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Pretreatment Of Cellulosic Biomass By Iron-Containing Magnetic Ionic Liquid Dissolution

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PRETREATMENT OF CELLULOSIC BIOMASS BY

IRON-CONTAINING MAGNETIC IONIC LIQUID DISSOLUTION

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ABSTRACT

The focus of this project is to determine the effectiveness, in the preprocessing of biomass when magnetic ionic liquids (MIL) (1-butyl-3-methylimidazolium tetrachloroferrate (Bmim[$FeCl_4$]) and 1-ethyl-3-methylimidazolium tetrachloroferrate (Emim[$FeCl_4$])) are used as a green solvent. Lignocellulose is a promising starting material for a plethora of products, ranging from biofuels to custom chemicals; however, lignocellulose is resistant to enzymatic degradation. Various biomass-preprocessing techniques such as microbial, mechanical, and chemical pretreatment are used for enhancing the digestibility of biomass to sugars for ethanol production. Varieties of ionic liquids have demonstrated the ability to fragment lignocellulose. However, after fragmentation, separation of biomass and ionic liquids has proven to present economic challenges for this pretreatment process. Research has proven that the addition of magnetic properties to the ionic liquid can be used to stabilize the ionic liquids and prevent its loss or other detrimental fluid/fluid interactions in the bioreactor. Therefore, this paper presents the outcomes of such MIL dissolution studies.

DEDICATION

This thesis is dedicated to my mother and my siblings who in one way or another have provided unquestionable support, encouragement, and life long memories.

And to all of my HOH Family, we see diamonds.

LIST OF ABBREVIATIONS AND SYMBOLS

ARP	Ammonia Recycle Percolation
ATR	Attenuated Total Reflectance
DP	Degree of Depolymerization
FTIR	Fourier Transform Infrared Spectroscopy
GSS	Gluclose Standard Solutions
IL	Ionic Liquid
LHW	Liquid Hot Water
MIL	Magnetic Ionic Liquid
MPMS	Magnetic Property Measurment System
NMMO	N-methyl morpholine N-oxide
NMR	Nuclear Magnetic Resonance
RTIL	Room Temperature Ionic Liquid
SCF	Supercritical Fluid
SQUID	Superconducting QUantum Interference Device
UV-VIS	Ultraviolet-Visible Spectrophotometer

ACKNOWLEDGMENTS

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CHAPTER I

1.0 INTRODUCTION

1.1 Biofuel Objectives

Some of the smallest known molecules fuel the world that we live in today. Our planet has developed a plethora of materials based upon amino acids, proteins, and polysaccharides. They range from DNA, cellulose, chitin, elastin, silk produced from worms, and more.[1] The converted biomass provides many sources of usable energy such as hydrocarbon fuels and chemical compounds like alcohols, gums, sugars, and lipid-based products.

The objectives stated in the 2006 research roadmap published by the United States Department of Energy were to place biomass energy conversion research on a fast-track, helping make biofuels an everyday resource and economically feasible by 2012, and by 2030 have the potential to offset 30% of the nation's current gasoline consumption.[2] Therefore, in recent years, there has been a renewed interest and increased research devoted to the development of biofuels made from lignocellulose biomass derived from agricultural byproducts, forest residues, and dedicated energy crops.[3–5]

With this new found push for renewable green-energy sources, important processes involved in the biochemical production of biofuels are being optimized. However, some of the

difficulties arise from the fact that the conversion to biofuel and type of biofuel produced depends greatly on the source/type of biomass. Consequently, most unit operations and processes for converting biomass to biofuel have four major steps: biomass handling, biomass pretreatment, hydrolysis, and fermentation.[6] Out of these steps, the pretreatment process proves to be the most difficult to optimize because complex structures in the biomass of choice are broken down into oligomeric subunits. These subunits are ultimately converted into monomers via hydrolysis and fermentation. Therefore, the development of a more universal pretreatment process is the goal of many bioresource researchers today.

1.2 Pretreatment of Lignocellulosic Biomass

The pretreatment strategies that have been developed thus far are used to improve the reactivity of cellulose and increase the yield of fermentable sugars.[7] Scientists have known that lignocellulosic biomass is a renewable and moderately carbon-neutral source of fuel that is readily accessible, with approximately 200 billion tons produced worldwide per year.[6] There are various pretreatment methods used today: mechanical (physical), chemical, physiochemical, and biological.

However, the full possibilities of cellulose-based biofuels have not yet been utilized for four main reasons: the use of petroleum-based polymers starting in the 1940s, the absence of an optimized green-process to extract cellulose, the difficulty in modifying cellulose properties, and the limited number of common solvents that easily dissolve cellulose.[8] To help achieve the full potential of cellulose based biofuels, one such proposed pretreatment process is the use of a green solvent, ionic liquids (ILs). The use of ionic liquids for lignocellulosic biomass processing

have recently gained more and more attention in science due to the solvent's ability to dissolve a diverse number of biomass types and also the tunability of the solvent chemistry.[7]

1.3 Ionic Liquids

Ionic liquids are defined as low melting points (<100^{o}C) salts, which hold many benefits such as very low vapor pressure, low flammability, recyclability, thermal stability, and low toxicity.[9] Because of these benefits, the ILs can be changed by varying the anion or cation in the liquid thereby creating different classes of ILs. Fredlake et al, stated that "ILs have been considered as solvents for reactions, as absorption media for gas separations, as the separating agent in extractive distillation, as heat transfer fluids, for processing biomass, and as the working fluid in a variety of electrochemical applications (batteries, solar cells, etc.)."[9]

Since, the discovery of ionic liquids in the early 1930s many researchers have looked at dissolution of cellulose in a variety of ILs. Nonetheless, little attention was given to the discovery of ionic liquids and was filed away. In 2002, a study published by Rogers and group proved that some imidazolium-based ILs could effectively dissolve cellulosic material at low temperatures.[10] In 2004, the Hayashi group reported a new class of magnetic fluids: magnetic ionic liquids (MIL). This new class has the same physical properties as non-magnetic ILs, but with the added characteristics of being paramagnetic. With the rediscovery of cellulose in ionic liquids, research in this field has taken off. Multiple research groups have published dissolution profiles for various ILs as shown in Table 1.[11]

Table 1. The dissolution of cellulose in some ionic liquids. The cellulose samples used in these studies commonly differed in degree of depolymerization (DP), molecular weight, or crystal structure.[11]

Ionic Liquid	Solubility (w/w%)	Experimental Condition
[Bmim]Cl	10	Heating at 100°C
[Amim]Cl	14.5	Heating at 80°C after a longer dissolution time
[Bmim]Ac	15.5	Heating at 70°C
[Bmim]Ac/LiAc	19	Heating at 70°C

This thesis examines the use of magnetic ionic liquids (MIL) for the dissolution of lignocellulosic biomass. We do this by first characterization of the MIL, the biomass, and finally the dissolution of biomass in the MIL. A brief introduction, summary of past work, and the structure of this thesis are in the first chapter. Chapters 2 and 3 present a thorough review of conventional biochemical conversion of biomass to fuels and then the current use of ionic liquids for biomass conversion. Chapter 4 describes the characterization of the MIL and biomass for use during the dissolution studies. Chapter 5 is written as a paper to be submitted for publication, it reports the results, conclusions, and a proposal for the future work that can be conducted to further the IL research field in understanding interactions between magnetic ionic liquids and biomass.

