A study of ionic liquids for dissolution of sugarcane bagasse

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Sugarcane bagasse, phosphonium ionic liquids, ATR-FTIR, pretreatment, delignification, acid in ionic liquid, acid catalysed delignification

Abstract

Over the last decade, Ionic Liquids (ILs) have been used for the dissolution and derivatization of isolated cellulose. This ability of ILs is now sought for their application in the selective dissolution of cellulose from lignocellulosic biomass, for the manufacture of cellulosic ethanol. However, there are significant knowledge gaps in the understanding of the chemistry of the interaction of biomass and ILs. While imidazolium ILs have been used successfully to dissolve both isolated crystalline cellulose and components of lignocellulosic biomass, phosphonium ILs have not been sufficiently explored for the use in dissolution of lignocellulosic biomass. This thesis reports on the study of the chemistry of sugarcane bagasse with phosphonium ILs.

Qualitative and quantitative measurements of biomass components dissolved in the phosphonium ionic liquids (ILs), trihexyltetradecylphosphonium chloride ([P66614]Cl) and tributylmethylphosphonium methylsulphate ([P4441]$MeSO_4$) are obtained using attenuated total reflectance-Fourier Transform Infra Red (FTIR). Absorption bands related to cellulose, hemicelluloses and lignin dissolution monitored *in situ* in biomass-IL mixtures indicate lignin dissolution in both ILs and some holocellulose dissolution in the hydrophilic [P4441]$MeSO_4$. The kinetics of lignin dissolution reported here indicate that while dissolution in the hydrophobic IL [P66614]Cl appears to follow an accepted mechanism of acid catalysed β-aryl ether cleavage, dissolution in the hydrophilic IL [P4441]$MeSO_4$ does not appear to follow this mechanism and may not be followed by condensation reactions (initiated by reactive ketones). The quantitative measurement of lignin dissolution in phosphonium ILs based on absorbance at 1510 cm^{-1} has demonstrated utility and greater precision than the conventional Klason lignin method.

The cleavage of lignin β-aryl ether bonds in sugarcane bagasse by the ionic liquid [P66614]Cl, in the presence of catalytic amounts of mineral acid. (*ca*. 0.4 %). The delignification process of bagasse is studied over a range of temperatures (120 °C to 150 °C) by monitoring the production of β-ketones (indicative of cleavage of β-aryl ethers) using FTIR spectroscopy and by compositional analysis of the undissolved fractions. Maximum

delignification is obtained at 150 °C, with 52 % of lignin removed from the original lignin content of bagasse. No delignification is observed in the absence of acid which suggests that the reaction is acid catalysed with the IL solubilising the lignin fragments. The rate of delignification was significantly higher at 150 °C, suggesting that crossing the glass transition temperature of lignin effects greater freedom of rotation about the propanoid carbon-carbon bonds and leads to increased cleavage of β-aryl ethers. An attempt has been made to propose a probable mechanism of delignifcation of bagasse with the phosphonuim IL.

All polymeric components of bagasse, a lignocellulosic biomass, dissolve in the hydrophilic ionic liquid (IL) tributylmethylphosphonium methylsulfate ([P4441]MeSO$_4$) with and without a catalytic amount of acid (H2SO$_4$, *ca.* 0.4 %). The presence of acid significantly increases the extent of dissolution of bagasse in [P4441]MeSO$_4$ (by *ca.* 2.5 times under conditions used here). The dissolved fractions can be partially recovered by the addition of an antisolvent (water) and are significantly enriched in lignin. Unlike acid catalysed dissolution in the hydrophobic IL tetradecyltrihexylphosphonium chloride there is little evidence of cleavage of β-aryl ether bonds of lignin dissolving in [P4441]MeSO$_4$ (with and without acid), but this mechanism may play some role in the acid catalysed dissolution. The XRD of the undissolved fractions suggests that the IL may selectively dissolve the amorphous cellulose component, leaving behind crystalline material.

List of Publications

1. Keskar S. S; Karatzos K.K; Edye L. A. The use of ionic liquids for the production of liquid fuels from lignocellulosic biomass. Biofuels (submitted) **2011**.

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3. Keskar S. S; Edye L. A; Doherty W. O. S; Bartley J. P. The chemistry of acid catalysed delignification of sugarcane bagasse in the ionic liquid trihexyl tetradecyl phosphonium chloride. Journal of Wood Chemistry and Technology (in press). **2011**.

4. Keskar S. S; Doherty W. O. S; Bartley J. P; Edye L. A. Role of acid in dissolution of sugarcane bagasse in tributylmethylphosphonium methylsulfate. Journal of Wood Chemistry and Technology. **2011**, (submitted).

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Dr. Leslie A. Edye, QUT

Dr. William O.S. Doherty, QUT

The work contained in this thesis has not been previously submitted to meet requirements of an award. To the best of my knowledge, the thesis contains no materials previously published or written by another person except where due reference is made.

Signature ……………

Date………..

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Abbreviations and Nomenclature

AMIMCl: 1-allyl-3-methylimidazolium chloride

AFEX: ammonia fibre explosion
AIL: acid insoluble lignin
ARP: ammonia recycle percolation
ASL: acid soluble lignin
ATR: attenuated total reflectance
BASF: BASF, the chemical manufacturing corporation

$BMIMBF_4$: 1-butyl-3-methylimidazolium tetrafluoroborate
$BMIMCF_3SO_3$: 1-butyl-3-methylimidazolium trifluoromethanesulphonate
BMIMCl: 1-butyl-3-methylimidazolium chloride
$BMIMPF_6$: 1-butyl-3-methylimidazolium hexafluorophosphate
EMIMCl: 1-ethyl-3-methylimidazolium chloride
EMIMAc: 1-ethyl-3-methylimidazolium acetate

DMA / LiCl: dimethylacetamide / lithium chloride
DMA: dimethylacetamide
DMSO: dimethylsulphoxide
DOE: Department of energy (United States)
DP: degree of polymerisation
FPU: filter paper units
FTIR: Fourier transform infrared spectroscopy
GHG: Green House Gases
HMF: hydroxymethylfurfural
HPLC: high-pressure liquid chromatography
IEA: International Energy Agency
IL: ionic liquid
LCB: lignocellulosic biomass
n.d: not determined
NMMO: *N*-Methylmorpholine-*N*-oxide
NMR: nuclear magnetic resonance

NREL: National Renewable Energy Laboratory (Golden, CO, USA)
[P4441]MeSO$_4$: tributylmethylphosphonium methylsulphate
[P44416]Cl: tributyltetradecylphosphonium chloride
[P66614]Cl: trihexyltetradecylphosphonium chloride

STEX: steam explosion
UND: undissolved fraction
XRD: X-ray diffractometry

Chapter 1 INTRODUCTION

Background

Fuels from lignocellulosic biomass

In recent years, there has been a significant interest in exploring renewable sources of energy. This has been attributed to several factors, *viz.*,

- Increase in world consumption of energy, both in the developed and developing countries.
- Instability in crude oil prices due to the concentration of almost half the world oil reserves in politically unstable countries.
- Concerns over the increase in Green House Gas (GHG) emissions resulting from fossil fuels, which have led to a significant rise in the temperature of our planet[1]

It is conceivable that the supply of stationary power can be supplemented or increased using renewable energy sources such as wind and nuclear power. It is also possible to electrify light passenger vehicles and public transport, such as rail, which is already an established practice. However, it is more likely that heavy road transportation will be powered by liquid petroleum fuel for the foreseeable future and there is no alternative to high density aviation fuel[2].

Hence a surge in commercial scale production and research activity has been witnessed in the field of biofuel (biodiesel, bioethanol and other renewable liquid fuels) in the last few decades[1].

Bioethanol was initially concentrated around production from starch and sugars; usually obtained from foodgrains such as corn and sugarcane, which is termed as ‘first generation bioethanol’[3]. However, first generation biofuels have been criticised for the utilization of arable land to grow feedstocks for fuel production and has now become a contentious issue[2,4]. The 3.4 billion gallons of first generation ethanol produced in the U.S. in 2004 amounted to about 2 % of all gasoline sold by volume[5]. Sooner or later the use of land that can otherwise be used for food production, for the production of biofuels, is going to be a real challenge. Consequently, production of ethanol from lignocellulosic biomass (LCB) such as forest residue, plant waste and sugar industry waste (bagasse) is currently being explored on a research level. Biofuels produced from lignocellulosic feedstocks are known as second generation biofuels.

Pretreatment of biomass

The structure of LCB is one of the most difficult technical barriers in the production of second generation biofuels[6]. Cellulose, lignin and hemicelluloses - the primary components of LCB - are connected in an intricate inter-molecular and intramolecular hydrogen bond network. The deconstruction of LCB in order to make ethanol from cellulose, is a complicated process that may require both chemical and enzymatic treatment[6].

Ionic Liquids for pretreatment of LCB

Since it was first reported that Ionic Liquids (ILs) can dissolve cellulose, there has been extensive research to explore if this ability to dissolve cellulose can be used in context of LCB pretreatment[7,8]. Significant research effort has been directed in understanding the role of imidazolium based ionic liquids in the dissolution of microcrystalline cellulose (MCC)[7,8], enhancement in saccharification of MCC after pretreatment with imidazolium ILs [9-11], dissolution of isolated lignin in imidazolium ILs[12] and dissolution of wood in imidazolium ILs[13,14]. Significant work has also been done in terms of enzyme hydrolysis of LCB treated using imidazolium ILs[10,15] and the addition of various catalysts in imidazolium ILs in order to accelerate hydrolysis of sugars [16,17]. However, there have been no detailed studies in the use of non imidazolium ILs and the possibility of their application in pretreatment of LCB. There have been some mechanistic approaches to the chemistry of cellulose with imidazolium ILs[18,19]. However, the dissolution of other components of LCB such as lignin and hemicellulose has not been sufficiently explored. There has not been sufficient investigation in the role of catalytic amount of impurities in ILs, such as acids that could bring about the dissolution of LCB.

This thesis reports on the investigation of the chemistry of LCB in two phosphonium ionic liquids – tetradecyl trihexyl phosphonium chloride and tributyl(methyl)phosphonium methylsulfate.

Research Aim

The overarching objective of this study is to investigate the chemistry of dissolution of sugarcane bagasse in phosphonium based ILs.

Objectives

The objectives of this work are to

- Investigate the dissolution of individual components of biomass i.e., MCC, isolated lignin and isolated hemicellulose and whole bagasse in phosphonium ILs
- Perform kinetic study on dissolution reactions to ascertain the optimum temperature for dissolution of bagasse.
- Study the mechanism of dissolution of bagasse using ATR-FTIR on reaction mixtures
- Study the effect of catalysts in phosphonium ILs.

Novelty

While imidazolium ILs have been extensively studied for dissolution of cellulose over the last decade, there are no accounts in the scientific literature of dissolution of either isolated components of biomass or whole LCB dissolution in phosphonium ILs. Until recently, there had been no attempt to study the kinetics of reactions *in situ* or attempts at using ATR-FTIR to study the mechanism of dissolution in phosphonium or imidazolium ILs. There has been no published work on either of the ILs used in this work and the authors have published the first paper on the dissolution of lignin in the hydrophobic tetradecyltrihexylphosponium chloride.

Summary of chapters

This chapter introduces the background, the motivation behind using ILs for pretreatment of LCB and the aims and objectives of this thesis in context of the reviewed literature.

Chapter 2 reviews the relevant literature that motivated this study and establishes a background for the results emerging out of this study. It covers the motivation for using LCB for the production of fuels and the political and environmental reasons behind this motivation. It provides a detailed review of the structure and chemistry of LCB and the reasons behind the critical pretreatment step required to access LCB for production of ethanol. It also covers a critical review of ILs in their application in biomass dissolution including techno-economic analysis of the feasibility of IL-biomass pretreatment scale-up.

Chapter 3 is a review submitted to the journal Biofuels. It elaborates on the use of ionic liquids for the production of liquid fuels from lignocellulosic biomass. Some of the information presented in Chapter 3 is already explained in Chapter 2. However, it has been condensed to meet the requirements and the style of the journal.

Chapter 4 elaborates on the initial expreriments carried out on both phosphonium ILs with individual components of biomass as well as whole milled bagasse. A detailed study of various FTIR bands observed in the reaction mixtures is presented and the FTIR bands arising from the individual components of biomass have been identified. Calibration of lignin dissolution in both ILs is presented and used to calculate the extraction of lignin from bagasse.

Chapter 5 is a detailed study on the dissolution of lignin in the hydrophobic phosphonium IL [P66614]Cl. It presents a kinetic study of dissolution of whole bagasse in [P66614]Cl, at 3 temperatures (130 °C, 140 °C and 150 °C), the calculation of rates of reactions and the study of the role of catalytic amount of residual acid in the IL. A mechanism of dissolution has been proposed using empirical evidence of high temperature kinetics and the formation of Hibbert's ketones, which are indicative of acid catalysed delignification.

Chapter 6 outlines a detailed study of dissolution of milled bagasse in the hydrophilic phosphonium IL [P4441]$MeSO_4$. It is an attempt to carry out mass balance on the dissolution reactions and isolating the dissolved and undissolved fractions. The effect of catalytic acid on dissolution of bagasse was studied and a detailed mass balance and compositional analysis of the dissolved and undissolved fractions are presented. The dissolved fractions from uncatalysed and catalysed experiments are compared to each other using FTIR and ^{31}P NMR. The undissolved fractions from different temperatures were analysed using XRD.

Chapter 7 presents the supplementary data for chapters 4 – 6 .

Chapter 8 elaborates on the overarching conclusions from this work and presents a discussion on the future of ILs in biomass processing.

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Chapter 2 LITERATURE REVIEW

Introduction

Petroleum refining underwent a revolution in the twentieth century. This was partly due to the low cost of petroleum based feedstocks, which feedstocks were converted to multiple products such as fuels, fine chemicals, pharmaceuticals, detergents and plastics, all of which human beings depend on heavily today. However, two environmentally limiting factors could slow the rate of technological advancement of the world, which is currently based almost exclusively on petroleum-based fuels and chemicals. Firstly, the detrimental effects of using carbon rich petroleum fuels on the environment are becoming a serious concern. These include the emission of Green House Gases (GHGs), and increased levels of carbon dioxide (CO_2) in the atmosphere, which has been linked to global warming[1]. The second, albeit more important reason, is the dwindling supply of petroleum, especially oil. Various models have been designed to predict the decline in oil production over the next few decades. The peak of oil production has been reached and presently, although there are gas, unconventional oil and coal reserves sufficient to last for centuries, liquid fuel production is certainly on a decline[2].

The world population is expected to increase by 50 percent by 2020, with more than half of that growth occurring in Asia and Latin Americ*a.* As more people migrate to the cities in the developing countries, more electricity and oil will be needed. This migration would directly affect the agricultural production of the developing world by demanding more field mechanization and transportation. A doubling of the proportion of China’s and India’s populations that lives in cities could increase per capita energy consumption by 45 percent, even if industrialization and income per capita remain unchanged[3]. In recent DOE/IEA report it is speculated that by 2035, amongst the developing countries China and India are likely to see an increase in liquid fuel consumption of up to 9.4 million barrels per day and 1.8

million barrels per day respectively, with the increase from China being largest for any single country worldwide[4].

The current use of fossil fuels is split with about 75 % for heat and power generation, 25 % for transportation fuels and just a few percent for materials and chemicals[5]. Alternatives such as solar energy, wind energy, hydropower and biomass could be applied for the generation of heat and power. However, the transportation sector has a limited choice. Renewable electricity is a prospective transport fuel option for the developed world, but at least in the medium term, transportation based on liquid petroleum fuels will remain the only option for the developing world. In countries such as China and India, the cheap internal combustion engine is the only affordable and profitable option (especially with ventures such as the large-scale production of US $2500 car, Tata Nano by Tata Motors, India have been set up[6]). For the aviation sector as well, high density liquid petroleum fuels are the only available option for a the foreseeable future[7].

