead of 25 µl.

Different enzyme loadings for both the cellulases and β-glucosidase relationships were also tested (cellulose loadings 10–50 µl and β-glucosidase loadings 4–10 µl).

Relative yields of the total reducing sugars and glucose out of TRS after enzymatic hydrolysis are presented in Figure 22. In this experiment the pH was adjusted to 5.5. Relative yields (%) were calculated by comparing the yield of sugars/glucose to the initial mass of fibre sludge.
Fig. 22. Relative yields of total reducing sugars and glucose during enzymatic hydrolysis (enzyme loadings: 50 µl enzyme mix and 6 µl β-glucosidase, pH 5.5) for 50 mg of [AMIM]Cl pretreated fibre sludge (FS) (paper III).

Compared to enzymatic hydrolysis performed with the previous enzyme mixture whose TRS yield after 90 minutes was around 30% and 35% after 120 min, the present enzyme mixture gave TRS yields of around 60% and 65% with the same hydrolysis times. TRS yields after a hydrolysis time of 240 minutes were around 90% (figure 22). Based on the analysis, two-third (66%) of these sugars were glucose which greatly suggests that Novozymes enzymes could be an efficient hydrolysis of pretreated fibre sludge. It should be noted that the focus of this study is the use of glucose yields as a significant platform chemical for biofuel production, and therefore, glucose specific enzymes would be favoured.

After approximately 180 minutes of hydrolysis, glucose yield of TRS was around 75%. However, when the TRS yield increased further the glucose yield was not able to follow and dropped to 68% of the achieved TRS% (see Figure 22). This drop could be caused due to a lack of β-glucosidase that cuts cellobiose into glucose or the formation of other sugar monomers by hemicellulose hydrolysis.
Different enzyme loadings were also tested. At pH 5.0 with enzyme loadings of 50 µl enzyme mix and 4 µl \( \beta \)-glucosidase (see figure 23), the yields for TRS and glucose were lower than with enzyme loadings of 50 µl enzyme mix and 6 µl \( \beta \)-glucosidase at pH 5.5.

Novozymes enzymes have showed very promising results in the enzymatic hydrolysis of dried fibre sludge. The yield of total reducing sugars (glucose) was significantly higher compared with the same pretreatment followed by acid hydrolysis (see figure 25).

### 5.5 One-step dissolution and hydrolysis of fibre sludge in [SBMIM]Cl

Fibre sludge contains water and it can be pressed in the process to obtain a moisture mass fraction of 50–60%. It is very critical, that this sludge can be used and depolymerised without any further drying which makes the process very cost-efficient.

It is energy consuming to dry fibre sludge and therefore this prevents the use of ILs on a larger scale, which leads to the fact that a more appropriate ionic liquid, non-sensitive to water should be selected. The aim with the task-specific ionic liquid was to combine the pretreatment steps together; dissolution with the hydrolysis step.
Wet fibre sludge (FSB in Figure 24) in [SBMIM]Cl dissolves very rapidly within 30 seconds. The dissolution of wet fibre sludge was much more rapid than for dry fibre sludge. The only difference between the fibre sludges was that FSB included water, and the water appeared to increase the dissolution rate. This is an excellent result because it is energy consuming to dry fibre sludge prior to the one-step pretreatment/hydrolysis reaction.

Contrary to [AMIM]Cl and [BMIM]Cl, this task-specific ionic liquid 1-(4-sulfobutyl)-3-methylimidazolium chloride [SBMIM]Cl, was able to dissolve and hydrolyse fibre sludge into reducing sugars in a single step. Based on the results, the yields of total reducing sugars are comparable to acid hydrolysis (maleic and sulphuric acid) (see figure 25). Wet fibre sludge (FSB) directly from the roll press at the pulp mill did not show equally high TRS yields as dried fibre sludge did when combined with distilled water (FSA) (see figure 24). This is probably due to some inhibitors from the pulping process.

![Fig. 24. TRS yields for dry respective wet fibre sludge pretreated and hydrolysed with [SBMIM]Cl with 15% water addition to the TSIL. FSA is FS made wet by distilled water and FSB is wet FS directly from the pulp mill (moisture ~ 50%).](image)

Optimisation is required to achieve TRS yields higher than a mass fraction of 4–5% using [SBMIM]Cl for dissolution and hydrolysis of fibre sludge in a one-step reaction. Furthermore, an important finding from the study is that [SBMIM]Cl also has the ability to dissolve and depolymerise wet fibre sludge (up to a moisture mass fraction of 50%) straight from the pulp mill. This is a significant advantage when fibre sludge is used as a raw material.
5.6 Summary of TRS yields by different hydrolysis methods

Figure 25 shows the yields of total reducing sugars (TRS, %) after different pretreatment / hydrolysis methods (acid hydrolysis, TSIL pretreatment and hydrolysis, and combined IL pretreatment and enzymatic hydrolysis). Yields of TRS after a combined dissolution and hydrolysis step over [SBMIM]Cl were not as high (a mass fraction of 4% of the initial dry fibre sludge) compared with theoretical yields. However, compared to the yields of reducing sugars after acid hydrolysis, the yield showed us that this task-specific IL works equally well in a one-step dissolution-hydrolysis reaction. Correspondingly, the yields after acid hydrolysis (30–180 minutes) were around the mass fractions of 1% (for 2–3mol L\(^{-1}\) maleic acid) and 8% (for 2–3mol L\(^{-1}\) sulphuric acid) without any IL pretreatment.