CHAPTER II

2.0 REVIEW: BIOMASS TO FUELS A BIOCHEMICAL CONVERSION PROCESS

2.1 Introduction

There have been many papers published about different pretreatment methods that can be applied to enhance the digestibility of lignocellulosic material. Biomass is either grown or acquired from various sources; then is transported to the production sites for biochemical conversion to fuels. Until the 1970s, the idea of agricultural residues such as straw grass or corn byproducts being potential sources of lignocellulosic biofuels was not well recognized. The fuel emergencies during the 1970-1980s was a significant reason for breakthroughs in alternative fuels and engines.[12] Biofuels are some of the most efficient alternatives thus far despite existing criticism, often incorrect, for an unfavorable net energy balance and significant arable land and water requirements.[13]

Biomass is categorized as all materials derived from plant, animal, and microbial origins (see Figure 1). The classification of biomasses used today in conversion to biofuels, are usually based on the animal or plant origin, woody or herbaceous carbon source and physical and chemical characteristics.[12]

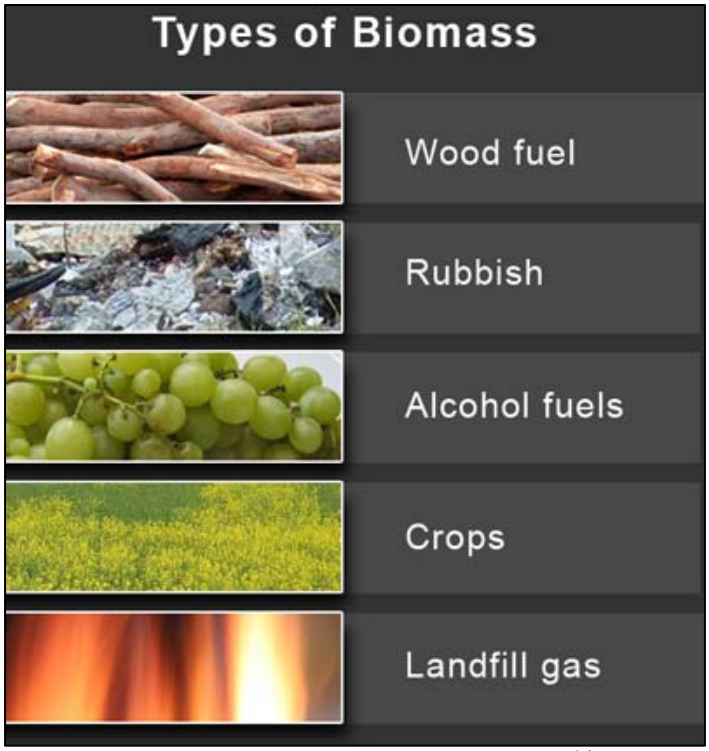

Figure 1: Types of biomass[14]

The plants are the preferred choice of biomass because they are abundant and have high potential to mitigate the emission of greenhouse gases. This section will present the structure of lignocellulosic biomass, an overview of the ethanol production processes, and summarize the different pretreatment methods used in today's industry.

2.2 Composition of lignocellulosic material

Any plant lignocellulosic material consists mainly of three different types of polymers closely associated with each other: cellulose, hemicellulose, and lignin.[15] These polymer components exist in the plant cell wall and vary in percentage by each green plant. Research has shown that, in the lignocellulosic material, cellulose, hemicellulose, and lignin are approximately

30-50%, 10-40%, and 5-30%, respectively.[16] All of these components are intertwined by covalent and hydrogen bonding, making the cell wall resilient to natural degradation.

2.2.1 Cellulose

Cellulose is a classic biopolymer existing in α-D-glucopryanoside subunits, linked by β-1,4 glycosidic bonds (see Figure 2).[15]

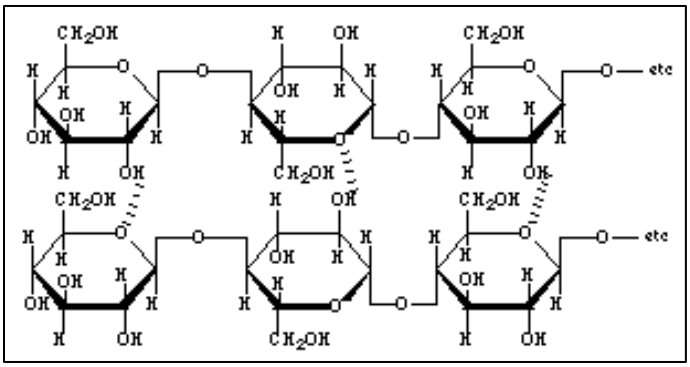

Figure 2: The structure of cellulose[12]

The degree of polymerization (DP) in cellulose ranges from 10^3 to $1x10^6$.[17] Cellulose has both organized (crystalline) and unorganized (amorphous) structure. This crystalline structure forms cellulose strands, which allow the cellulose to be very tightly packed and complex with hydrogen bonding, making the bonds rather difficult to break. This network of cellulose strands form cellulose fibrils (or cellulose bundles), which are again held together by hydrogen bonding. Therefore, the cellulose fibrils are insoluble in most commonly used solvents.

2.2.2 Hemicellulose

Hemicellulose is both a branched polymer and a heteropolymer consisting of different monomers like uronic acids, pentoses (i.e. xylose & arabinose), hexoses (i.e. galactose, mannose,

and glucose).[18] Hemicellulose has a relative low molecular weight when compared to cellulose, short lateral branches, and different sugars that are easily hydrolyzable.[15] This part of the plant cell wall is amorphous in structure, giving little strength to the wall.

Although this component of lignocellulose is not covalently bound to the surface of each cellulose fibril, it is still tightly bound to the surface. Hemicellulose serves as a connection between the lignin and cellulose fibers, thereby creating the cellulose-hemicellulose-lignin network making the plant cell wall more rigid.[19] Therefore, the digestibility of the cellulose somewhat depends on the percent of hemicellulose content present in the sample.