The 1970s energy crisis saw many developed countries face a shortage of petroleum[8]. The two worst crises of this time were the 1973 oil crisis caused by the Arab Oil Embargo of OPEC, and then the 1979 energy crisis caused by the Iranian revolution[3]. The politics surrounding oil are widely seen as the root cause of the recessions that the developed world has faced since 1970s[9]. The politically unstable Middle East still holds about 55 % of the world's oil reserves[4]. These political crises emphasized the importance of alternative energy sources, ethanol among them, resulting in dedicated initiatives such as National Alcohol Program in Brazil and the 'gasohol' program in the USA[10]. Since then, the research activity in alternative sources of energy has shown a steady rise all over the world.

Fuels from biomass

The generic definition of biofuels is fuels derived from biomass *viz.* plants and organic waste. Biomass derived from trees, forest residues, grasses, plants, aquatic flora and food crops are renewable sources of energy, which human beings can cultivate or produce again and again. The general scheme of producing bioenergy, either in the form of liquid fuels or heat, is based on utilizing energy stored in biomass. Through photosynthesis, plants utilize light energy to convert water and carbon dioxide to biomass. This biomass comprises carbohydrates in both polymeric and monomeric forms, lignin, proteins and lipids. The carbohydrate fraction of plant biomass exists in various forms such as cellulose, starch, sucrose and simple sugars, which, among other things can be fermented to produce ethanol[1]. There are many established pathways of utilizing energy from biomass. The process of converting biomass to valuable fuels and products by using advances in process chemistry, genetic engineering and biotechnology is termed 'biorefinery'[11]. Biorefineries can further be classified in a number of ways, depending on feedstocks, process flexibility and the desired end products. Although, in the short term, ethanol is seen as the most valuable product of biorefining, in the long term, co-products would underpin the profitability of processing biomass into fuels. The ultimate goal of biorefining is to utilize the biomass feedstock in its entirety to make multiple products; similar to petroleum refining.

From the process perspective, biomass can be treated using a number of processes. This includes physical treatments, chemical treatments, biological treatments and thermal treatments. All of these treatments eventually lead to fuels, platform chemicals or value added products[1]. Figure 2-1 gives an overview of different treatments involved in converting biomass into fuels.

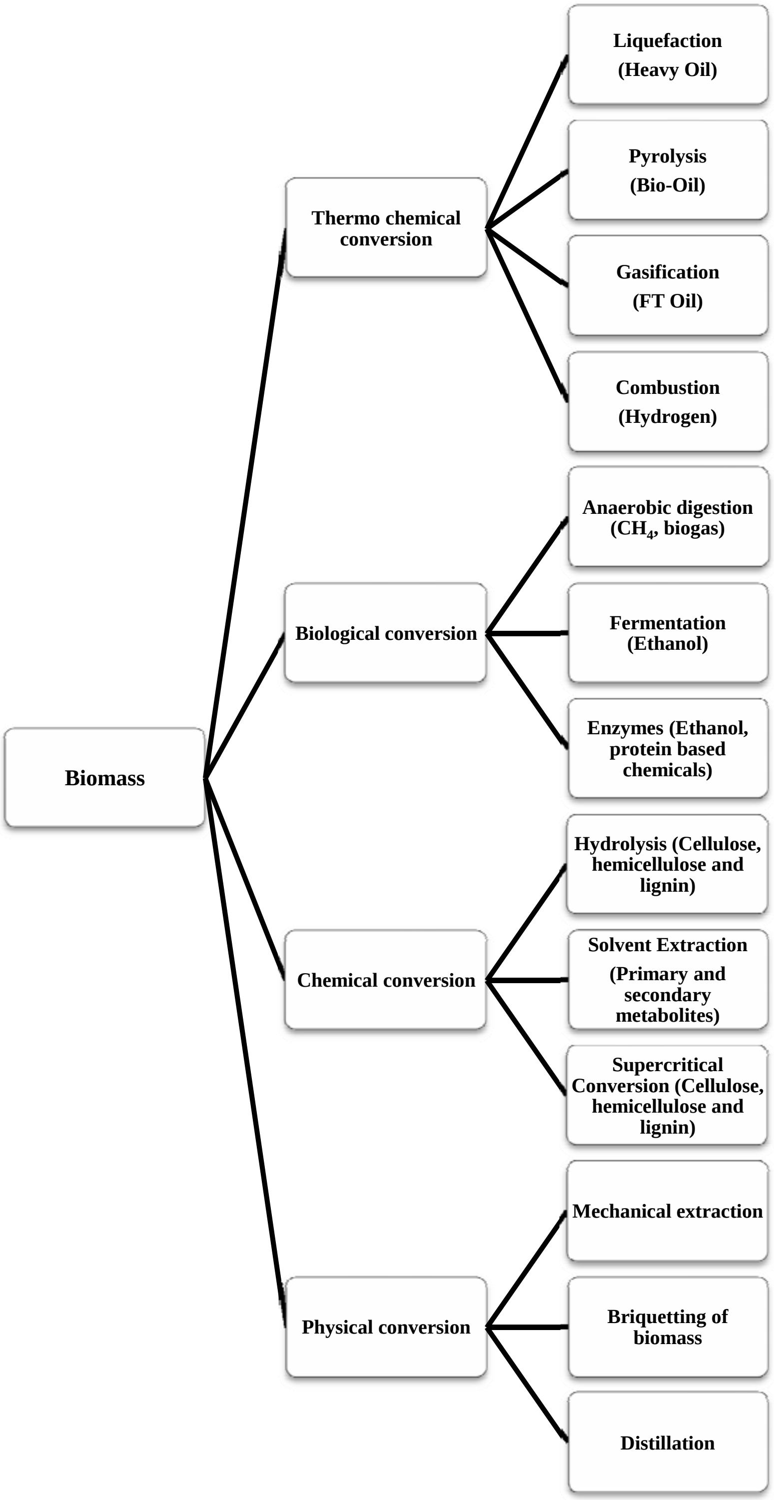


Figure 2-1 Classification of biomass conversion processes[1]

Biofuels can be loosely classified as fuels derived from feedstocks that can be used as human food and fuels derived from non food sources such as forest waste, agricultural waste and municipal waste[1]. Figure 2-2 gives a detailed outline of the fuel feedstocks currently in use or under research.

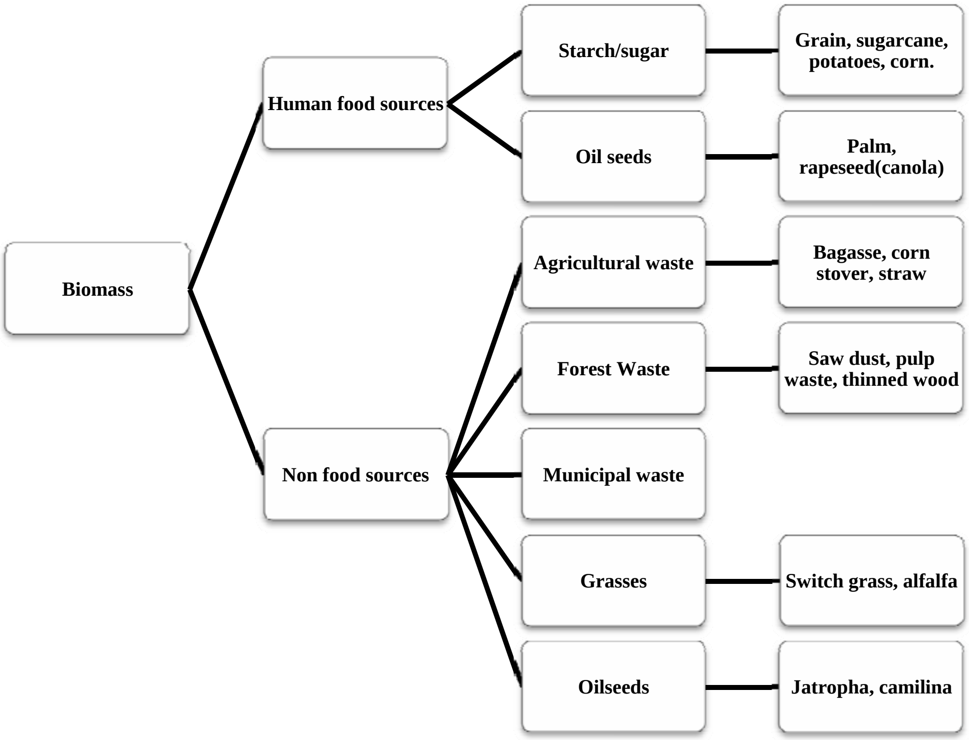


Figure 2-2 Classification of biofuel feedstocks[1]

Most biofuels today, come from the starch, sucrose and oil rich parts of plants. In United States, corn is used to produce ethanol, while in Brazil it is sugarcane. Feedstocks such as canola seeds, palm fruits and soybeans are also used as feedstocks. The biofuels (ethanol) derived from starch rich grains that could otherwise be used as food, are termed ‘first generation biofuels’[12]. On the other hand, biofuels produced from lignicellulosic waste such as wheat straw, corn husk, discarded rice hulls, cellulosic refuse from the sugar industry – bagasse, are termed 'second generation biofuels'.

As it can be seen from Figure 2-2, the feedstocks that can be used as food for human consumption are products of existing agricultural enterprise directed at food production. Apart from the fact that starch is easier to ferment than cellulose, which will be dealt with in this review, it is also worth noting that the first generation biofuel technology heavily relies on the agricultural technology and experience that is familiar to humanity and has matured over hundreds of years. The non-food resources however, are a new challenge both in terms of agriculture and physical and chemical composition.

Need for second generation biofuels

Food versus fuel debate

The process of making first generation biofuels from starchy foodgrains usually follows seven steps: milling, liquefaction, saccharification, fermentation, distillation, dehydration, and denaturating. When sugarcane is used instead of corn (*e.g.* Brazil), only four or five steps are required: milling, pressing, fermentation and distillation, and dehydration in case of alcohol blends[13].The technology for making fuel from starch is simple, has been established commercially and is operating for almost a decade. However, first generation biofuels have been severely criticized for a number of reasons, of which the food versus fuel issue has been the strongest argument against the technology. The crux of this argument is, that in order to increase biofuel production while accounting for the basic purpose of foodgrains as food, arable land that could be used for food production is being dedicated to growing grains for fuels[14]. The rising demand for liquid transportation fuel and the rising hunger compete for the same feedstocks. Hence first generation biofuels have come under scrutiny.

All ethanol currently produced in the U.S., is first generation biofuel. The 3.4 billion gallons of ethanol blended into petrol in 2004, amounted only to about 2 % of all gasoline sold by volume[15]. A 10 % substitution of petrol and diesel fuel is estimated to require 43 % of the current cropland area in the country[16]. Even at this substitution, the current cropland is not sufficient to meet the biofuel and food demand. To keep manufacturing first generation ethanol, it would become essential to clear forests and grasslands. Clearance would result in depletion of carbon stores in the vegetation and increased CO_2 emissions, which would mitigate the avoided emissions of biofuel use. Both food versus fuel and the use of arable land for production of fuels are politically charged and highly contentious issues. Although the discussions around these issues are highly polarized[6,15,17-19], it can be said with certainty that in considering the elasticity in agricultural production (i.e., possibility of significantly increasing the productivity of existing croplands), there is a point beyond which the diversion of food to fuel production would marginalize poorer nations. At some point, there are direct and indirect consequences of using arable land formerly committed to food production for fuel production.

Net energy balance considerations

Another argument against first generation biofuels is the net energy analysis of biofuels. This includes energy and cost analysis of each step in manufacturing 1L of biofuel from corn. The final value is calculated as the energy value of ethanol minus the sum of all the fossil energy inputs. The end result indicates that ethanol made from corn is highly energy intensive, as it requires a number of unit operations like milling, fermentation, double distillation and purification to 99.9 % ethanol before blending with petrol[20]. However, of all the arguments against first generation fuels, this argument is somewhat misleading. Although Pimentel *et al*. calculate the energy required to produce 1L of ethanol and deem it energy intensive, they do not compare it with the energy required to produce 1L of petrol. Farrell *et al.*[16], compare the net energy required to produce 1L of petrol with the net energy required to produce the same quantity of first generation ethanol. When the net energy of ethanol (+27 %) is compared to that of petrol (-18 %), it is evident that petrol is much more energy intensive than ethanol[6]. The actual outcome of the energy balance calculation also depends on the models that the authors of these works employ. Significant efforts have been made to standardize the Life Cycle Analysis (LCA) calculations. However, there is a lack of understanding in the scientific community about the purpose of LCA. LCA studies are not generic or absolute. They are always carried out to gain a deeper understanding of specific and comparable processes. They are based on common assumptions. Hence, the paper by Farrell *et al*.[16] presents a clearer case for the net energy argument of ethanol and petrol. For the purpose of this review, it is sufficient to understand that all the arguments against first generation biofuels are contentious, and perhaps not well understood yet by the larger scientific community. However, it is clear from the existing data that there is a need to proceed from the first generation technology to a more sustainable alternative.

The most logical step forward from first generation biofuels is making fuels from sources that do not challenge world food supply or the availability of arable land, i.e., second generation biofuel production. The turn of events in the recent times, such as, China's commitment of not allowing further increase in the production of starch based ethanol due to competing use as food, construction of the first commercial lignocellulosic ethanol plant by Broomfield (Colorado) and more recently, the French Agency for Environment and Energy Management, in Paris, announcing its plan to test three lignocellulosic biomass gasification processes is an indication that second generation biofuels indeed could be a part of the answer to the impending global fuel crisis[21].

Advantages of second generation biofuels

Second generation biofuels are produced from biomass in a more sustainable way. Lignocellulosic biomass appears to be an attractive feedstock for biofuels for three main reasons. First, it is a renewable resource that could be developed sustainably in the future. Second, fuels derived from biomass have positive environmental properties such as no net releases of carbon dioxide into the environment and very low sulfur content. Lignocellulosic biofuels have low CO_2 release because the carbon that was sequestered in growing the feedstock is only being re-released into the atmosphere.Third, it appears to have significant economic potential provided fossil fuel prices increase in the future[22]. Lignocellulosic material makes up the majority of cheap and abundant nonfood materials available from plants. This includes feedstocks such as corn husks, forest residues and sugarcane bagasse in sugarcane producing countries like Brazil, India and Australia. The primary difference between first generation biofuels and second generation biofuels is the source of sugars used to make the ethanol. While in first generation biofuels, ethanol is produced by fermenting starch (using yeast) or by fermenting sugars from sugarcane juice, in second generation biofuels, the source of sugars is cellulose, a polymer of glucose, that is abundantly available in lignocellulosic residue[11].

Although second generation biofuels seem to answer most questions about land, food versus fuel issue and carbon emissions, at present the production of such fuels is not cost-effective because there are a number of technical barriers that need to be overcome before their potential can be fully realized[23]. One of the most significant of these barriers is the structure of lignocellulosic biomass.

The structure of lignocellulosic biomass

For the purpose of this thesis, it is also essential to review the structure and use of the individual polymers celullulose, hemicellulose and lignin in biomass. Lignocellulosic biomass typically consists, on a dry basis, of about 20 – 50 % cellulose, 10 – 30 % lignin and about 15 – 20 % hemicellulose[24]. Traditionally, lignocellulosic biomass has been used across the world as

- Construction material after various forms of processing (*e.g.,* whole logs, lumber and particle boards).
- As a feedstock for heating and power generation, generally after chipping and pelleting.
- Paper board and paper (after pulping which removes most of the lignin and some or most of the hemicellulose).
- Animal feed (and to a much lesser extent, human feed).

Cellulose

Cellulose makes up for about 50 % of dry weight of biomass. It is the dominant carbohydrate in biomass, and is certainly the most abundant material on the planet[25]. In spite of these properties that would make cellulose a logical feedstock, the effort of utilizing cellulose, especially by the pathway of hydrolysing it to simple sugars, has been riddled with technical barriers; many of these are related to the molecular structure and orientation of cellulose in biomass. The main reason why grain based ethanol is easier to produce is the solubility of starch in hot water and its subsequent accessibility to enzymes. Starch is a mixture of two homopolymers, composed of α-D-glucopyranosyl units, namely amylose(linear) and amylopectin (branched).The structure of amylose is shown in Figure 2-3. The DP of amylose ranges from 200-20,000; however the DP of common starches found in grains such as rye, wheat and maize is < 300. Hence, when compared to highly crystalline cellulose found in lignocellulosic waste, starch is easier to break down and ferment. Starch granules are composed of amylose and amylopectin molecules arranged radially. They contain both crystalline and amorphous regions in alternating layers in the granule. Starch granules have the ability to swell temporarily in cold water. However, with increase in temperature this effect becomes irreversible and the starch granule bursts to form a paste[24,26].