Higher yields and better selectivities were observed in combined IL pretreatment and enzymatic hydrolysis. At best, TRS yields with mass fraction of over 80% of the initial dry fibre sludge were observed.

![Fig. 25. Yields of total reducing sugars (TRS, %) after different pretreatment / hydrolysis methods. Dissolution and hydrolysis was performed in acidic media in the presence / absence of ionic liquids (paper IV).](image)

5.7 Improvements suggestions for pretreatment with ILs and hydrolysis with enzymes

The chlorine ion in ILs is corrosive and it could then be questioned if it is wise to scale-up and use ionic liquid pretreatment on a larger scale. The chlorine ion in
ILs should be substituted, even if the solubility of cellulose in chloride based ILs is high (Bose et al. 2012). Preliminary experiments with a chloride free ionic liquid, glycercylimidatzolium hydroxide [GLYMIM]OH, have already been made and with some promising results. If the total reducing sugar yields with chlorine-free ionic liquids are high then it would be a more green and sustainable choice to use halide-free ionic liquids for the pretreatment step of fibre sludge.

To carry this research further pretreatment with a chlorine-free ionic liquid should be combined with the enzymatic hydrolysis step. Pretreatment with ILs and enzymatic hydrolysis step should be combined (Bose et al. 2012).

ILs should also be recyclable. In this study the focus was not on the recyclability of used ionic liquids. The [BMIM]Cl used for dissolution of pulp can be recovered from ethanol using vacuum distillation and subsequent drying. The performance of the recovered [BMIM]Cl could be maintained at its original level (Ichiura et al. 2011). According to our unpublished data, [AMIM]Cl did show reusability possibilities.
6 Conclusions

Sustainable energy sources for the future are lignocellulosic materials which include wood, grass, forestry waste, agricultural residues and municipal solid waste. The implementation of a pretreatment step is necessary to release the components of lignocellulosic materials for use. The effectiveness of lignocelluloses pretreatment is one of the key factors to a successful conversion of the originally low-cost material into a sugar platform and from there, into biofuels or a biofuel intermediate.

The dissolution of cellulose using ionic liquids has provided a platform for the utilisation of cellulose in addition to providing a comprehensive application of lignocellulosic materials in a green way. The excellent chemical and physical properties of task-specific ionic liquids make them effective and rapid cellulose solvents.

The main conclusions of this thesis are that ionic liquids 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-allyl-3-methylimidazolium chloride ([AMIM]Cl) and the task-specific ionic liquid 1-(4-sulfobutyl)-3-methylimidazolium chloride, [SBMIM]Cl, possess the necessary properties for fibre sludge pretreatment. [BMIM]Cl and [AMIM]Cl are able to make structural changes in the fibre sludge to improve the hydrolysis rate and yields of total reducing sugars. With the ionic liquid [AMIM]Cl used for pretreatment in this study, we were able to recover 85% of the initial dry fibre sludge.

The task-specific ionic liquid [SBMIM]Cl was able to dissolve and hydrolyse fibre sludge in a one-step reaction. The dissolution was very fast and can be considered as an advantage for [SBMIM]Cl. This ionic liquid was also able to dissolve wet fibre sludge (moisture up to 50%) straight from the roll-press from a pulp mill. Although the hydrolysis yields of total reducing sugars with the TSIL wasn’t that high (4–5%) its capability to dissolve and polymerise wet fibre sludge is a huge advantage.

TRS yields both in acid and enzymatic hydrolysis were higher compared to the relative TRS yield of untreated fibre sludge samples. It was also shown that there was no significant difference in using [BMIM]Cl or [AMIM]Cl in the pretreatment step of fibre sludge. In both cases ionic liquids made the cellulose structure more accessible to acid or enzymatic degradation. However, enzymatic hydrolysis was much more efficient than acid hydrolysis when fibre sludge was used as a feedstock for the conversion of cellulose to reducing sugars. Most of these sugars in the final solution were supposed to be glucoses.
Enzymatic hydrolysis over Novozymes enzymes showed very promising results. The outcome of the pretreated dry fibre sludge was over 95% of the initial mass of fibre sludge, which indicates that we have been able to make a good working ionic liquid in [AMIM]Cl and that the Novozymes enzymes are suitable to fibre sludge hydrolysis.
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Original papers


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