2.2.3 Lignin and structure

The most abundant and last biopolymer after cellulose and hemicellulose is lignin. This unit of the cell wall is a complex amorphous heteropolymer composed of three main phenylpropane units: *p*-coumaryl, coniferyl- and sinapyl acids (see Figure 3).[20] The monomers in lignin have different strong chemical bonds and complex compositions thus providing a cross-linked structure. This in turn makes lignin very strong providing excellent plant structural support thereby making it resistant to many external forces (microbial attack, physical, or oxidative stress), chemicals, and degradation.

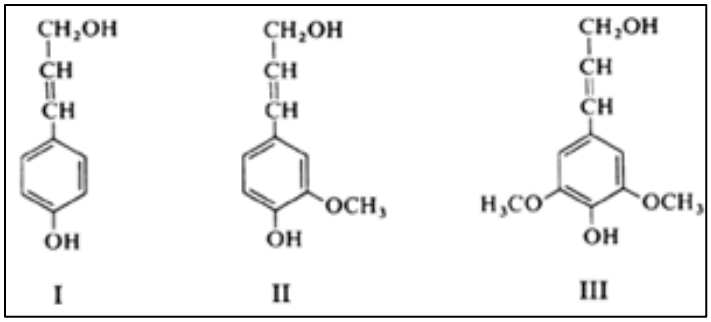

Figure 3: Structural monomers of lignin: *p*-coumaryl alcohol (*I*), coniferyl alcohol (*II*), and sinapyl alcohol[20]

Lignin, the amorphous heteropolymer, is very insoluble in water and optically inactive; again making degradation difficult.[15] The cellulose to lignin ratio is an important factor affecting the conversion process of the lignocellulosic material.

These three components of cellulose, hemicellulose, and lignin bond to make the cell wall (see Figure 4). When these components are combined they have natural factors that bioresource researchers believe make feedstock recalcitrance to degradation [21]:

- the degree of lignification
- the structural heterogeneity and complexity of cell-wall constituents, such as the cellulose microfibrils, the matrix polymers and cross-linkages between these components
- the difficulty enzymes have in acting on an insoluble substrate
- crystallinity and restricted solvent accessibility

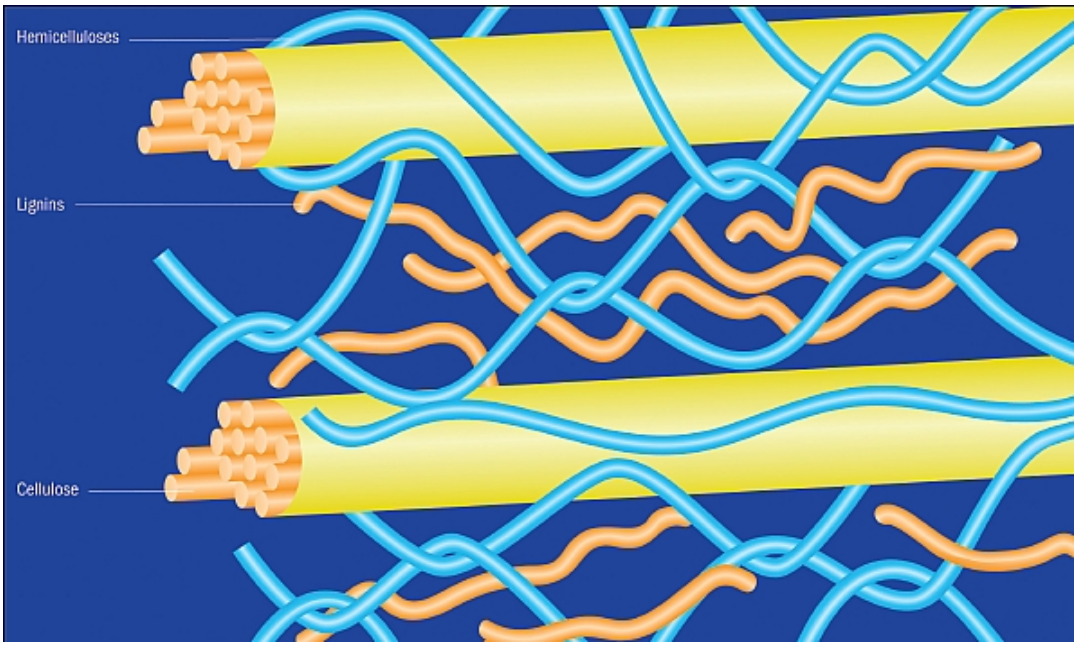

Figure 4: A schematic of a plant cell wall showing cellulose fibrils (brown) laminated with hemicellulose (turquoise) and lignin (orange) polymers. [21]

These well-designed structural and chemical benefits in lignocellulosic biomass affect the enzyme accessibility and activity and/or liquid penetration. Therefore, the crucial point to an efficient and economical production of biofuel is the separating of these complex structures into their subunits.

2.3 Process Overview

Ethanol produced from lignocellulosic biomass is one of the most abundant biofuels on Earth. However, despite its abundance, the biomass material is complex and requires a significant amount of processing before being a usable biofuel. Some of the common unit operations and processes to all biomass converted to biofuel have these major steps: biomass handling, biomass pretreatment, hydrolysis, and fermentation.[6] Depending on the biomass used, the route to biofuel production can vary. Figure 5 provides a graphical representation of the biochemical conversion of biomass to end products.