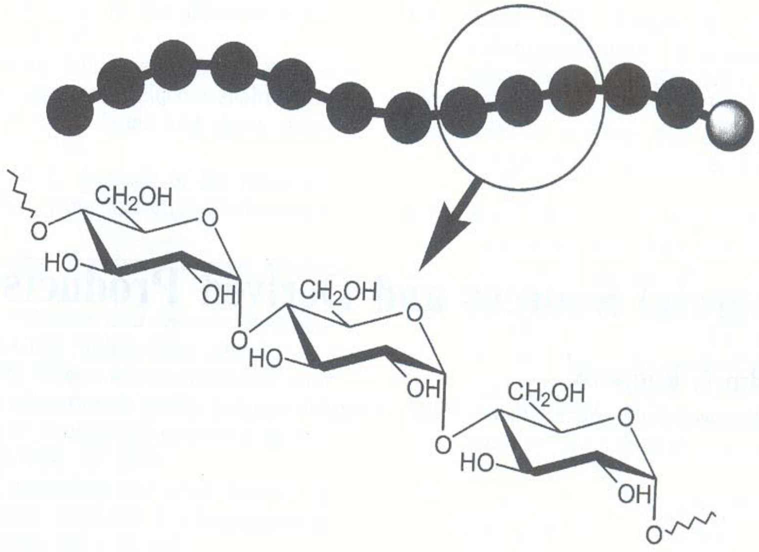


Figure 2-3 Structure of amylose[24]

The structure of cellulose, unlike starch, is more complex and hence difficult to hydrolyse. Natural cellulose is a partial crystalline polymer of 1 – 4 linked β - D glucose units. The primary structure of cellulose has been established by a number of investigations as a linear homopolymer of glucose residues having the D configuration and connected by the β-(1-4) glycosidic linkages[24,27]. The glucan chains in cellulose tend to form sheets. These sheets are hydrogen bonded to each other forming fibers with regions of crystalline cellulose[24]. Depending upon the source of cellulose, this DP varies from about 300-1700 for wood, to about 800-10000 for various plant fibers. It is also known that from a stereochemistry aspect, the constraints posed by the glycosidic linkage create a linear extended glucan chain in which every other glucose unit is rotated ~180° with respect to its neighbor. Consequently cellobiose, and not glucose is the basic repeating unit of the molecule[28] (see Figure 2-4).

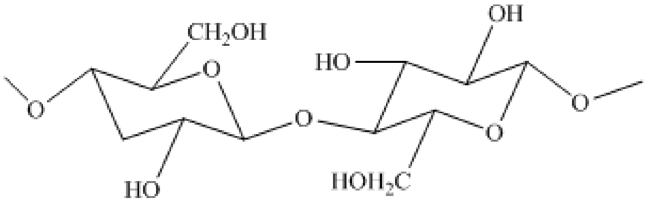


Figure 2-4 Basic repeating unit in a cellulose chain [32]

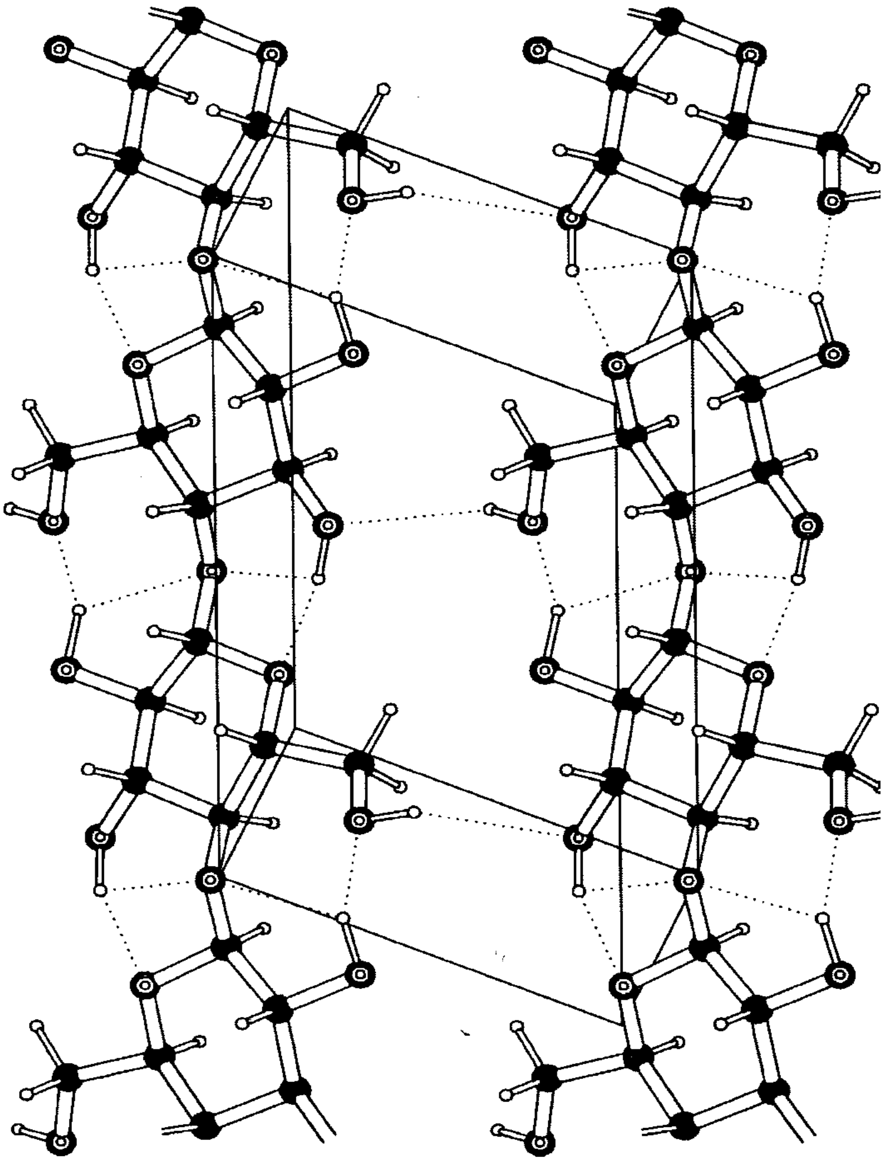


Figure 2-5 Hydrogen bonding in cellulose chains[29]

Figure 2-5 shows the crystal structure of cellulose. It can be seen that cellulose forms extensive chains and a rigid network through secondary hydrogen bonding between the chains, with a high degree of polymerization (DP). In fact crystalline cellulose exists in several morphological forms, the highly crystalline cellulose I (shown in Figure 2-5), also known as native cellulose as this is the crystalline morphology found in higher plants[25]. However, cellulose occurring in plant fibers is highly crystalline, especially in the secondary cell wall. The cellulose of the secondary cell wall has a DP of about 14000. The typical arrangement of glucan chains running in parallel direction, the individual chains connected to each other by extensive hydrogen bonding, makes the structure highly resistant to depolymerization[29].

This complex molecular structure imparts cellulose its characteristic properties. Although cellulose can absorb water, it is insoluble in water due to its high DP and extensive intra and inter-molecular hydrogen bonding[26].

Hemicellulose

Hemicelluloses are a heterogeneous group of polysaccharides and constitute about 20-25 % of biomass on a dry basis. The term "hemicelluloses" was coined at the time when the structure of these compounds was not elucidated clearly. They can be grouped into xyloglucan, xylans, mannans and glucomannans. Some polysaccharides such as galactans, arabinans and arabinogalactans are also included in the hemicelluloses group. Some of the side chains may also contain acetyl groups of ferulate[30].

These cell wall polysaccharides cannot be characterized as cellulose or pectin and comprise of xylose, arabinose and glucose. There are numerous configurations of these individual sugars in lignocellulosic biomass and those configurations are not in the scope of this thesis[31]. The structure of glucuronoarabinoxylan found in sugarcane bagasse is shown in Figure 2-6, as it is relevant to the source of biomass (bagasse), studied here.

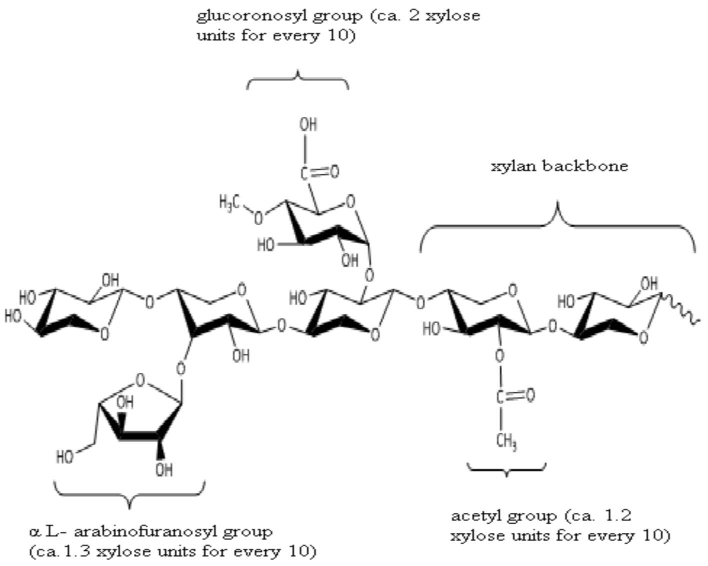


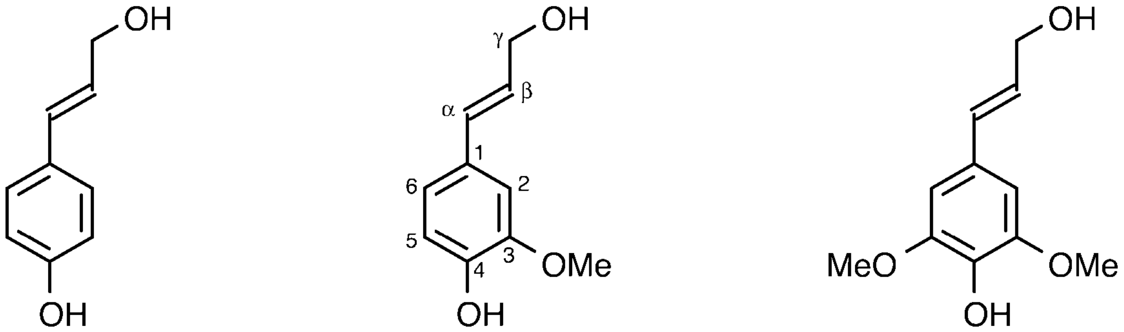
Figure 2-6 Structure of bagasse hemicellulose[32]

Xyloglucan probably has a structural role in plant cell wall, since most of the xyloglucans are hydrogen bonded to cellulose. Xyloglucan will bind rapidly and strongly to cellulose, in vitro[29].

Lignin

Lignin is a phenolic polymer consisting of phenyl propane units. On a dry basis, lignocellulosic biomass roughly consists of about 20-25 % lignin[30]. The structure of lignin has been a subject of research for over a century and even today, many questions about the conformation of lignin in plant cell wall remain unanswered. The structure of lignin varies between plant species, between cell types within a single plant and between different parts of the wall of a single cell. The exact structure in all these positions has not been established[32]. Unlike many biopolymers, lignins are not simple linear polymers of repeating subunits, linked together by certain bonds in fixed positions within the macromolecule. For the purpose of this dissertation, it is essential to understand the chemical structure of lignin. However, even today, there remains a considerable ambiguity around the exact structure of lignin. The main impediment in understanding lignins is the fact that as polymers, lignins display a considerable amount of diversity. The composition as well as the amount of lignin varies between taxa, cell types as well as individual cell wall layers. For example, dicotyledonous angiosperm (hardwood) lignins consist principally of **G** and **S** units and traces of **H** units, whereas gymnosperm (softwood) lignins are composed mostly of **G** units with low levels of **H** units. Lignins from grasses (monocots) incorporate **G** and **S** units at comparable levels, and more **H** units than dicots[33].

Lignins are traditionally considered to be polymers of three alcohol monomers or monolignols: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol as shown in Figure 2-7. Figure 2-8 illustrates the structure of phenyl propane units that are the building blocks of the lignin macromolecule. and Figure 2-9 shows various carbon-carbon and carbon-oxygen linkages found in the lignin molecules. Note the β-aryl ether linkage as this is a common linkage and cleavage of this bond is implicated in lignin dissolution.



MH *p*-coumaryl alcohol

MG coniferyl alcohol

MS sinapyl alcohol

Figure 2-7 Basic units (monolignols) in the lignin macromolecule[33]

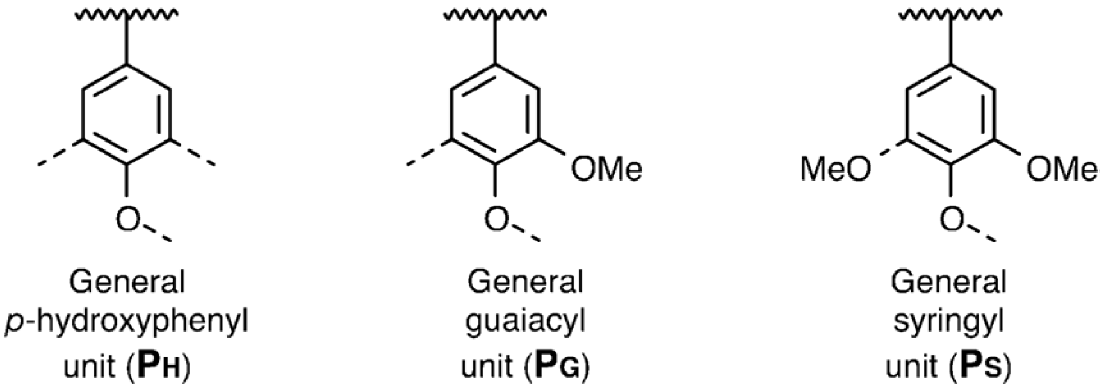


Figure 2-8The lignin macromolecule[33]

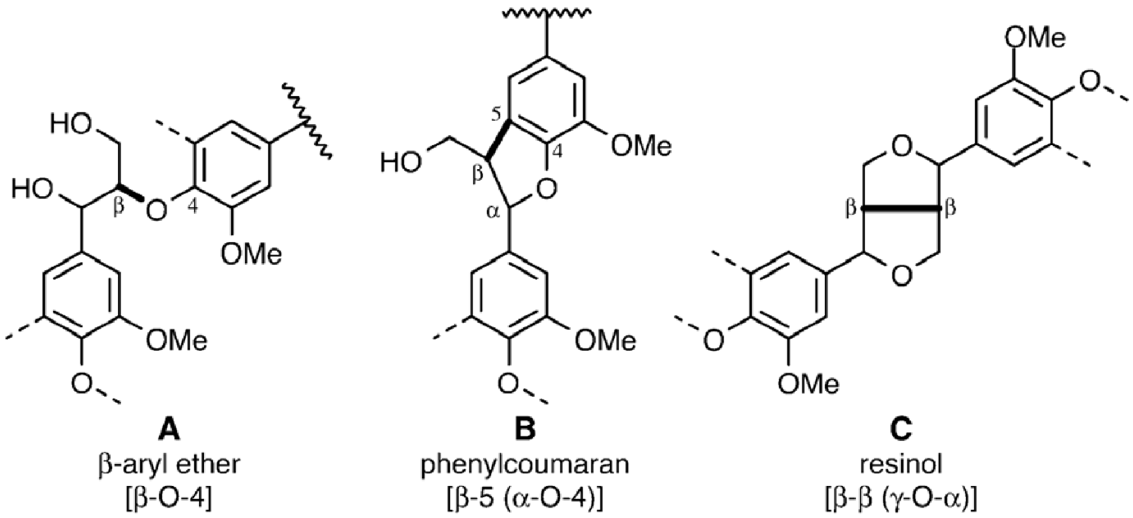


Figure 2-9 Likages between phenyl propane units[33]

Removal of lignin from biomass is one of the considerably complicated chemical process. For one, it is difficult to isolate lignin from its native source without significant alteration in its chemical structure. Furthermore, lignins isolated by using different processes differ in physical and chemical properties. This is mainly due to three reasons:

- Strong covalent bonds exist between lignin and carbohydrates
- Lignin has a high molecular weight and possibly, forms a three dimensional network around the carbohydrates
- Hydrogen bonding may be involved in the retention of lignin in the wood fiber wall[34]

FTIR studies on isolated hardwood and softwood lignins have confirmed the existence of intramolecular hydrogen bonding within the lignin fragments such as between the aliphatic hydroxyl groups[35]. In order to use the cellulose in lignocellulosic biomass, removal of lignin, also termed as delignification is of significant importance. The β -aryl-ether linkages, also known as β-ether linkages are of a critical importance in delignification[36]. The removal of lignin from biomass has been linked to the decrease in the β-aryl ether linkages in the lignin isolated from the treatment[36]. However, the cleavage of α-aryl ether and β-aryl ether bonds leads to formation of unstable intermediates such as carbocations (in case of acid catalysed delignification) which lead to a repolymerization or condensation of lignin fragments, where instead of being linked by oxygen molecules, the phenyl propane units are linked by carbon-carbon bonds which are extremely difficult to cleave using conventional chemical methods[36,37].

Interconnectivity of lignocellulose components

Hemicellulose hydrogen-bonds to cellulose microfibrils, thus forming a network that provides the structural backbone to plant cell wall. Cellulose and hemicellulose are potential sources of fermentable sugars. Lignin forms covalent bonds with hemicellulose.This cross linking of polysaccharides and proteins by lignin forms a very complex three dimensional network[32]. The crystallinity of cellulose, hydrogen bonding between the three polymers and the protection of cellulose by inter dispersed lignin in the cell wall makes lignocellulosic biomass highly recalcitrant to enzymatic treatment. Hence, a process to strip the recover fermentable sugars, reduce the crystallinity of cellulose in order to make it easier to access for enzymes is needed. This process of making the biomass amenable to enzymes is known as “pretreatment of biomass”.

Pretreament of lignocellulosic biomass

In plants, the cell wall acts naturally as a barrier to degrading organisms. Some of these organisms carry hydrolytic enzymes that target different plant cell wall components promoting disruption of fibres. From the evolutionary perspective, the plant cell wall is designed to protect against pathogen degradation[38]. The goal of making ethanol from lignocellulosic biomass is therefore against the plant's natural defence mechanism. Hence a pretreatment step is required to alter the macroscopic as well as microscopic size and structure of biomass, so that the hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved rapidly and with greater yields. Further processing of ethanol from lignocellulosics can be done by hydrolysis, fermentation and product separation/purification. If the pretreatment is effective, the unit operations in later part can be carried out with the same efficiency as that with first generation feedstocks[30].

An effective pretreatment is characterized by several criteria. It avoids the need for reducing the size of biomass particles, it preserves the hemicellulose fractions, it limits formation of degradation products that would inhibit growth of fermentative microorganisms and it minimizes energy demands[39]. Different pretreatments have different chemistry and pathways of interacting with the components of lignocellulosic biomass. Besides, there is variety in the feedstocks as well, ranging from grasses to softwoods and hardwoods, each differing from the other in terms of carbohydrate to lignin ratio. These variations create interdependence between the pretreatment and the biomass used as feedstock. The choice of biomass and type of pretreatment will affect the physicochemical properties of the pretreated substrate[38]. Hence a number of pretreatment processes have been designed.

Types of pretreatment

Pretreatment methods are predominantly physical or chemical. Physical pretreatments include comminution (mechanical size reduction), steam explosion, and hydrothermolysis. Size reduction in the form of dry, wet and vibratory ball milling and compression milling is sometimes needed to make material handling easier through subsequent processing steps. Acids and bases are added to the biomass to facilitate the removal of certain components. For example, acid concentrated processes promote polysaccharide hydrolysis and yield soluble monosaccharides and a lignin residue, while alkaline processes work by removal of hemicellulose and lignin while leaving a cellulosic solid[30]. Some of the close to commercial, cost-effective pretreatment strategies are discussed in this section and summarized in Table 2-1.

Table 2-1Types of pretreatments

Type of pretreatment	Mode of action	Advantage	Disadvantage
Uncatalyzed steam explosion	High pressure steam and acids from biomass hydrolyse polysaccharides.	Low residence times (seconds and minutes) Low neutralizing chemicals required as no acid is added.	High temperature and pressure required.
Hydrothermolysis	Hydrolysis at high temperatures catalysed by hydronium ions in water.	Low residence times. Flexible process design (co-current, counter-current, flow through)	High temperature and pressure requirements. pH has to be monitored to reduce the hydrolysis of sugars.

Concentrated acid hydrolysis	Hydrolysis of carbohydrates to release oligomers and monomers.	Break down of carbohydrates into monomers.Low residence times.	High cost acid resistant vessels.Formation of degradation products.Need for neutralization before enzyme treatment or separation of acids from sugar.
Dilute acid hydrolysis	Similar to conc. Acid hydrolysis but at lower temperatures.	Cleavage of lignin-hemicellulose bonds resulting in lignin removal. Low temperatures as compared to conc. acid.	High cost infrastructure due to acidic reagents. High water-solid ratios make handling of biomass difficult.
Lime or caustic Pretreatment	Removal of lignin from the biomass thus improving the reactivity of the polysaccharides.	utilize lower temperature and pressure conditions compared to other pretreatments	Higher reaction times. Remove the acetyl and uronic acid substitutions on hemicellulose making biomass less accessible to enzymes.
Ammonia Fibre Explosion (AFEX)	Lignin solubilisation , cleavage of lignin-carbohydrate bonds by ammonolysis.	Reduces lignin content and removes hemicellulose.	The cost of ammonia and its recycling efficiency dictates the economics of AFEX.

Uncatalyzed steam explosion

A lot of dedicated research has been conducted in this area of pretreatment. The basic principle of uncatalyzed steam explosion, or ‘auto hydrolysis’ as it is called, is to use high pressure steam to bring about hydrolysis of hemicelluloses. It is considered to be the lowest cost option. The biomass is rapidly heated with steam, the heated mixture is held under high pressure for a few minutes and it is terminated by rapid decompression[30],[38]. Although no external chemicals are added to the biomass, the high temperature and steam leads to the release of organic acids, such as acetic acid from the biomass that help in catalysing hydrolysis of polysaccharides. The main physicochemical changes occurring in the steam exploded biomass is the swelling and dissolution of hemicellulose. This improves the accessibility of cellulytic enzymes to cellulose[38].

Liquid hot water pretreatments

Liquid hot water pretreatments use pressure to maintain hot water in a liquid state at elevated temperatures (200 °C – 230 ° C)[30]. Depending on the technical design of the process, these treatments are termed as hydrothermolysis[40], aqueous or steam aqueous fractionation[41], and uncatalyzed solvolysis[42]. At temperatures greater than 100 °C. water activity is 100 fold greater than at room temperature. Consequently, lignocellulosic biomass undergoes hydrolysis reactions in the presence of hydronium ions generated due to high pressure ionization of water molecules, which act as catalysts. The glycosidic bonds of hemicelluloses are easily cleaved under these reaction conditions to form oligosaccharides. Deacetylation also occurs and the resulting acetic acid further catalyses polysaccharide hydrolysis.

Depending on operating conditions, polysaccharides (mainly hemicelluloses) are depolymerised to oligomers and monomers, and the corresponding sugars may dehydrate to form furfural and hydroxymethylfurfural (HMF)[40].

Acid pretreatment (concentrated acid hydrolysis)

In concentrated acid pretreatment, mixture of acid and biomass is heated indirectly through the vessel walls or by steam injection, the latter operated in the same manner as steam explosion. The acid is added through a bed, sprayed onto the residue after which the residue is heated or agitated with the biomass in a reactor[30]. Acid hydrolysis releases oligomers and monosaccharides. The added acid catalyses the breakdown of cellulose into glucose. This process has a number of limitations including the need for acid resistant materials. The acid must also be either neutralized or separated from the sugars by simulated moving bed chromatography, which adds to cost, before fermentation of sugars is carried out. Formation of degradation products and release of natural biomass fermentation inhibitors are also important barriers that make this process uneconomical.

Dilute acid hydrolysis

Addition of very dilute sulphuric acid (about 0.07 % versus the 0.6-3.0 % for the previously described pretreatment) in a flow through reactor configuration is effective at acid levels lower than 0.1 %. Lower temperatures are applied to hydrolyze reactive hemicellulosic components. Temperatures of 140, 150 °C are used for the first stage, using fresh steam and in the second stage temperatures are taken higher up to 200 °C. This typically results in the solubilization of hemicelluloses and cleavage of lignin-hemicellulose bonds, resulting in separation of lignin from the bulk lignocellulosic material. The pretreated cellulose is thus highly digestible to enzymes[43]. Despite having excellent sugar yields and highly digestible cellulose with low acid, the high water to solids ratio and equipment configuration requires significant energy for product recovery. Hence this process has not been successfully commercialized. However, when combined with steam explosion this process is favored.

Lime pretreatment

Alkali pretreatments utilize lower temperature and pressure conditions compared to other pretreatments. It could be carried out at ambient temperature, but pretreatment time is measured in hours or days as against minutes or seconds with the other pretreatments. Pretreatments of various lignocellulosic residues with lime have been carried out in the past[44],[45]. Lime has been used to treat various biomass feed stocks such as wheat straw[45], switch grass[46] and corn stover[47]. However, the reaction time for these pretreatments is significantly higher (2h – 13 h) than to the acidic pretreatment. Lime also tends to form irrecoverable salts which are left as residues in the biomass[30]. Besides lime, other alkali pretreatments use sodium, potassium, calcium and ammonium hydroxide as reactants. Sodium hydroxide has received significant attention[45]. However, lime has the additional benefit of low reagent cost and safety as it can be recovered from water as insoluble calcium carbonate by bubbling CO_2[30].

Ammonia pretreatment

Ammonia fiber/freeze explosion (AFEX) has been under development for the past two decades[48], [49], [50]. The latest study on the effectiveness of the AFEX process shows a significant reduction in enzyme loadings for ammonia pretreated biomass[48]. In this pretreatment, an aqueous solution of ammonia (5-15 %), is contacted with biomass in a flow through reactor at temperatures of up to 160-180 °C, with a residence time of 14 min[30]. The ammonia is separated after the reaction and recycled. This pretreament works exclusively by solubilising lignin, as ammonia cleaves the lignin-carbohydrate bonds through a reaction called as ammonolysis[51]. By cleaving these lignin-carbohydrate bonds, AFEX pretreatment simultaneously reduces lignin content and removes hemicellulose. It has also been found to affect the crystallinity of cellulose[30]. However, the cost of ammonia is a significant barrier to making this pretreatment economical. Hence ammonia recycling and reuse is of importance. This method of pretreatment works well on grasses but is not so effective on hardwoods and softwoods due to higher lignin content[30].

Pretreatments using solvents

As stated earlier in this review, the individual components of the lignocellulosic matrix have distinct and different functional properties. While cellulose forms long hydrogen bonded chains of high crystallinity, imparting strength to the plant, lignin provides resistance against pathological attack[28]. Lignin is the second most abundant polymer on the earth after cellulose. It is a major carbon sink in the biosphere, accounting for about 30 % of the more than 1.4 X 10^{12} kg of carbon sequestered into terrestrial plant material each year[52]. Unfortunately, it is the most undesirable element of biomass as far as the production of bioethanol from biomass is concerned, as it hinders the accessibility of enzymes to the biomass and the recovery of enzymes from the process.

Attempts have been made previously to selectively dissolve components of biomass in order to harness the cellulose. These efforts have lead to the discoveries of many organic solvents that can separate either lignin or cellulose preferentially from the biomass. Although, solvent extraction brings its own challenges in terms of volatility and toxicity of the solvents, use of pressure vessels and the energy requirements to recycle the solvents, it is worth reviewing them to understand biomass chemistry through its interaction with different solvents.

Solvents for cellulose

Cellulose has been a polymer of commercial importance even before the conception of biorefining. The viscose process was invented in 1892 and has been commercially used for a considerable period of time. It employes CS_2 and NaOH to prepare cellulose xanthogenate[53]. In addition, aqueous solutions of metal complexes such as cuproethylene diamine or cuprammonium hydroxide (Cuam) are also well-established cellulose solvents. Broadly, cellulose solvents can be classified into two groups. The non-derivatizing solvents, which dissolve cellulose by disruption of intermolecular hydrogen bonds only, without chemically altering the monomeric units or the polymeric linkages, and the derivatizing solvents, which bring about dissolution by formation of unstable ethers, esters or acetal derivatives[53]. Figure 2-10 shows the classification of cellulosic solvents with selected examples. Table 2-2 shows the multicomponent aqueous salt systems that have been used in cellulose dissolution.

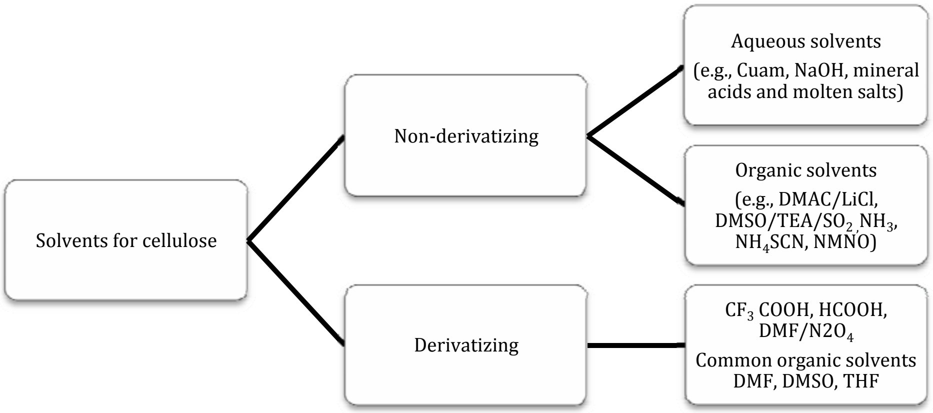


Figure 2-10 Characterization of cellulose solvents[55]

Table 2-2 Aqueous non-derivatizing solvent systems for cellulose

Type of Salt	Swelling of cellulose	Dissolution of cellulose
Pure salt	$LiCl\ H_2O$ $Zn\ (NO_3)\ 6H_2O$ $Na\ (ClO_4)_2\ H_2O$ $Mg\ (ClO_4)_2\ H_2O$	$ZnCl_2\ 4H_2O$ $LiClO_4\ 3H_2O$ $Zn\ (NO_3)_2\ xH_2O\ (x<6)$ $FeCl_3\ 6H_2O$
Salt Mixture	$2LiClO_4\ 3H_2O/CaCl_2\ 6H_2O$	$LiClO_4\ 3H_2O/MgCl_2\ 6H_2O$ $LiClO_4\ 3H_2O/Mg(ClO_4)_2/H_2O$ $LiClO_4\ 3H_2O/NaClO_4\ H_2O$ $LiCl/ZnCl_2/H_2O$

Commonly used aqueous non derivatizing solvents for cellulose include solutions of inorganic compounds and salts such as cuprammonium hydroxide (Cuam) and cupriethylenediamine hydroxide (Cuen). These were mainly used for regeneration of cellulose for the production of cellulosic membranes[53]. Aqueous solutions and/or mixtures of inorganic salts as solvents for cellulose have also been studied. Salts with the general formula $LiX*H_2O$ ($X=$ I, NO_3^-, CH_3COO^-, ClO_4^-) have been recorded for their ability to dissolve cellulose of upto 1500 DP[53]. While some salts dissolve cellulose while others aid in swelling of cellulose. In case of salt mixtures that dissolve cellulose, the dissolution has been attributed to the polarizing anions and a small cation[53].