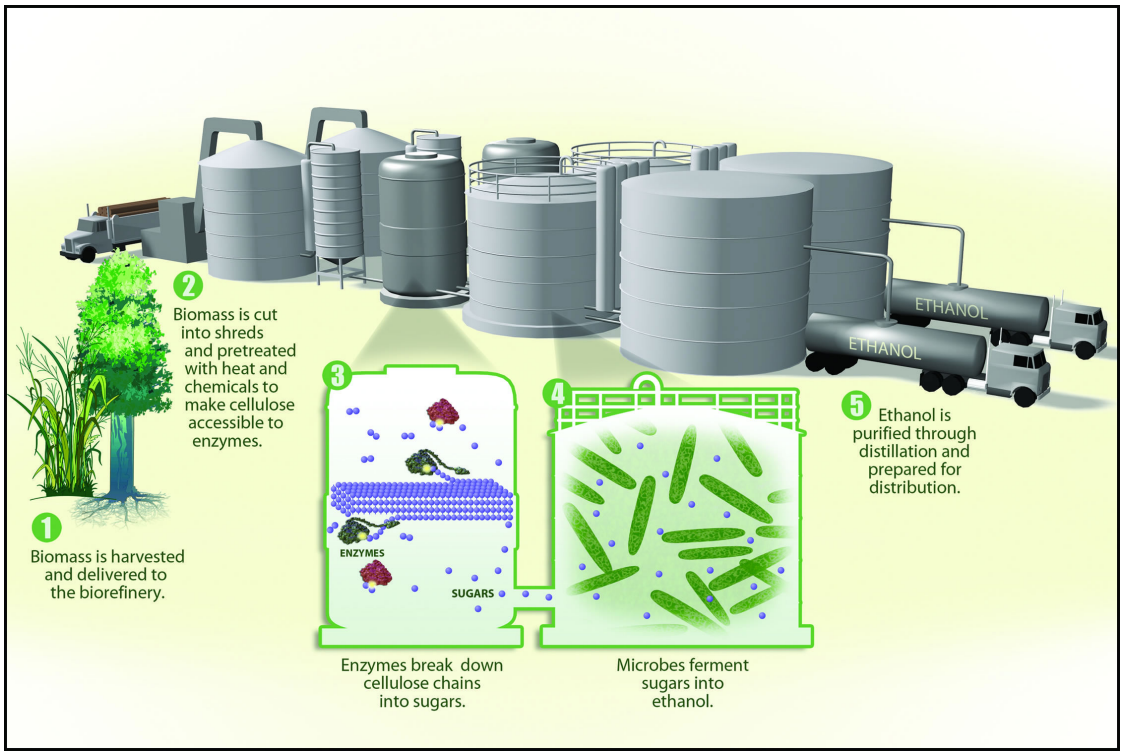

Figure 5: From Biomass to cellulosic ethanol.[22]

2.4 Main Categories of Pretreatment

For the production of biofuels, pretreatment is an important step in the process. During this step, complex structures in biomass are broken down into oligomeric subunits. Monomeric subunits are produced from oligomers during hydrolysis and fermentation. Pretreatment becomes vital to increasing the product yields by disrupting and solubilizing the lignin and hemicelluloses structures in biomass (see Figure 6).

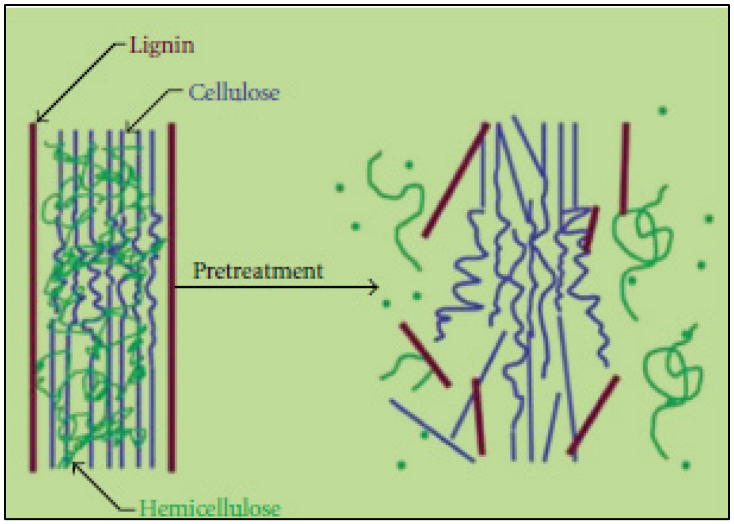

Figure 6: Schematic representation of the matrix of polymers before and after pretreatment. [7]

There are several biomass properties that affect the conversion of lignocellulose: 1. crystallinity of the cellulose, 2. degree of polymerization, 3. moisture content, 4. available surface area, and 5. lignin content.[23] The pretreatment categories used today are classified into four different types: physical, biological, chemical, and physicochemical. Therefore, the goal of any pretreatment process is to have[12]:

- High yields for multiple crops from young and mature sites with varying harvesting times
- Highly digestible solid
- Minimum number of toxic compounds
- Biomass size reduction not required
- Operation in reasonable size and moderate cost reactors
- Non-production of solid-waste
- Effective at low moisture content
- Obtains high sugar concentration (from hydrolysis)
- Fermentation compatibility (minimal production of inhibitors)

- Lignin recovery
- Minimum heat and power requirements

2.4.1 Physical Pretreatment

Physical pretreatment is any pretreatment method not involving chemicals. This method involves the breakdown of biomass size and crystallinity by milling or grinding. As a result, the surface area of the sample is increased and the degree of polymerization is decreased.[12] There are multiple methods used to achieve this goal: vibro-energy milling, ball, roller, hammer, ultrasonic, etc. The main advantages and disadvantages to this method are principal investment costs, operating costs, scale-up options, and depreciation of equipment.[24]

2.4.2 Biological Pretreatment

Biological pretreatment uses nature to help with the degradation of biomass. The microorganisms of choice are generally, brown and soft rot-fungi that degrade hemicellulose and lignin. The main advantages of this pretreatment are relatively mild operating conditions and low energy consumption. The main disadvantage to this method is the required long residence time.[24]

2.4.3 Chemical/ Physiochemical Pretreatment

There is an extensive collection of chemical/ physiochemical pretreatments with documented details on the mechanism of the reactions used. All of these chemical pretreatments initiate by chemical reactions for the disruption of biomass structures (lignin, hemicellulose, etc.)

(See Appendix A, Table A.1). There are multiple advantages and disadvantages of each pretreatment method discussed (see Appendix A, Table A.2).

In 2010, Harmsen et al. published a review paper summarizing the different physical and chemical pretreatment processes for lignocellulosic biomass; their brief summary is presented in Table 2. The overall objective of this section is to review the various preprocessing techniques used in the industry for the pretreatment of biomass, such as microbial, mechanical, and chemical/physiochemical processing, which improve the digestibility of biomass to sugars for biofuel production. In this paper, the chemical pretreatment of choice are a new class of ionic liquids- magnetic ionic liquids (MIL). MILs are the model pretreatment for all biomass studies conducted. A more in-depth investigation of MILs are presented in the next chapter.