Non-aqueous, non-derivatizing solvents (see Table 2-3) for cellulose can be loosely classified into three groups again: unicomponent, bicomponent and tricomponent systems. Unicomponent systems usually consist of a single molten salt, *e.g.*, N-ethylpyridinium chloride (alkylpyridinium halogenides). The most powerful non-derivatizing nonaqueous solvent for cellulose is N-methylmorpholine-N-oxide

(NMNO). However, NMNO is solid at room temperature and could lead to the formation of explosive peroxides, which is a significant limitation to this process[53].

Dicomponent solvent systems are combinations of salt and a polar, aprotic, non-derivatizing organic solvent, such as, dimethyl sulfoxide (DMSO) or N,N-dimethyl acetamide (DMAC). In the tricomponent system, salts and polar aprotic solvents are combined with NH_3 or amines[53].

Table 2-3 Non aqueous non derivatizing solvent systems for cellulose

Number of components	**Substance group**	**Examples**
Unicomponent	N – Alkylpyridinium halogenices	*N*-Ethylpyridinium chloride
	Oxides of tertiary amines	*N*-Methylmorpholine-*N*-oxide
		Triethylamine-*N*-oxide
		N-Methylpiperidine-*N*-oxide
Bicomponent	Dimethyl sufoxide (DMSO) containing solvents	DMSO/methylamine
		DMSO/KSCN
		DMSO/$CaCl_2$
		DMSO/TBAF
	Liquid ammonia/ sodium or ammonium salts	NH_3/NaI (NH_4I)
		NH_3/NaSCN (NH_4SCN)
	Dipolar aprotic solvents/ LiCl	N, N-dimethylacetamide/ LiCl
		N-methylpyrrolidone/ LiCl
		Pyridine/ resorcinol
Tricomponent	NH_3 or amine/ salt/ polar solvent	NH_3/ NaCl/DMSO
		Ethylenediamine/ NaI/ N, N-dimethylformamide
	NH_3 or amine/ SO_2 or $SOCl_2$/ polar	Diethyleneamine/SO_2/DMSO

An alternative pathway to cellulose dissolution is to use solvents that dissolve cellulose by forming unstable esters, ethers and acetal derivatives during the process of dissolution. Table 2-4 lists some of the derivatizing solvent systems and the corresponding cellulose derivatives obtained in the process.

Table 2-4 Non aqueous derivatizing solvents for cellulose

Solvent system	Derivatization
N_2O_4 /DMF	Cellulose nitrite
HCOOH/ H_2SO_4	Cellulose formate
CF_3COOH	Cellulose triflouroactetate
$Cl_2CHCOOH$	Cellulose dichloroacetate
$(CH_2O)_5$/ DMSO	Methyl cellulose

A major disadvantage of derivatizing solvent systems is the numerous side reactions and products. However, combinations such as dimethylformamide (DMF)/N_2O_4 have found industrial applications in synthesis of organic cellulose esters despite its toxicity[53].

Solvents for lignin

The aqueous pretreatments previously described in this section are effective in removing lignin to varying degrees. The aqueous AFEX process exclusively removes lignin via ammonolysis of the ether linkages between the phenylpropane units[49]. The alkaline Kraft process is predominantly used to remove lignin from lignocellulosic material in the Unites States. It is an aqueous process that employs sodium hydroxide and sodium sulfide, also known as white liquor, which is used to delignify biomass at 170 °C for 2 h. The mechanism of delignification involves cleavage of ether bonds in lignin by the anions in the white liquor[54].

Addition of organic solvents to acidic and alkaline processes is known as organosolv pulping[37]. Aqueous processes such as the Kraft process and aqueous bisulphite pulping, carry out delignification by fragmenting the larger lignin macromolecule into small hydrophilic water/alkali soluble fragments[54]. The resulting lignin therefore has a very low molecular weight which limits its applications in production of value added products[11]. Due to presence of an organic solvent, organosolv processes have been successful at recovering relatively high molecular weight lignin fractions[37].

Organosolv processes can be further classified into neutral, acidic and alkaline processes. The neutral organosolv process is analogous to the uncatalysed steam explosion process described previously in this review. It is still essentially an acid catalysed process where the acid is generated from the biomass. Solvents such as ethanol and ethylene glycol have been employed in the neutral and acidic processes with the reaction liquor containing up to 50 % of organic solvent[37]. The alkaline organosolv process uses alkaline sulfite anthraquinone methanol (ASAM) – an organic addition to the aqueous alkaline sulfite anthraquinone (ASAQ) process[37].

Delignification in organosolv processes follows three distinct phases: the initial phase, the bulk phase and the residual phase. The initial phase is predominantly governed by diffusion and occurs at lower temperatures (<150 °C) while the bulk phase consists of chemical reactions. Most of the lignin is removed in the bulk phase. In the residual phase, the rate of delignification decreases and residual (up to 10 %) lignin becomes insensitive to chemical attack[37], [54].

The chemical reactions in organosolv processes can be divided into two groups, *viz.*, degradation reactions and condensation reactions. Degradation reactions involve acidic or alkaline attack on the alpha and beta ether linkages on the phenyl propane units in the lignin macromolecule. Amongst these bonds, the cleavage of beta aryl ether is desirable for maximum delignification. However, during the cleavage of β-aryl ether bonds, unstable intermediates such as carbo cations, in case of acid catalysis and quinone methides in case of alkaline catalysis are formed. These intermediates initiate the condensation reactions, which involve reassembling the lignin fragments with carbon-carbon bonds. These reactions are undesirable for effective removal and reuse of lignin[37], [54].

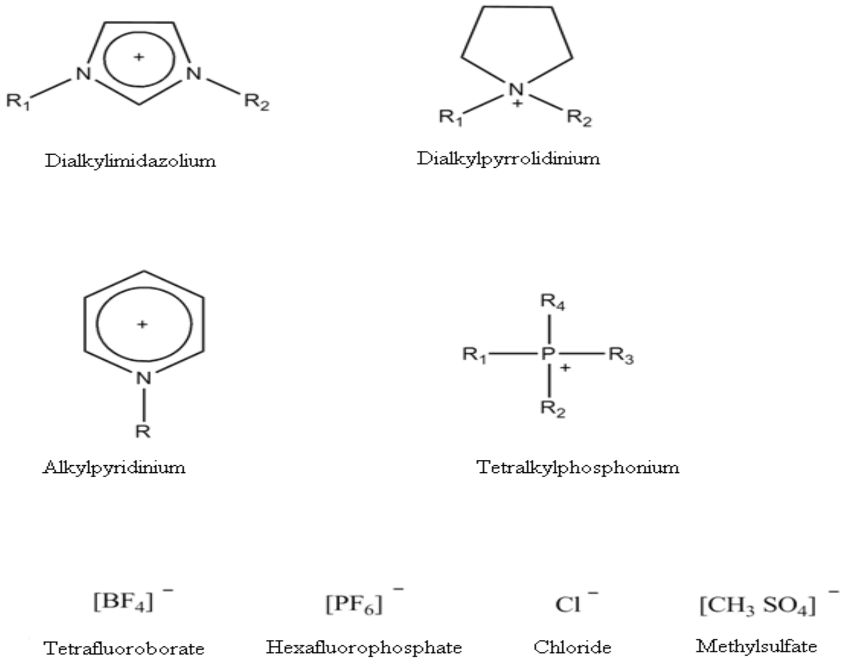
Although processes involving organic solvents are less popular as pretreatments as compared to the aqueous process, the chemistry of the primary polymers of biomass has unfolded largely through their interaction with organic solvents. Since cellulose is the highly desired polymer, the use of cellulosic solvents has attracted a lot of attention. The investigations in understanding the use of molten salts in dissolution of cellulose has led to the unraveling of an entirely novel solvent system – Ionic Liquids (ILs).

ILs for dissolution of biomass

Ionic liquids (ILs) have been the centre of enormous research activity in the past decade. In the previous decade (1990-2000), there were 138 published articles about ILs in the scientific literature. This number has reached a staggering 21,021 between 2000-2010 (source: SciFinder). An IL or a Room Temperature Ionic Liquid (RTIL) is defined as a material containing only ionic species, without any neutral molecules and having a melting point of lower than 298K[55], [56].

Initially, ILs were investigated primarily for their use as an alternative to Volatile Organic Compounds (VOCs) in catalysis[57]. Since then, these novel solvents have found numerous applications in organic chemistry for a number of reasons: they are good solvents for a range of organic and inorganic compounds, they are composed of poorly co-ordinating ions and can be used as highly polar yet non-coordinating solvents, they have negligible vapour pressure, a broad liquidus range and depending upon their physical properties, can form biphasic systems with aqueous and organic solvents[57]. Different cations and anions can be combined to form ILs with different physical and chemical properties (see Figure 2-11). The number of such potential combinations of all organic cations and inorganic anions that can be used to engineer ILs is computed to be about 10^{12}[58].

The proponents of the use of ILs contend that these properties make ILs applicable in various chemical processes, *e.g.*, organic synthesis, liquid-liquid extraction and electrochemical studies.



R= alkyl group

Figure 2-11 Examples of IL cations and anions

Another important characteristic of ILs is the ability of some ILs to dissolve cellulose, which has led to the formation of a subgroup of interest within the vast literature relating to ILs[57].

In 1934, Graenacher suggested that molten N-ethylpyridinium chloride could dissolve cellulose in presence of nitrogen containing bases[59,60]. This was further explored on a research level by the use of molten salts such as LiCl in salt/organic solvents combinations[53]. However, since the discovery of the ability of certain imidazolium based ionic liquids to dissolve cellulose, dedicated research has been directed towards establishing ILs as solvents for cellulose[61-65].

From a biomass chemistry point of view, ILs can be viewed as a hybrid of the organic and inorganic pathways of dissolving up cellulose, as they are composed of large organic cations such as alkyl imidazolium, tetraalkyl phosphonium, alkyl pyridinium and small inorganic anions– halides, sulfates and phosphonates[60].

Since the turn of the last decade, scientists have been trying to link the ability of some ILs to dissolve cellulose to the pretreatment and/or fractionation of lignocellulosic biomass for ethanol production[59]. The purpose of this review is to categorize current scientific literature concerning ILs and the individual components of biomass and to critically review the body of published literature from a chemistry as well as process perspective.

Classification of IL-biomass interactions

As shown in Figure 2-12, for the purpose of this review, the interactions of biomass and ILs have been characterized as: solute-based interactions, solvent-based interactions and catalysis reactions using ILs as solvents. The studies of IL-biomass interactions are scattered in the scientific literature loosely around these criteria.

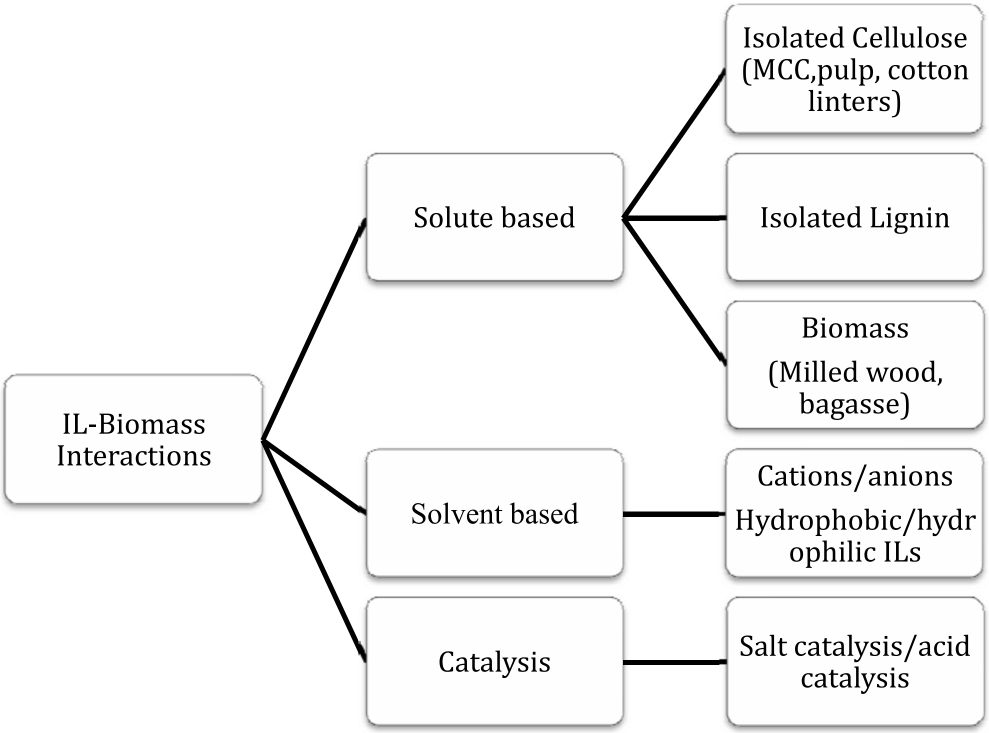


Figure 2-12 Classification of biomass-IL interactions

Microcrystalline cellulose (MCC), isolated lignin and whole biomass

The solute based interactions can be divided into three groups: a. isolated cellulose, b. isolated lignin and c. lignocellulosic biomass (Figure 2-12).

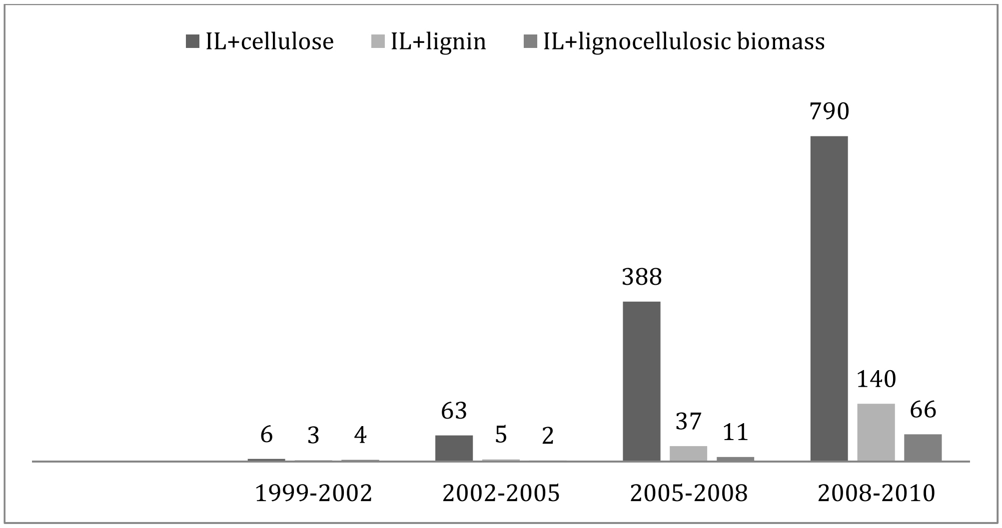


Figure 2-13 Publication patterns in IL-biomass research

It is evident from Figure 2-13, that dissolution of cellulose has attracted much more attention than the other components of biomass. However, if ILs are to be used in any industrially relevant, biomass related process in the future, a thorough understanding of the interaction of ILs with all the biomass polymers, *viz.*, cellulose, hemicellulose and lignin needs to be achieved. The interactions of imidazolium ILs with pure cellulose have been a subject of rigorous study, however, there needs to be further investigation into the use of ILs for the processing of whole biomass.

Dissolution of MCC in ILs

One of the earlier attempts to understand the mechanism of dissolution of isolated cellulose in ILs was done by Swatloski *et al.*[59]. In their work, MCC was dissolved in nine imidazolium ILs with different properties. In these experiments 1-Butyl-3-methylimidazolium chloride (BMIMCl) emerged as the most efficient IL in dissolving up isolated cellulose, as it dissolved 10 % (mass) cellulose at 100 °C and 25 % (mass) at 80 °C with microwave heating. The authors attributed the chloride activity in the IL to the rapid dissolution of cellulose, which was analogous to the mechanisms proposed for the most effective traditional solvent system for cellulose LiCl/DMAC[53],[66].