Table 2: Biomass Pretreatment Method and Description of Method[24]

Pretreatment	Description of method
Alkaline	NaOH, $Ca(OH)_2$, ammonia or lime
Acid	Concentrated and diluted acids
Wet Oxidation	Utilizes oxygen as an oxidizer for compounds dissolved in water
Green Solvents	Room temperature ionic liquids (RTIL), N-methyl morpholine N-oxide (NMMO), or other solvents
Steam Explosion	Most commonly used as it employs both chemical and physical techniques, high-pressure saturated steam
Liquid Hot Water (LHW)	Liquid water at an elevated temperature and pressure
Ammonia Fiber Explosion (AFEX)	Much like the steam explosion pretreatment, however, this utilizes liquid anhydrous ammonia under high pressures and moderate temperatures, which is then rapidly depressurized
Ammonia Recycle Percolation (ARP)	Aqueous ammonia in a flow-through column reactor (packed with biomass), with high temperatures and pressures (2.3 MPa)
Supercritical Fluid (SCF)	Uses a supercritical CO_2 or a biphasic CO_2-H_2O mixture

CHAPTER III

3.0 REVIEW: DISSOLUTION OF CELLULOSE WITH IONIC LIQUIDS

3.1 Introduction

Ionic liquids (ILs) are a class of organic salts that exist as liquids at temperatures below 100^{o}C. There are a plethora of different ILs, though, they all have common characteristics of being composed of an inorganic anion and organic cation making a very heterogeneous molecular structure. Most common liquids (water, oil, etc.) are predominantly composed of electrically neutral molecules. However, ions, ionic bonds, and Van der Waals dispersion forces help to create ILs unique properties. The difference between the anion and cation molecular structure makes the bonding of the ions weak enough for the salt to act as a liquid at moderate temperatures.[24]

ILs have a wide variety of applications from being electrical conducting fluids to being deemed as powerful green solvents. Most of the current use for ILs are kept in a laboratory setting due to several uncertainties; lack of experience, ability to recover the ILs, the toxicity of the compounds, and the combination of water with the ILs. Since this is a growing area of research relatively little information is known about lignocellulosic interaction with ILs.

In 2004, the Hayashi group reported a new class of magnetic fluids: magnetic ionic liquids (MIL). This new class has the same physical properties as non-magnetic

ILs, but with the added characteristics of being paramagnetic.[25] This chapter will provide a brief overview of ILs, and the new class of MILs for the dissolution of lignocellulosic materials.

3.2 Structure and Physiochemical Properties of Ionic Liquids

Based on the structure of cations, these salts are divided into four different types of ionic liquids: imidazolium-, pyridinium-, quaternary ammonium-, and quaternary phosphonium (see Figure 7).

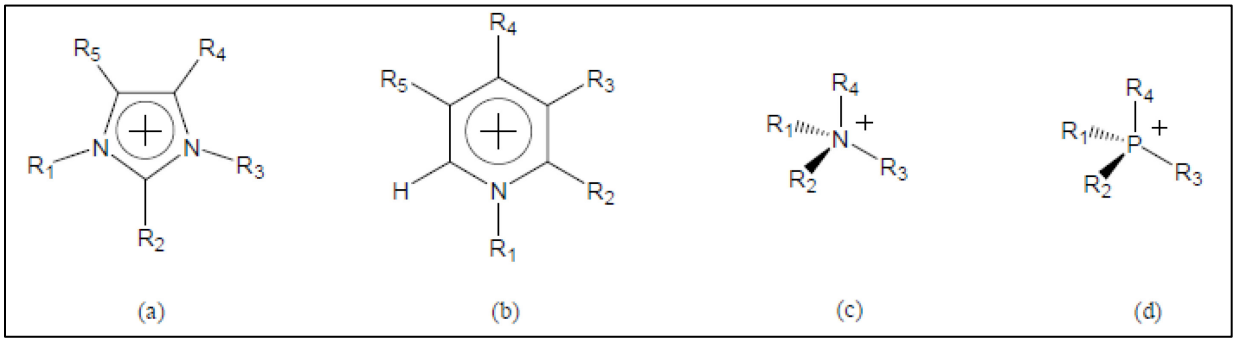

Figure 7: Common structures of ionic liquid cations (a) imidazolium, (b) pyridinium, (c) quaternary ammonium, (d) quaternary phosphonium.[11]

Some of the physicochemical properties of ionic liquids are summarized below.[11]

- High thermal stability. The decomposition temperatures of many ionic liquids can be more than 300°C.
- Broad liquid range from -200 to 300°C, and excellent dissolution performance for organic, inorganic compounds and polymer materials.
- Immeasurable vapor pressure and non-flammability under common conditions.
- High conductivity and wide electrochemical window of 2~5 V.
- Designable structures and properties for various practical applications.

Research thus far has proven that better dissolution of lignocellulosic biomass occurs in imidazolium based ILs when compared to other ILs at same operating conditions. Fort and researchers were able to demonstrate with minimal sample preparation that $[C_4mim]Cl$ is fully capable of dissolving structured polysaccharide-based natural matrices (see Figure 8).[26]

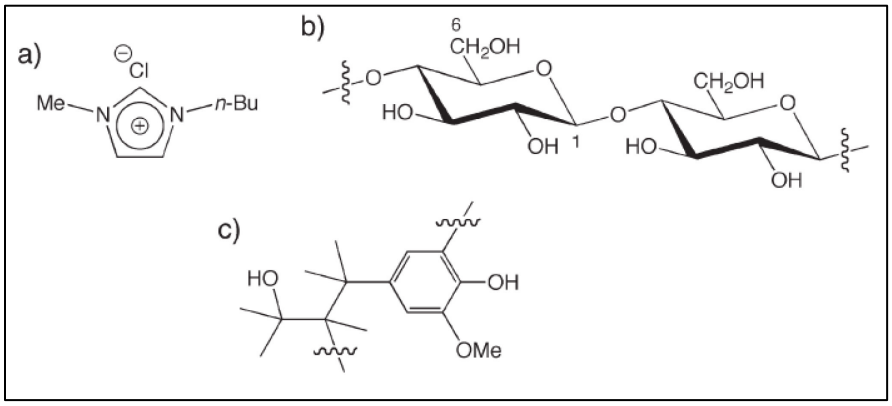

Figure 8: Structures of (a) $[C_4mim]Cl$, (b) cellulose, and (c) lignin.

Other studies have found that ILs are most efficient with dissolution and separation of lignocellulosic biomass when they contain Cl^- (chloride), $[HCO_2]^-$(formate), $[CH_3CO_2]^-$ (acetate, Ac^-), $[NH_2CH_2CO_2]^-$ (aminoethanic acid), $[CH_3SO_4]^-$(methylsulfate), $[RR'PO_2]^-$ (phosphonate), $[Me_2C_6H_3SO_3]^-$ (xylenesulphonate) anions.[11]

3.3 Dissolution of Cellulose in ILs

In the mid 1930s, Charles Graenacher first discovered that cellulose in the presence of nitrogen-containing bases is dissolved into molten N-ethylpyridinium chloride.[27] Little attention was given to this discovery. As technology and energy demand increased, there was a growing need for economical alternative fuels. Therefore, researchers revisited the idea of molten salts as a pretreatment process for biofuel production. In 2002, Dr. Robin Rogers & associates at the

University of Alabama published a study demonstrating the ability of select imidazolium-based ionic liquids dissolve cellulose (up to 25 wt%) efficiently at low temperature ($\leq 100^{o}C$).[10]

Bioresource researchers have looked at a wide variety of imidazolium salts to see if the same effective dissolution of cellulose is possible. It was later discovered that there is a correlation between water content (in the ILs) and the solubility of the cellulose. Vitz and group noted that the solubility of cellulose was reduced when non-dried ILs were used thus, making it necessary to dry all ILs carefully before use[28] Vitz's findings and others are listed in Table 3.

Researchers Zhang and Fukaya successfully synthesized allyl-based ILs: 1-allyl-3-methylimidazolium chloride ([Amim]Cl) and 1-allyl-3-methylimidazolium formate ([Amim][HCO_2]).[29,30] They both noted that these ILs have lower viscosity, lower melting points, and relative stronger dissolution capabilities for cellulose than those of the common imidazolium-based ionic liquids with the identical anions. Approximately 5% of cellulose (DP-650) could be dissolved easily in [Amim]Cl at 80°C within 30 minutes; with an increase in time dissolution increased to 14.5%.[29] If [Amim][HCO_2] was used as a solvent, the solubility of cellulose was as high as 10% at 60°C.[30]

Table 3: The dissolution of cellulose in ionic liquid solvents. The cellulose samples used in these studies commonly differed in degree of depolymerization (DP), molecular weight, or crystal structure.[11]

Ionic Liquid	**Solubility (w/w%)**	**Experimental Condition**	**Reference**
[Bmim]Cl	10	Heating at 100°C	Swatloski et al., 2002
[Bmim]Cl	25	Microwave heating	Swatloski et al., 2002
[Amim]Cl	5	Heating at 80°C within 30 min	Zhang et al., 2005
[Amim]Cl	14.5	Heating at 80°C after a longer dissolution time	Zhang et al., 2005
[Amim][HCO_2]	10	Heating at 60°C	Fukaya et al., 2006
[Emim][(MeO)HPO_2]	10	Heating at 45°C within 30 min	Fukaya et al., 2008
[Emim][(MeO)HPO_2]	2~4	Room-temperature within 3~5 hour	Fukaya et al., 2008
[Emim][Et_2PO_4]	14	Heating at 100°C within 1 hour	Vitz et al., 2009
[Bmim]Ac	15.5	Heating at 70°C	Xu et al. 2010
[Bmim][HSCH**2**CO_2]	12	Heating at 70°C	Xu et al. 2010
[Bmim]Ac/LiAc	19	Heating at 70°C	Xu et al. 2010

Other researchers have looked at changing the anions and cations to 1-ethyl-3-methylimidazolium methyl methylphosphonate ([Emim][(MeO)$MePO_2$]), 1-ethyl-3-methylimidazolium dimethyl phosphate ([Emim][$(MeO)_2PO_2$]), 1-ethyl-3-methyl-imidazolium methyl phosphate ([Emim][(MeO)HPO_2]), 1-ethyl-3-methylimidazolium diethyl phosphate ([Emim][Et_2PO_4]) and 1,3-dimethylimidazolium dimethyl phosphate ([Dmim][Me_2PO_4]).

Xu and group noted that with the addition of lithium salts such as LiAc, LiCl, LiBr, $LiClO_4$, and $LiNO_3$, dissolution of cellulose increased from 15.5% to 19% when [Bmim]Ac is the ionic liquid of choice. These finding and more suggests that the addition of lithium salts have the possibility to increase the dissolution of cellulose. In summary, thus far researchers have proven the following:

- Cellulose, hemicellulose, or lignin, refined or natural can be dissolved by disrupting the extensive hydrogen-bonding network in the crosslinking polymers
- [Bmim]Cl and [Amim]Cl are the main ionic liquids of choice (see Figure 9), other ILs exist with varying dissolution percentages
- Cellulose solubility can be controlled by the selection of ionic liquid constitutes[10]
- Microwave irradiation or sonification can significantly facilitate better dissolution[31]
- Lithium salts can enhance the dissolution of cellulose in ILs[8]

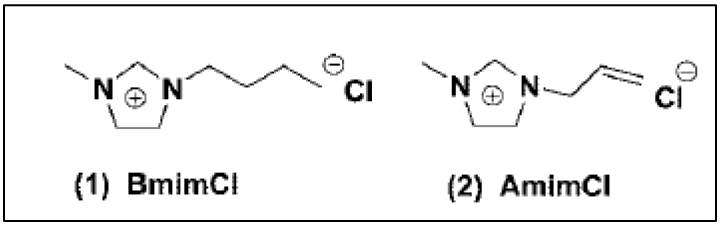

Figure 9: Structure and abbreviation of ionic liquids (1) 1-butyl-3-methyl-limidazolium chloride, (2) 1-allyl-3-methylimidazolium chloride, [32]

3.3.1 Dissolution Mechanism of Cellulose in ILs

Sun and colleagues published work noting that even thought the dissolution is greatly affected by: 1. source of the cellulose; 2. different DP; and 3. dissolution conditions (heating

method, irradiation, time, pressure, etc.), commonly, with the same cation the solubility of cellulose in ionic liquids decreased in the following order[33]:

$$[(CH_3CH_2O)_2PO_2]^- \approx [OAc]^- > [SHCH_2COO]^- > [HCOO]^- > Cl^- > Br^- \approx [SCN]^-$$

Before, ILs research developed, it was believed that the ions, especially the anions of the ILs could effectively break the extensive inter- and intra- molecular hydrogen bonding network of lignocellulosic material. With this thought process, the interactions between cellulose and ILs were investigated using modern Nuclear Magnetic Resonance (NMR) relaxation spectroscopy (^{13}C and $^{35/37}Cl$). Remsing et al., discovered that carbons C-4' and C-1" of the $[Bmim]^+$ cation displayed a small variation in the relaxation times as the concentration of cellobiose (two glucose molecules linked by a β-(1→4) bond) in [Bmim]Cl increased (see Figures 10 and 11). The value changes in the ^{13}C T_1=70°C and T_2=90°C eluding that $[Bmim]^+$ does not have a specific interaction with the cellobiose. On the other hand, in the $^{35/37}Cl$ relaxation rates it appears that the anion Cl^- has a strong interaction with the cellobiose molecules.[34] Further research is being conducted to discover exactly what occurs and how to best modify ionic liquids to achieve optimal dissolution of lignocellulosic biomass.