In 2005, Zhang *et al.*[67], published work on the IL 1-Allyl-3-methylimidazolium chloride (AMIMCl) as a non-derivatizing solvent for MCC, delignified pulp and cotton linters. The solvent reportedly caused swelling of cellulose at lower temperatures (60 °C) and dissolution of cellulose at temperatures above 80 °C. It was also observed in the same study that AMIMCl could dissolve cellulose with a DP of 650 in 30 minutes.

Dadi *et al.*[68], conducted an experiment to assess the saccharification of cellulose regenerated from ionic liquids. Regeneration of cellulose refers to the process of dissolving crystalline cellulose in a solvent and recovering it by addition of an atisolvent, such as water. This study took IL-cellulose research further by demonstrating that cellulose regenerated from cellulose dissolving ILs such as BMIMCl is akin to better saccharification than crystalline cellulose[68]. This finding fuelled the prevailing idea of using ILs for dissolution of cellulose from whole biomass.

However, dissolution of isolated cellulose has led to many other applications of ILs in cellulose functionalization such as acetylation[64], and carboxymethylation of cellulose[69], which is of industrial relevance because of the toxicity and flammability of the traditional solvents such as CS_2 used for functionalization of cellulose[53]. The mild reaction conditions and the ability to be able to recycle the ionic liquid after functionalization is an important finding for designing environmentally friendly reactions[70]. The production of bioactive and reconstituted cellulose films has also received significant attention in the field of cellulose-IL chemistry[71], [72].

Dissolution of whole biomass in ILs

The ability of certain imidazolium ILs to successfully dissolve highly crystalline isolated cellulose consequently led to the investigation of ILs for the dissolution of native cellulose from biomass. Kilpelainen *et al.*[73], carried out experiments with a range coarsely milled (0.1-2 mm mesh size), unmilled wood and sawdust in BMIMCl and AMIMCl at temperatures ranging from 80 °C to 130 °C. The resulting wood-IL solutions were precipitated using water as an antisolvent similar to the procedure of regenerating cellulose. It was observed that in case of whole biomass as well, the regenerated wood was more amorphous as compared to the starting material even though clear solutions were not obtained. It is worth noting however, that dissolution of wood in ILs required higher temperatures as well as longer reaction times – as high as 15 h for unmilled wood chips[73]. These higher temperature and time requirements can be attributed to the complexity of the plant architecture[30], that has been amply elaborated in studies conducted in the field of pretreatment of biomass. The phenolic polymer lignin, which consists of about 20 % (mass, dry basis) of biomass, poses the primary impediment in the dissolution of whole biomass in ILs[30].

Fort *et al.*[74], investigated the ability of BMIMCl to dissolve finely divided wood by employing carbon NMR and comparing the NMR profiles of the wood-IL solutions to the NMR profiles of MCC-IL and Indulin-IL (lignin standard). They concluded that BMIMCl could partially dissolve wood. Unlike the pulping technologies that depend on the insolubility of polysaccharides and focus on selective delignification, certain imidazolium ILs can dissolve lignin and carbohydrates simultaneously[74]. It is evident in all the studies related to dissolution of whole biomass in ILs, whether finely or coarsely milled, that it takes significantly longer times and higher reaction temperatures than the dissolution of MCC or delignified pulp[73], [74].

Singh *et al.*, carried explored biomass-IL interaction using fluorescence spectroscopy using the IL 1-ethyl-3-methylimidazolium acetate (EMIMAc). It was observed in this study that the IL brings about swelling of cellulose and dissolution of lignin. On precipitation with an antisolvent (water), the lignin remains in solution while the cellulose precipitates. This was evident from reduced fluorescence in the precipitated material[75]. All these investigations lead to the question whether ILs can be used successfully as an alternative biomass pretreatment. The patent literature has been enriched in recent years with inventions that employ ILs as a mode of pretreatment of lignocellulosic biomass[76-78], and ILs as a fractionation strategy[79-82]. This was followed by publications that used commercial enzymes for saccharification of biomass that had been treated with ILs[83-85].

Even though the concept of recyclability of ILs, their ability to decrystallise wood and their superiority to the toxic VOCs used in the pulp and paper industry place them favourably as cellulosic solvents, when interactions of whole biomass and IL are considered long reaction times and fine milling add considerable costs to the economic modelling of IL-biomass processes. Lee *et al*.[86], published their results on the ease of enzyme saccharification of wood treated in eight imidazolium based ILs. This study is a realistic step towards actual IL-biomass process since it involves thorough characterization of pretreated material using standard methods in carbohydrate chemistry[86]. However, it is worth noting that the digestibility of the untreated material is reported as 46 % in this study, owing to the fact that the wood was milled to flour before pretreatment. The digestibility of unmilled biomass is much lower than that of milled biomass. This has been demonstrated in studies investigating milling of biomass as a form of pretreatment in itself[87],[88], as it significantly increases the surface area available to the enzymes[89]. Hence converting wood to flour prior to pretreatment with ILs can be equated to using two cost-intensive pretreatments – the first being milling.

Another significant hurdle in upgrading isolated cellulose-IL reactions to biomass-IL is the difficulty in achieving higher biomass:IL ratios. Although up to 25 % (mass) of MCC can be dissolved in BMIMCl[59], when handling whole biomass-IL solutions, the biomass loading invariably drops to about 5- 8 % (mass)[74], [73]. Whole biomass is significantly more difficult to handle than isolated cellulose in terms of: a. reaction times b. reaction temperatures, c. particle size and d. loading of substrate. Consequently in order to make IL-biomass processes cost-effective, the need to find an ionic liquid that can dissolve both lignin and cellulose became imperative.

Dissolution of lignin in ILs

It is evident (from Figure 2-13) that lignin dissolution is the least investigated aspect of IL-biomass interactions, while lignin appears to be the primary impediment in IL-biomass reactions. In order to make IL based biorefineries economical, it is essential that revenue be generated from the lignin recovered from the pretreatment. About 26 million tonnes of lignin are manufactured annually as a by-product of the environmentally harsh Kraft pulping process. This lignin is thiolated and mainly used as combustion fuel. Since it has a relatively high initial water content, its fuel value is low, producing less than ¼ as much energy for an equivalent mass as middle distillate (diesel, jet and boiler) fuels[90]. Moreover, it has been demonstrated that lignin can be converted to value-added products such as adhesives, coatings, polymer blends, and carbon fibre composites[90-92].

In 2005, Myllymaeki and Aksela[93] filed a patent that addresses dissolution and delignification of biomass with microwave irradiation. Since then, some contributions have been made to the scientific literature concerning lignin dissolution or delignification. Pu *et al.*[94], studied the dissolution of isolated lignin in imidazolium based ILs using a range of anions with the imidazolium cation. In their studies, it was found that anion [MeSO4]- was most effective in dissolving isolated lignin and that anions dictate the selectivity of lignin dissolution. However, there have not been any studies that used different cations to confirm the finding.

Tan *et al.*[95], used imidazolium alkylbenzenesulfonates to extract lignin from bagasse, which was inspired by the traditional hydrotropic pulping that uses sodium xylene sulfonate. The IL was successfully recovered and showed no structural changes. Up to 93 % delignification was reported. Here, it is worth taking into consideration that to achieve a 93 % yield of lignin, temperatures as high as 190 °C were used. This supports the observations made from previous studies involving biomass IL interactions with respect to high temperatures. 1-Ethyl-3-methylimidazolium acetate (EMIMAc) has been investigated for simultaneous dissolution of both cellulose and lignin[75,96]. In 2008, Edye and Doherty[97] filed a patent that addresses the fractionation of lignocellulosic material by employing a biphasic system of lignin and cellulose solvents. Rodirguez *et al.*[98], recently published their work with biphasic systems of ILs with Poly Ethylene Glycols (PEG), taking advantage of the exclusive solubility of lignin in PEG and the solubility of both lignin and cellulose in ILs such as BMIMCl.

The chemistry of IL-biomass degradation reactions

In any biorefinery process that aims at producing ethanol as an end product, a careful analysis of the desired and undesired reactions becomes necessary. ILs have been sought for properties such as high liquidus range, negligible vapour pressure and thermal stability[57]. However, the combination of a highly ionic reaction medium, elevated at high temperatures for long reaction times also inevitably leads to many side reactions that are undesirable to fermentation. It is a well known fact that numerous fermentation inhibitors are produced when biomass is pretreated using dilute acid[99], hydroxymethylfurfural (HMF) being one of the most important of these.

Although a detailed analysis of the degradation reactions during IL-biomass pretreatments has not been published yet, numerous publications have covered the use of ILs to produce HMF for platform chemicals using biomass in metal chloride catalysis[100] and acid catalysis[101], [102] in ILs. Although these publications suggest an entirely different pathway for producing high value fuels, production of HMF in reactions intended for fermentation also comes into the picture due to these studies. It is essential to evaluate the yield of ethanol using ionic liquids especially for reactions above 80 °C as higher temperatures also favor production of degradation products. Apart from HMF, other byproducts such as acetic acid, formic acid, levulenic acid are formed when biomass is treated in hydrophilic environments.

While the carbohydrate portion of biomass leads to the formation of acids and furans, the lignin monomer is often deconstructed in situ due to acid/base catalysed reactions[37]. These reactions have been studied in traditional pulping and solvent systems such as, acid bisulphite pulping and Organosolv pulping. However, it is worth understanding whether the lignin macromolecule breaks down in the same way in ionic liquids as it does in traditional reactions. Xie *et al.*[103], have investigated the chemical modification of wood in ILs. In their study, it becomes evident that all the phenolic OH groups in the lignin macromolecule are consumed during the extraction process of lignin in IL probably owing to condensation reactions. These studies were based on expanding the understanding of biomass modification, *e.g.*, carbanilization, and bezolyzation using ILs. However, it is worth noting that even a catalytic amount of impurity could cause cleavage of ether bonds in the lignin considering the sensitivity of ILs to these reactions. Ebner *et al.*[104], have investigated the inertness of imidazolium ILs to cellulose and found convincing evidence that numerous side reactions, including degradation of cellulose as well as IL occur during IL-cellulose interaction. They also questioned the inertness of butylmethylimidazolium ILs as the outcome of the experiment demonstrates that ILs react with the C2 position with the reducing ends of cellulose and aldopyranose model compounds.

Interaction of biomass with different ionic liquids

Experimental studies have been carried out using a number of cations and anions[105,106]. The ILs that have been already investigated for dissolution of cellulose can be characterized loosely based on: a. cation (*e.g.*, alkylimidazolium/tetraalkylphosphonium) b. anion (*e.g.*, chloride, hexafluorophosphate, methylsulfate), c. length of alkyl substituent on cation (methyl, butyl, hexyl, octyl) d. hydrophobicity or hydrophilicity of the IL used.

Imidazolium based ionic liquids

Most papers published in the scientific literature focus on the study, application and optimization of the imidazolium cation[59,60,68,86,107]. Various theories on why this particular class of ILs works in dissolving up cellulose have been proposed. We shall try to review them in this section.

Cation and anion pairs for cellulose dissolution and biomass pretreatment

In the initial investigations in this area, the combination of 1 butyl-3-methylimidazolium cation with a chloride anion was considered most effective for cellulose dissolution[59], as it could dissolve up to 25 % mass cellulose with microwave heating. In this study it was observed that non-chloride anions, *e.g.*, BF_4^- and PF_6^- were ineffective at dissolving cellulose[59]. AMIMCl was able to swell cellulose at room temperatures and dissolve it at 60 °C with stirring. In this study, AMIMCl is reported to have dissolved a maximum of 14.5 % (mass) of cellulose at 80 °C , without microwave heating[67].

In their review published on the study of cellulose dissolution using different cation-anion pairs, Simmons *et al.*[108], present seven cations in combination with eighteen anions. According to this review 1-Allyl-3-methylimidazolium formate (AMIMHCOO) can dissolve to 21 % cellulose. Surprisingly, the IL benzyl-dimethyl tetradecyl ammonium chloride, which is comprised of a bulkier cation than AMIMHCOO shows a higher dissolution of cellulose (39 % mass). Lignin dissolution appears to be predominant in ILs with the methyl sulphate or methylsulfonate anions, the highest dissolution of 34.4 % (mass) being in the IL 1,3-dimethylimidazolium methylsulfonate[108]. This is also supported by the work carried out by Pu *et al.*[94]

which shows that 1-methyl-3 methylimidalozium methylsulfate (MMIMMeSO_4) and BMIMMeSO_4 could dissolve a maximum of 344 g/L and 312 g/L of isolated kraft pulp lignin[94].

Zavrel *et al.*[107], published their work on high throughput screening of six ionic liquids for cellulose dissolution. Their work suggest that EMIMAc is the most efficient solvent for dissolution of cellulose as demonstrated by the in situ microscopy studies, while AMIMCl was successful in dissolving completely, biomass from four different sources in addition to crystalline cellulose.

As alternatives to the corrosive and viscosity-inducing halides, halide-free ILs with high hydrogen bond basicity have been successfully employed in the dissolution of cellulose. For example, Fukaya *et al.*[109] found that the low viscosity [AMIM]HCOO dissolves *ca.* 20 % mass of microcrystalline cellulose (*DP ca.* 250) at 80 °C, whereas AMIMCl dissolved only *ca.* 2 % under the same conditions. The same team continued the investigation for halide-free ILs by synthesizing and testing some $[MMIM]^+$ ILs with varying phosphonate anions[110]. Their results indicated that these anions where exceptionally good cellulose solvents at very mild temperatures (i.e. 45 °C). All ILs tested had high dipolarity, high hydrogen bond basicity (β, a Kamler-Taft solvation parameter) and low viscosity to which their success was attributed. [MMIM]methylphosphonate dissolved cellulose at high concentrations (10 % mass) at 45 °C and 30 min and could also dissolve lower concentrations (2 % to 4 % mass) at room temperature. The closely analogous [MMIM]diethylphosphate was reported as a good solvent since cellulose degradation during dissolution was low[111].

Hydrophobic and hydrophilic ILs

The explorative studies and screening experiments carried out using various hydrophobic ILs (anions such as BF_4^- and PF_6^-) suggest that hydrophobic ILs are do not dissolve cellulose as effectively as hydrophilic ILs (with anions such as Cl^-, $HCOO^-$)[59]. Whether an IL is hydrophobic or hydrophilic depends on the length of alkyl chain on the cation and the anion[112]. The presence or absence of water in ILs has been linked to its ability to dissolve cellulose. High amount of water leads to the competition between the hydroxyls in water and the hydroxyls in cellulose. However, it is also interesting to know that hydrophobic ILs are not effective in dissolving cellulose either[59].

Size of the cation

The effect of dialkylimidazolium cation structure on cellulose solubility has been systematically studied. Generally, cellulose was found to be soluble in chloride salts of imidazolium with ethyl, butyl and allyl side chains, with decreasing solubility as alkyl chain length increases. It was also observed that even numbers of carbon atoms show higher cellulose dissolution in the series C_2 to C_{20} as compared to odd numbers[113]. This unexpected and unexplained outcome was later corroborated by Vitz *et al.*[111].

In the first study of this kind, Swatloski *et al.*[59], conducted screening experiments for cellulose dissolution in nine imidazolium ILs. The selection of ILs included a range of alkyl substituents on the cation; starting from the smaller 1-butyl-3-methylimidazolium to 1-octyl-3-methylimidazolium cations. It was observed that the length of the alkyl chain, which also increases the size of cation, influences dissolution of cellulose in ILs. This hypothesis was corroborated by the experiments published by Zhang *et al.*[67], with AMIMCl for cellulose dissolution. They argue that AMIMCl is more effective at dissolving cellulose at lower temperatures than BMIMCl because of the smaller size of the cation. Similar results have been obtained in subsequent studies[107,109], making the AMIMCl as well as AMIMHCOO, a better cellulosic as well as lignocellulosic solvent than BMIMCl.