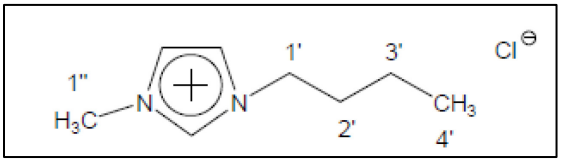

Figure 10: Structure and numbering of [Bmim]Cl.[34]

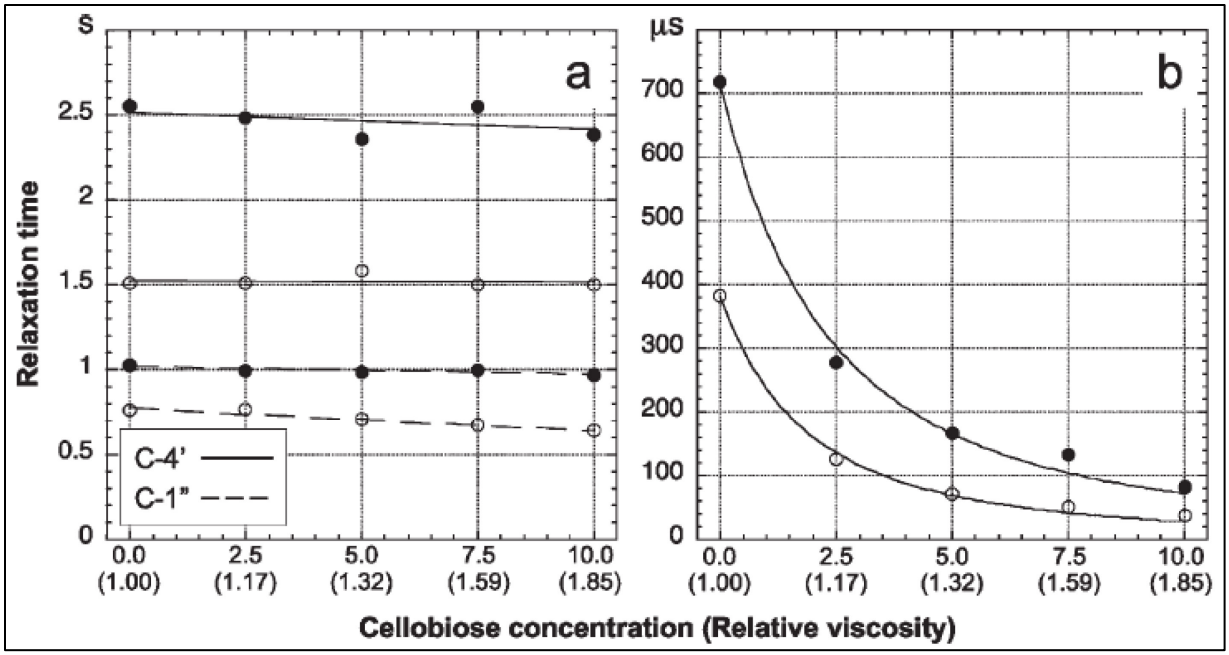

Figure 11: ^{13}C and ^{35}Cl when T_1 (●) and T_2 (○) relaxation times as a function of cellobiose concentration (wt%) for the C-4' and C-1" carbons (a) and chloride ions (b) in neat [C_4mim]Cl at 90°C.[34]

3.3.2 Dissolution Separation of Cellulose in ILs

Dissolution separation is an important step in the pretreatment of lignocellulosic biomass. Therefore, multiple techniques are employed to facilitate with the separation of cellulose in ILs after it has been dissolved. The method for most regeneration of lignocellulosic material is simple in theory (see Figure 12).

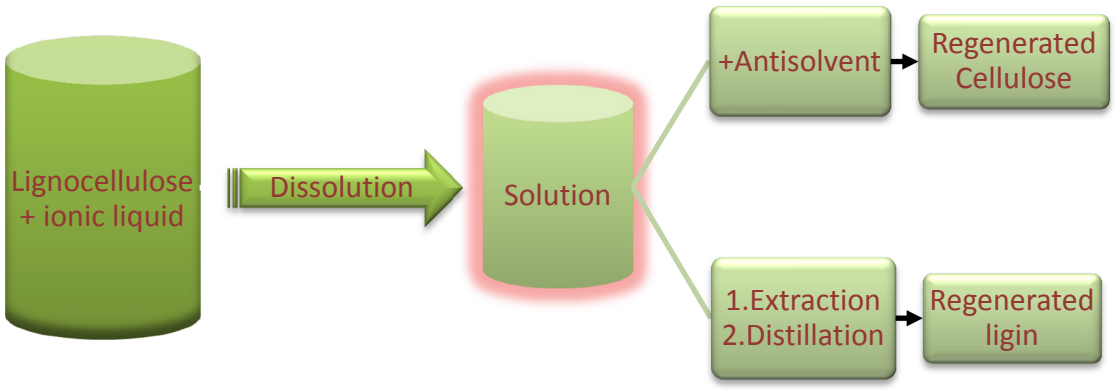

Figure 12: Classic process used for the separation of lignocellulose from ionic liquids

Kilpeläinen and researchers reported that wood could be easily regenerated from imidazolium-based ionic liquids with a common anti-solvent such as simple water.[32] Other anti-solvents are used with varying but similar results such as 1:1, acetone-water solutions, organic solvents, or mechanical methods (i.e. rapid mechanical stirring). Using these methods and other the yields of the reconstituted cellulose ranged from 30 to 60%.[11] Different studies have discovered that the main components (cellulose, hemicellulose, and lignin) of lignocellulosic material can be separated by various post-treatment techniques.

Even though the separation process of ionic liquids and biomass are relatively simple, a limitation in using ILs is the known, but under looked fact that they tend to inactive cellulose. Turner and researchers studied the hydrolysis of cellulose by T. reesei cellulose in [Bmim]Cl and [Bmim]BF_4 that contained 5% of cellulose and discovered that the hydrolytic rate in the ILs was poor; at least 10-fold less than under standard reaction conditions.[35] This was because the ILs leads to the unfolding and permanent inactivation of the enzymes, thus preventing the subsequent

steps of the biofuel process. Turner also noted that complete regeneration of cellulose after pretreatment and removal of all ionic liquid before hydrolysis is necessary for the enzymes to function.

Broduer et al. said that, "…introduces a regeneration and separation step into the process which increases the overall cost and precludes the development of a single stage continuous process for conversion of lignocellulosic biomass. Thus, selection of a solvent for pretreatment in which cellulases and microorganisms are active is a key step in the development of the "biorefinery concept" or "consolidated bioprocessing" schemes, which try to develop a single-stage continuous process for biomass conversion."[7]

3.4 Magnetic Ionic liquids

Magnetic ionic liquids (MIL) are just like any other kind of ionic liquid. In 2004, Satoshi Hayashi and coworkers discovered the magnetic properties and pioneered the way for MIL research today. The only main difference between MIL and ILs is that MILs are magnetic. MILs have the usual ILs properties of high ionic conductivity, high thermal stability, non-flammability, and extremely low volatility; additionally, they show a strong response to a magnetic field. What makes this liquid a new class and sets them apart from other magnetic liquids is that MILs are a "single-phase" liquid whereas others consist of "dispersed magnetic micro-particles in fluids"[36].