Temperature and heating

Swatloski *et al.*, reported up to 25 % (mass) dissolution of isolated cellulose in BMIMCl. This reaction was aided by microwave pulses[59]. Dissolution of whole biomass, typically requires temperatures higher than 100 °C. Kilpalainen *et al.*[73] carried out dissolution of wood in fourteen different imidazolium chloride ILs. They reported highest dissolution of sawdust of 8 % with AMIMCl and BMIMCl at 110 °C for 8h. However, the effect of temperature and milling of biomass is evident in this study as reaction times drop to half when milled wood is used instead of chips at 130 °C. Also, at 130°C and 8h, all ILs could dissolve 2-7 % of wood.

The higher temperatures required for dissolution of wood could be due to the fact that most lignins have a glass transition between 130 °C and 150 °C[114,115]. Initially, studies involving MCC were carried out at milder temperatures of below 100 °C[59,67,107,116]. For regeneration of cellulose as well as biomass, temperatures above 100 °C have been used[83-85,108,117,118]. It has been observed that sugar yields show drastic improvements at higher temperatures (around 140 °C -150 °C) and lower reaction times[84,102]. Studies with whole biomass invariably use temperatures in the range of 120 °C-150°C[68,83,119]. At this juncture it is critical to ask the question whether complete dissolution of biomass is essential for an effective pretreatment. While reaction times of up to 15 h have been used to dissolve wood completely in ILs[73], it is worth noting that IL pretreatment (partial dissolution) at 120 °C and 0.5 h can improve the saccharification of treated material significantly[83,84].

Addition of antisolvents

If ILs are to be used in a commercial process, a closer look at the antisolvents has to be taken. Some laboratory studies have used a combination of water and organic solvents for precipitation of dissolved biomass from ILs[68,83,120]. In some of these publications, the amount of antisolvent used is not quantified[83,121]. However, the amount of quantified antisolvent varies from 1:1[84] , 1:2[68], 1: 3[74] to 1:10[120] IL:antisolvent ratio. There is a need to quantify and optimize the use of water as an antisolvent in IL:biomass processes as it would dictate the economics of the process.

Biomass loading in ILs

Improving biomass:IL ratios is also imperative to achieving better economics in biomass-IL processes. All the laboratory scale studies on biomass have been limited by a maximum loading of only 5 % (mass) biomass:IL ratios[68,74,83,84,103,120,121]. All studies have been carried out on imidazolium ILs. This is true even in case of BMIMCl and AMIMCl, which can dissolve more than 10 % (mass) MCC[59,67]. Hence, it can be said with certainty that analogous to the temperature effect where higher temperatures are required to solubilize biomass as against MCC, biomass loading is also important. This is often attributed to the viscosity of imidazolium ILs, which is significantly higher than aqueous or organic solvents[122]. The added complexity of intermolecular bonds between lignin, hemicellulose and cellulose, with the existing intramolecular hydrogen bonding of cellulose[39] appears to complicate interactions of whole biomass with ILs.

Mechanism of dissolution of cellulose and lignin in imidazolium ILs

The mechanism of cellulose dissolution in ILs is not fully understood, but there is evidence to suggest that both the cation and the anion take part in the hydrogen bond disruption of the cellulose chains. ^{13}C NMR and $^{35/37}$Cl NMR relaxation studies indicated that there is a 1:1 stoichiometric interaction between the chloride ions in BMIMCl and the hydroxyl groups in cellulose[123]. Although initially the combination of imidazolium cation and chloride anion was linked to cellulose dissolution drawing a correlation from the LiCl/DMAC solvent system, the dissolution of biomass by EMIMAc that effectively dissolves lignin as well as cellulose[75,124,125] has led to reassessment of the chloride activity theory.

The work conducted by Zhang *et al*.[67], also challenges the theory that anion dictates the dissolution of cellulose in ILs. The authors argued that the dissolution of cellulose is not exclusively a property of anion as concluded by the initial studies. The chemical properties of small cations also contribute to the dissolution of cellulose, which is true even in the case of the traditional LiCl/DMAC solvent system. Lithium is the only alkali metal that led to the dissolution of cellulose in combination with a chloride anion. Other metals such as sodium, potassium, barium, calcium and zinc were ineffective in dissolving cellulose, probably owing to their larger atomic size. The correlation of temperature and higher dissolution rates was attributed to the increase in the conductivity of the IL with temperature. It was speculated that higher ionic activity of the solvent was responsible for enhanced rates of dissolution[67]. Figure 2-14 shows one of the proposed mechanisms of cellulose dissolution in imidazolium ILs.

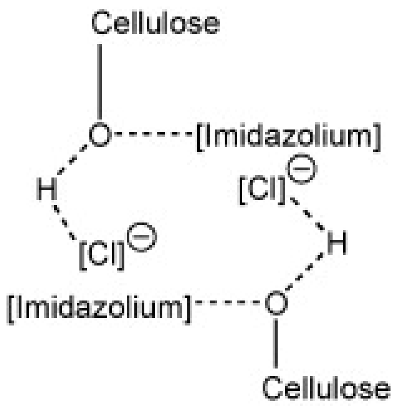


Figure 2-14 Mechanism of dissolution of cellulose in chloride ILs[132]

Hydrogen bond basicity (β, a Kamler-Taft solvation parameter) and strong dipole moments of the IL have been reported as pivotal for the performance of ILs as both cellulose and biomass solvents[126,127]. Conventional non-aqueous cellulose solvent systems such as DMA / LiCl exhibit notably high hydrogen bond basicity and high polarity. Not surprisingly the same is observed in cellulose solvating ionic liquids. BMIMCl, the most cited IL for cellulose dissolution, is a highly polar IL with a high β value[128] and [AMIM]HCOO solvated higher amounts of cellulose than [AMIM]Cl due to the hydrogen bond basicity of the former being 1.2-fold that of the latter[67,126].

Amongst the imidazolium ILs that have been explored for dissolution of either cellulose or whole biomass, BMIMCl has been most effective in dissolving up MCC[59] and EMIMAc has been most effective in dissolving wood, i.e., partial delignification along with dissolution of cellulose[125]. EMIMAc has received significant attention as a solvent for both cellulose and lignocelluloses[120,129,130]. Its low toxicity, relative compatibility with cellulose enzymes and high solvating capacity are its most prominent attributes. However, it has been demonstrated that EMIMAc does not act solely as a solvent but also covalently interacts with the reducing ends of cellulose chains as investigated by Liebert and Heinze.[60] (see Figure 2-15). This chemistry was further confirmed by means of ^{13}C-isotopic labelling experiments carried out by Ebner *et al.*[104]. Surprisingly these covalent interactions do not form with BMIMCl[60], which leads to speculation that either the glycoside formation is catalysed by the basicity of the acetate anion, or the stronger ion pairing network that chlorides form with the imidazole ring prevents covalent bond formation[60].

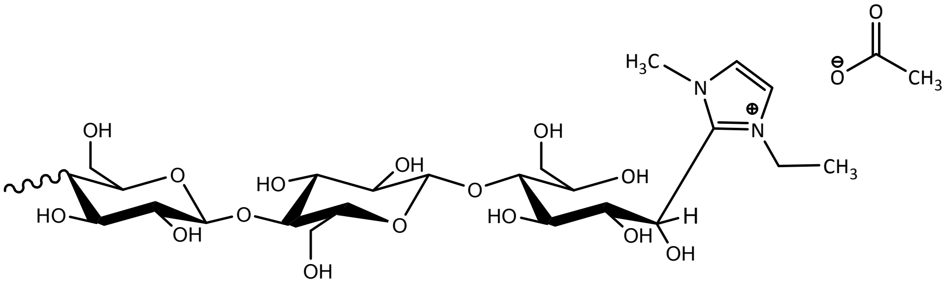


Figure 2-15 Structure of proposed covalent binding of EMIMAc to cellulose[60]

A recent study by Liu *et al.*, hypothesizes that the cation and anion both take part in the dissolution of cellulose. Their dynamics calculations was based on EMIMAc and the study found that there is a change in conformation of the oligomers and the rotamers in cellulose. This explains the 'regeneration' of cellulose in imidazolium based ionic liquids[131]. Binder *et al.*[132] proposed mechanisms for acid catalysed delignification in a range of ILs. The conversion of eugenol to guiacol was monitored to measure dealkylation of lignin like compounds in ILs. An acid catalysed delignification mechanism that proceeds via the formation of Hibbert's ketones has been proposed along with the mechanism for condensation reactions has been proposed in this study[132]

Phosphonium ILs for dissolution of biomass

Phosphonium ILs have been successfully utilized various chemical processes[133-135]. However, they have not been sufficiently explored in the field of biomass chemistry. Fraser and MacFarlane have published their findings on the physical and chemical properties of various phosphonium ILs[136]. Considering their significantly high thermal stability, they would make an excellent medium for high temperature biomass dissolution reactions.

Catalysis reactions in ILs

Due to the high temperature window ILs offer, they can be utilized as solvents in which to carry out catalysed reactions. One such reaction is the acid catalysed hydrolysis of carbohydrates from biomass. *In situ* hydrolysis of sugars in ILs and subsequent separation has been investigated by several groups in the past few years [116,137]. However, the hydrolysis of sugars in ILs complicates the recovery process of both the solvent and the sugars. Rinaldi *et al.*, have reported a pathway of depolymerization of cellulose by utilizing an acidic resin Amberlyst 15DRY. The resins break down the cellulose into oligomers and subsequently sugars. If the reaction is stopped at the right time, depolymerised cellulose can be obtained for further processing[138]. Zhao and co-workers[100] utilized acid in ILs to convert the cellulose to HMF using metal chlorides as catalysts. Although this discovery goes against the production of cellulosic ethanol, HMF can be utilized to prepare other platform chemicals. Binder *et al.*[132] have reported acid catalysed depolymerization reactions of lignin in ionic liquids. The study involved high throughput screening of a wide range of Lewis and Brønsted acid catalysts in combination with reduction catalysts in a variety of ionic liquids using a model lignin compound eugenol[132].

In a similarly motivated study, Binder *et al.*[102] studied the reaction of conversion of fructose to HMF in the traditional solvent combination of LiCl/DMA using Bronsted acids (e.g., H_2SO_4) and metal chlorides as catalysts. In this study, it was observed that the addition of 5-20 % (mass) of various ILs, improved the yield of HMF. This was attributed to the fact that the chloride ions in ILs are loosely bound as compared to LiCl/DMA. It was also observed in the same study that the HMF yields for untreated biomass using this system were similar to the AFEX pretreated biomass[102]. However, the study does not address the recovery of all the solvents and catalysts for reuse.

It is evident that dissolution of isolated cellulose and isolated lignin in ILs does not paint a clear picture of IL-biomass interactions. The complex structure of plant architecture still poses a significant challenge in IL-biomass reactions. It is essential to understand IL-biomass interactions both from a chemistry and sustainability perspective. In a broader sense, ILs appear to be a potentially ‘green’ solution to the alternatives that are currently available. However, clarity in their mode of action is essential if they are to be used in a large scale process.

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Chapter 3 Review: The use of ionic liquids for the production of liquid fuels from lignocellulosic biomass

Author's Statement

This review article has been submitted to the journal Biofuels.

The first author of this paper, Sai S. Keskar is responsible for reviewing and compiling relevant literature as well as its interpretation in terms of chemistry of ionic liquids in biomass dissolution.

The interpretation of possible mechanisms of wood dissolution, IL recovery and possible acetylation of biomass in EMIMAc was kindly shared by Sergios K. Karatzos from the literature review and results of his PhD dissertation. Les A. Edye provided insights into the economic viability of ILs and carried out the economic evaluation presented in section 6.

Chapter 4 . ATR-FTIR measurement of biomass components in phosphonium ionic liquids

Author's Statement

This paper has been submitted to the Journal of Wood Chemistry and Technology.

The first author of this paper and the author of this thesis (Sai S. Keskar) is responsible for the experimental design, execution, interpretation of data and writing the first, complete draft and subsequent revisions of the later drafts until the final submission.

Dr. Leslie A. Edye provided valuable insights into interpretation of the available data and organizing the results to strengthen the central argument. He was also responsible for coaching the first author towards a literal, scientifically unambiguous expression.

Dr. Christopher Fellows provided guidance in early conceptual design of this paper by reviewing raw data and extending his interpretation.

Dr. William Doherty supervised the initial experimental design.

Chapter 5 The chemistry of acid catalysed delignification of sugarcane bagasse in the ionic liquid trihexyltetradecylphosphonium chloride

Author's Statement

This paper has been accepted for publication by the Journal of Wood Chemistry and Technology.

The first author of this paper and the author of this thesis (Sai S. Keskar) is responsible for the experimental design, execution, interpretation of data and writing the first, complete draft and subsequent revisions of the later drafts until the final submission.

Dr. Leslie A. Edye provided guidance in the interpretation of the FTIR data and the design of rate kinetics experiments.

Dr. William Doherty provided valuable guidance in the entire experimental design of this paper.

Dr. John Bartley made important contributions into the design of high temperature consorted mechanism for delignification.

Chapter 6 Role of acid in dissolution of sugarcane bagasse in tributylmethylphosphonium methylsulfate

Author's Statement

This paper has been submitted to the Journal of Wood Chemistry and Technology.

The first author of this paper and the author of this thesis (Sai S. Keskar) is responsible for the experimental design, execution, interpretation of data and writing the first, complete draft and subsequent revisions of the later drafts until the final submission.

Dr. William Doherty provided guidance in experimental design.

Dr. John Bartley provided valuable guidance in the design, execution and interpretation of ^{31}P NMR.

Dr. Les Edye provided coaching in interpretation of results and strengthening the core argument of the paper with a critical review of the available literature.

Chapter 7 SUPPLEMENTARY INFORMATION

Introduction

This chapter presents the significant results produced during the course of this PhD that have not been included in the submitted work but have been crucial in giving the experimental work direction.

Chapter 4: ATR-FTIR measurement of biomass components in phosphonium ionic liquids

Screening experiments with imidazolium and phosphonium ILs

Choice of ILs

Crystalline cellulose (Avicel, Sigma Aldrich) amorphous cellulose (Solkafloc, International Fiber Corporation, New York) and isolated soda lignin (prepared in the laboratory using standard procedure[1]) were dissolved in 8 ILs (in Table 7-1) at a range of temperature and time measurements.

Table 7-1 Initial screening experiments with ILs

IL	Acronym	Properties
1-Butyl-3-methylimidazolium chloride	BMIMCl	Hydrophilic IL known to dissolve cellulose [2]
1-Allyl-3-methylimidazolium chloride	AMIMCl	Hydrophobic IL known to dissolve cellulose[3]
1- Ethyl-3-methyilmidazolium chloride	EMIMCl	Hydrophilic IL known to dissolve lignocellulose[4]
1-Ethyl-3-methylimidazolium acetate	EMIMAc	Hydrophilic IL known to dissolve lignocellulose[5]
1-Butyl-3-methylimidazolium hydrogen sulfate	$BMIMHSO_4$	Hydrophlic IL used in catalysis reactions[6]

Tributyltetradecyl)phosphonium chloride	[P44414]Cl	Hydrophobic IL, solid at RT.
Trihexyl(tetradecyl) phosphonium chloride	[P66614]Cl	Hydrophobic IL, liquid at RT.
Tributylmethylphosphonium methyl sulfate	[P4441]MeSO$_4$	Hydrophilic IL, liquid at RT.

Screening experiments were carried out at a temperature range of 80 °C to 150 °C. and a time range of 30 min to overnight. While dissolution of crystalline cellulose was possible in in BMIMCl, EMIMCl, EMIMAc and especially in AMIMCl at temperatures below 100 °C, lignin and whole bagasse only dissolved when temperatures were taken above 120 °C. Phosphonium ILs [P44414]Cl and [P66614]Cl did not dissolve cellulose at any temperature or time, while [P4441]MeSO$_4$ could not form a clear solution with cellulose.

However, lignin was soluble in all phosphonium ILs. When dissolution experiments were carried out with milled bagasse, a change in color was observed in all ILs above 120 °C (30 min).

There is a significant body of already published data on imidazolium ILs [2,4,5,7,8]. However, there was no recorded publication in the use of phosphonium ILs for biomass processing. Hence a detailed study of these ILs was more significant for this thesis. The experimental work for this dissertation commenced in December 2007. Most of the references in Table 7-1 have appeared in the public domain after the commencement of these experiments. There was no published record of dissolution of wood in EMIMAc until late 2008. The motivation to study phosphonium ILs stemmed from their absence in published literature, their thermal stability and their lower cost compared to imidazolium ILs.

ATR-FTIR in imidazolium ILs is difficult to carry out due to IL-related absorbances at wavenumbers associated with biomass. By comparison, ATR-FTIR of phosphonium ILs was clear and straightforward.

FTIR study in [P44414]Cl

In situ ATR-FTIR study was performed on phosphonium ILs. The hydrophobic [P44414]Cl showed FTIR absorbances identical to [P66614]Cl, presented in Chapter 2. However, [P44414]Cl is solid at RT, which made the handling of reaction mixtures difficult. This posed significant problems in the later steps of the dissolution experiments such as filtration and centrifugation of the dissolved and undissolved fractions. Hence, the experiments with [P44414]Cl were not pursued.

Polarity measurements of phosphonium ILs

Polarity studies of imidazolium and pyridinium ILs using Kamlet-Taft parameters has been carried out by other researchers to study the dissolution of polysaccharides in ILs [9,10]. To better understand the observed dissolution of lignin in phosphonium ILs, a study of polarity was attempted using n-n-diethyl-4-nitroaniline, Reichardt's dye and 4-nitroaniline following the method described by Fukaya *et al.*[9]. However, the hydrophobicity of [P44414]Cl and [P66614]Cl made determination of these values cumbersome. Hence, a clear calculation of the polarity of ILs could not be achieved.

Chapter 5: The chemistry of acid catalysed delignification of sugarcane bagasse in the ionic liquid trihexyl tetradecyl phosphonium chloride

Recovery of dissolved lignin from [P66614]Cl

Recovery of dissolved lignin from [P66614[Cl was a challenge that could not be overcome during the course of this work.

It is possible that the lignin that was extracted into the IL was fragmented and condensed due to acid catalysed cleavage of ether bonds[11].

The extraction of dissolved lignin from the IL was complicated further by the hydrophobic nature of the IL, which limited its miscibility to organic solvents such as acetone and ethanol. However, a number of methods were designed to recover dissolved lignin.

Aqueous alkali biphasic system

A solution of 14 % NaOH (200 mL) was mixed with the IL-lignin solution (10 g) and shaken vigorously in a separating funnel. The mixture was allowed to separate into two phases – aqueous phase and IL phase. It was anticipated that the dissolved lignin would be soluble in strong alkali solution and hence can be recovered in the aqueous phase. However, the lignin preferred the IL phase to the aqueous phase. The concentration of NaOH was subsequently increased to 20 % with no change in outcome.

Organic-aqueous biphasic system

Mixtures of acetone:water (1:1, 200 mL), ethanol:water (1:1, 200mL), acetone:14 % NaOH (1:1, 200 mL) ethanol: 14 % NaOH (1:1, 200 mL) were mixed with the IL-lignin (10g) solution. The aim of this experiment was to find out whether the dissolved lignin was easier to recover into an aqueous phase with the aide of a strong organic solvent. However, the biphasic systems separated into an organic layer (containing IL, lignin and organic solvent) and aqueous layer (containing water or NaOH solution).

It was not possible to use organic solvents to recover lignin due to the solubility of the IL in organic solvents.

Chapter 5 and Chapter 6: Study of undissolved fractions

Enzyme hydrolysis of the undissolved recovered fractions

Bagasse and pretreated bagasse samples (2 % glucan equivalents) were suspended in citrate buffer (10 mL, 50 mM, pH 4.7) and equilibrated on a temperature controlled rotary shaker (150 rpm, 50 ˚C). Accelerase 1000 (Genencor) was added to achieve an enzyme activity of 15 FPU g^{-1} (50 µL of Accelerase as received). Samples (0.5 mL) were removed periodically, placed in ice, centrifuged at 4 ˚C and then frozen. After thawing of the samples at room temperature, glucose concentrations were measured by HPLC (Waters, RI detector, Bio-Rad HPX-87H column operated at 85 ˚C with a 5 mM H_2SO_4 mobile phase at a flow rate of 0.6 mL min^{-1}). The glucose results were converted to glucan mass equivalents equivalents using appropriate multiplication factors (Figure 7-1).

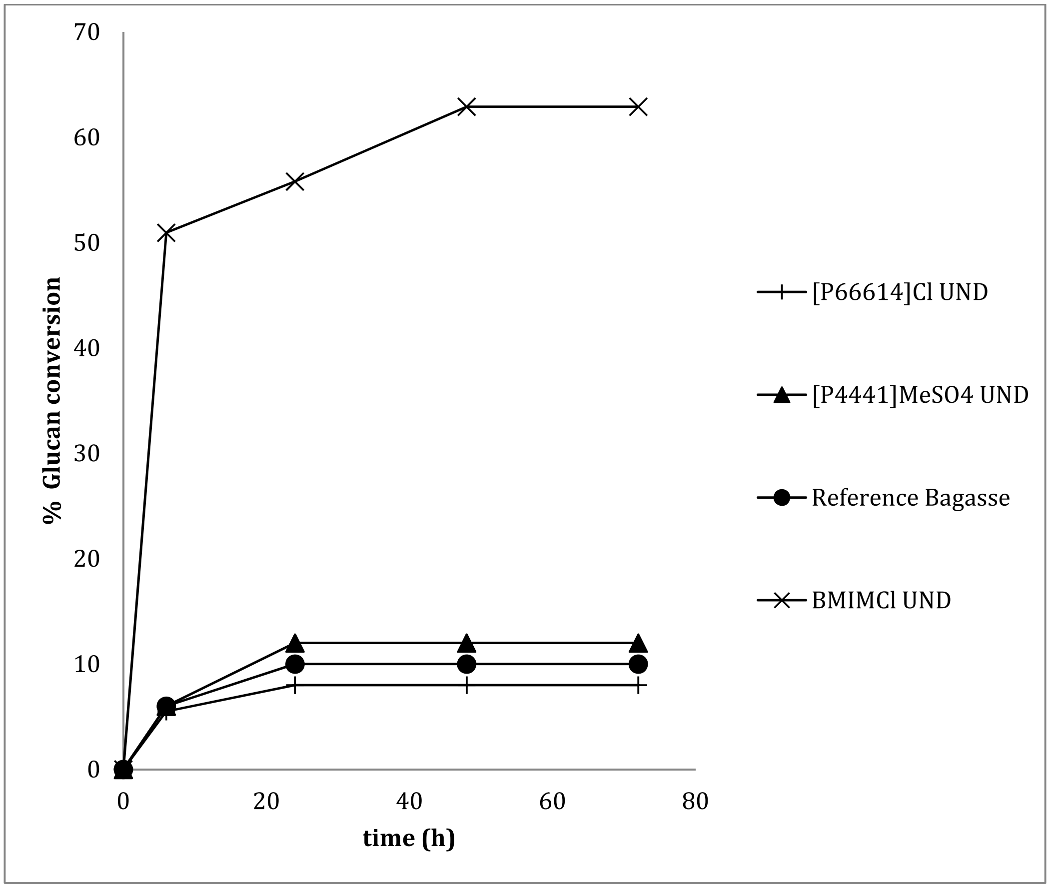

Figure 7-1 Enzyme hydrolysis of undissolved fractions

It can be seen that although there is lignin removal in both ILs, they perform poorly in enzyme saccharification. To compare the performance in terms of glucan saccharification, the glucan conversion of the undissolved fraction after the treatment of bagaase with BMIMCl (150 °C, 90 min)[12] has been included in Figure 8-1. While, at 72 h, more than half of the glucan in the undissolved fraction from BMIMCl has been converted, the conversion is close to that of untreated material in the samples treated with phosphonium ILs.

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Chapter 8 CONCLUSIONS

The overarching objective of this study was to understand the chemistry of dissolution of biomass in phosphonium ILs. The approach towards achieving this was, to initially identify ILs that were able to dissolve isolated components of bagasse *viz.* MCC, lignin and hemicellulose and then identify if these components were successfully extracted from biomass. The biomass-IL reactions were studied taking into consideration the effect of a range of parameters. Some of the findings of this work are presented here with conclusions.

In situ ATR-FTIR measurement of IL-biomass mixtures

Phosphonium ILs offer an opportunity to study reactions of biomass by the ATR-FTIR measurement of biomass components that have been dissolved and are in solution. The lack of straight forward ATR-FTIR measurement of dissolution in imidazolium ILs (without employing chemometric analysis) was an impediment in understanding the chemistry of biomass in ILs. Although the effect of ILs on cellulose and lignin dissolution has been unravelled using post-pretreatment analysis such as carbohydrate characterization, XRD, ^{13}C NMR, the analysis of reaction mixtures has not been presented.

Lignin related absorbance bands were clearly observed in the reaction of milled bagasse with phosphonium ILs, which was confirmed by dissolving isolated lignin. Quantitative measurement of aromatic skeletal vibrations at 1510 cm^{-1} from the lignin in solution were obtained for [P66614]Cl and [P4441]MeSO$_4$. The appearance of 1510 cm^{-1} in the hydrophobic [P66614]Cl as well as hydrophilic [P4441]MeSO$_4$ was indicative of lignin dissolution. However, the presence and absence of 1705 cm^{-1} (indicative of acid catalysed β-ether cleavage) and the subsequent understanding of mechanism it offers, proves the utility of the ATR-FTIR measurement of reaction mixtures. That the delignification of bagasse in [P66614]Cl is acid catalysed would not have been discovered without the measurement of absorbance band at 1705 cm^{-1} and this led to the realizatisation that [P66614]Cl contained an acid impurity carried over from its manufacture. Furthermore, the discovery of the origin of acidic protons in an IL that does not have any ring protons that could be susceptible to deprotonation, as in the case of imidazolium ILs, was possible due to the evidence of acid catalysis in the ATR-FTIR experiments.

The other advantage of *in situ* monitoring of reaction mixtures was the opportunity it offered to study the onset of undesirable condensation reactions (Chapter 4). The drop in the absorbance at 1705 cm^{-1} is indicative of formation of carbon-carbon bonds in the fragmented lignin as the reactive ketones are consumed in condensation reactions. The absence of absorbance at 1705 cm^{-1} even after adding catalytic amounts of acid to the hydrophilic [P4441]$MeSO_4$ indicates that the presence of acidic protons does not always bring about β-ether cleavage of the lignin bonds in biomass.

Catalytic impurities in ILs

It was discovered in the course of this work that the delignification observed in [P66614]Cl was catalysed by acidic protons due to the residual HCl in IL, which originated from the process of IL manufacture. The IL was ineffective in the absence of acid. This brings us to the important issue of the role of catalytic amounts of impurities in ILs. Due to the wide temperature window ILs offer as solvents, it is possible to carry out reactions that would take longer times in aqueous solutions at lower temperatures. However, this also has a significant disadvantage of accelerating undesirable reactions such as condensation of lignin fragments in [P66614]Cl and formation of HMF in hydrophilic ILs[1,2] with catalytic amount of acid. Hence, it is necessary to know and to measure any trace level impurities that could cause undesirable reactions (or desirable reactions that are incorrectly attributed to the properties of the solvent alone).

Although [P66614]Cl was ineffective without acid, the experiments in aqueous solution of the same amount of acid prove that the IL was responsible for selective removal of lignin. The enrichment of glucan and subsequent reduction in lignin content in the undissolved fractions is an outcome that is specific only to the combination of acid and hydrophobic IL. In the experiments with hydrophilic [P4441]MeSO$_4$ it was observed that in presence of acid, dissolution of all the components of bagasse was increased, however showing a strong preference to lignin dissolution. Hence, the role of catalytic amount of acid differs with the solvents it is added to. A combination of [P66614]Cl with catalytic HCl is a better solvent system for selective lignin removal than acid in water or acid in hydrophilic [P4441]MeSO$_4$.

Temperature and kinetics of dissolution

In this study, the highest delignification was observed at 150 °C in [P66614]Cl. The kinetic study presented in Chapter 5 and the Arrhenius plot for a wide range of temperatures (120 °C to 170 °C) indicates that the rate of cleavage of β ethers at temperatures < 150 °C is significantly faster than at lower temperatures. We hypothesize this shift in the rate to the crossing of glass transition temperature of lignin. A faster concerted mechanism at higher temperatures is plausible because at temperatures above the glass transition of lignin, there is a greater freedom around the carbon- carbon bonds in the lignin molecules.

The faster rate at higher temperatures is a finding of key importance in terms of filling the existing knowledge gaps in the scientific literature.

There has been a significant body of published work on the dissolution of MCC in imidazolium ILs at lower temperatures (80 °C to 110 °C). However, in case of bagasse, dissolution reactions at lower temperatures require significantly higher reaction times. Crossing the glass transition temperature of lignin would reduce the reaction times in the reactions with whole biomass.

The argument around why high temperatures are more effective in dissolving biomass in ILs has not been well supported with experimental evidence. In some publications, enhanced sachharification kinetics of recovered material has been linked to delignification[3]. However, in our studies, we found that removing lignin from biomass does not necessarily enhance saccharification rates. It is likely that enhancement of saccharification is due to decrease in crystallinity of cellulose because of its dissolution in the IL[4].

Acid catalysis in hydrophobic and hydrophilic IL

In chapter 4, we investigated the effect of catalytic amount of residual acid in [P66614]Cl and in Chapter 5, the effect of added acid in the hydrophilic phosphonium IL [P4441]MeSO$_4$ was studied.

The effect of catalytic amount of acid in hydrophilic imidazolium ILs has been shown by other researchers, to effect hydrolysis of polysaccharides *in situ.*

However, due to the inability of the hydrophobic [P66614]Cl to dissolve carbohydrates, catalytic acid caused preferential lignin dissolution by cleaving the β-aryl ether bonds.

When the same amount of acid was added to the hydrophilic [P4441]MeSO$_4$, cleavage of β-aryl ether bonds was not observed (evident from the absence of 1705 cm^{-1} peak). This was also supported by the lignin characterization of dissolved fractions obtained from the reactions with and without added acid. Although significantly more lignin was extracted quantitatively from the bagasse in presence of acid, the structure of the lignin obtained with and without acid was not significantly different.

The acid increased the total dissolution of biomass from 30 % to 70 %. Although there was some glucan and xylan in the dissolved fractions, the IL still demonstrated preferential lignin dissolution, with 56 % lignin in the dissolved fractions obtained with added acid. This is different to the observations made with hydrophilic imidazolium ILs, which dissolve and even hydrolyse more carbohydrates in presence of acid[2].

Future Work

In terms understanding the chemistry of biomass in ILs, there is still exists a knowledge gap in the literature in explaining the mechanism of dissolution in ILs. While in the earlier works, chloride activity in ILs had been strongly linked with cellulose dissolution[5], that claim has been refuted (ILs such as EMIMAc that dissolve cellulose and ILs such as [P66614]Cl that do not dissolve cellulose under any conditions[6,7]). Our studies with phosphonium ILs have led to the speculation that perhaps preferential cellulose dissolution is a property of the imidazolium cation since both phosphonium ILs used in this study showed a strong preference towards lignin dissolution.

In terms of the application of ILs in biomass processing, we believe that at the current cost of the ILs, an IL-biomass process seems unlikely to be economical in the near future. Studies also need to be conducted on the sustainability of IL manufacture. LCA studies need to be conducted on ILs to understand the sustainability of the process of manufacturing ILs. In order to make IL-biomass processing completely 'green', low cost ILs manufactured from renewable sources of carbon are needed. Choline based salt mixtures, also known as Deep Eutectic Salts (DES)[8], perhaps have the potential to be used in place of hydrocarbon based ILs that are currently used in biomass research. The choline cation can be combined with anions from naturally occurring acids such as acetic acid, lactic acid, amino acids and tartaric acids[9]. Using naturally derived materials such as these would make the process of IL manufacture more sustainable.

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