Researchers Hayashi and Hamaguchi published papers indicating that in addition to the other known MILs, a new class of magnetic fluids were discovered: 1-butyl-3-methylimidazolium tetrachloroferrate (Bmim[$FeCl_4$]) and 1-butyronitrile-3-methylimidazolium

tetrachloroferrate (nBmim[$FeCl_4$]).[25] Bmim[$FeCl_4$] looks like the non-magnetic ILs but with the addition of $FeCl_4$ (see Figure 13). Where nBmim[$FeCl_4$]) is synthesized by replacing the butyl group of the $bmim^+$ cation with the butyronitrile group. Hayashi and et al. noted "if magnetic anions are aligned locally in an ILs, it would show interesting magnetic properties (see Figure 14)."[25]

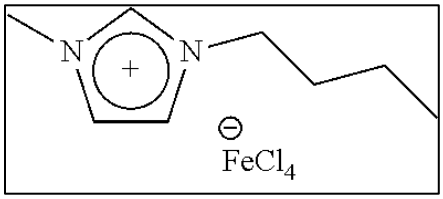

Figure 13: Structure of Bmim[$FeCl_4$]

The Hayashi group performed MPMS SQUID measurements and discovered that the MILs are indeed paramagnetic, having a large effective magnetic moment (μ_{eff}) of 5.80 (Bohr magneton).[37] Misuk and coworkers used visible absorption spectroscopy (VIS) to verify "the reason for the magnetic properties of this compound is provided by the possible local ordering and high-spin of the $FeCl^-$ anions"[38]

Figure 14: Pictures showing the response of Bmim[$FeCl_4$] to a small Nd magnet (0.55 T)[25]. Water is added to the sample to better show the displacement and distortion of Bmim[$FeCl_4$] (I):No magnetic field, two layers of liquids, water, and Bmim[$FeCl_4$], upper and lower respectively. (II and III): Magnetic field is applied Bmim[$FeCl_4$] is attracted and moves toward the magnet.

Some researchers such as Sang Hyun Lee recently investigated the possibility of using a magnet to recover MILs after they were used in a reaction system. Lee and group discovered "they can be used as alternatives for organic solvents and separated by magnetic field after use in a reaction system.[39]" Lee also stated that the separation factor of magnetic ionic liquids in a solution could be increased via several convention methods such as ultracentrifugation, filtration, and adsorption by exploiting the ILs properties such as high molecular weight, density, and conductivity.[39] This proved to be a step in the right direction for the pretreatment of biomass using ionic liquids.[8]

In 2010, Wang and associates developed the use of MILs as a catalyst. Conventionally, the glycolysis of poly(ethylene terephthalate) (PET) is catalyzed by a variety of compounds, metal acetates, titanium-phosphate, solid superacids, metal oxides (i.e. copper oxide), etc.. They published a study that proved Bmim[$FeCl_4$] could behave as the efficient and eco-friendly catalyst for the depolymerization of PET when compared to $FeCl_3$, metal salts, or an ionic liquid.[40] This finding is significant because it provides the basis that Bmim[$FeCl_4$] can depolymerize compounds. However, there is no report on the use of MIL in the catalytic depolymerization of lignin, hemicellulose, or cellulose. In this present study, magnetic ionic liquids, Bmim[$FeCl_4$] and 1-ethyl-3-methylimidazolium tetrachloroferrate (Emim[$FeCl_4$]), are synthesized and examined to discover the effectiveness of preprocessing lignocellulosic biomass. If possible, magnetic ionic liquids offer high potential for markedly reducing the costs of pretreatment and will facilitate in the development of a consolidated biorefinery process.

CHAPTER IV

4.0 PREPARATION AND CHARACTERIZATION OF MAGNETIC IONIC LIQUIDS

4.1 Introduction

The synthesis of the magnetic ionic liquids (MILs) is a vital step to the dissolution studies; therefore, in depth research was conducted for the best method. This section will present the methods used to synthesize and characterize 1-butyl-3-methylimidazolium tetrachloroferrate (Bmim[$FeCl_4$]) (Liquid 1) and 1-ethyl-3-methylimidazolium tetrachloroferrate (Emim[$FeCl_4$]) (Liquid 2) the MILs used in the dissolution studies.

4.2 Experimental Procedure

4.2.1 Materials

Commercial chemicals were of reagent or analytical grade and were used without further purification. 1-butyl-3-methylimidazolium chloride [Bmim]Cl (CAS#: 79917-90-1), 1-ethyl-3-methylimidazolium chloride [Emim]Cl (CAS#: 65039-09-0), acetone, Iron(III) chloride hexahydrate (CAS#: 10025-77-1) were obtained from Sigma Aldrich (sigmaaldrich.com). Molecular structures and properties of the ionic liquids are shown in Appendix B.

4.2.2 Synthesis of Magnetic Ionic Liquids (MILs)

In the present study, Bmim[$FeCl_4$] was prepared via a similar method previously described by Hayashi and et al.[25] Equimolar amounts of crystal powder [Bmim]Cl and $FeCl_3 \cdot 6H_2O$ were weighed out in a N_2 enriched glove box. The two compounds are mixed to produce a dark brown two layer liquid from an endothermic solid-state reaction. The lower layer, hydrophobic MIL, was purified by repeated washing with deionized water. Acetone was added to the washed MIL and was dried in a rotary evaporator system at 18 torr, and 80^{o}C for 6 hours. The same procedure was performed for the synthesis of Emim[$FeCl_4$].

4.2.3 Characterization of MIL

In order to spectroscopically characterize the liquids, first the visible absorption (VIS) spectrum was recorded on an Evolution 201Thermo Scientific spectrometer. Fourier transform infrared spectroscopy (FTIR) were measured using an Agilent Tech Cary 660 Series FTIR Spectrometer with a potassium bromide Attenuated Transmitted Reflectance (ATR) crystal. IR spectra over a 4000-700 cm^{-1} range were collected at 40 scans, 2 cm^{-1} resolution using the normal Happ-Genzel function.

4.3 Results and Discussions

It is known that elemental Iron-containing ionic liquids demonstrate an intense VIS absorbance which comes from the intra-configurational d-transition Fe^{3+} in a tetrahedral ligand field.[41] The spectra for Liquids 1 and 2 are presented in Figures 15 and 16, respectively. The visible absorption spectrum for both samples were compared to that of related literature and they

both resemble the three characteristic bands of $FeCl_4^-$ ion at 532, 603, and 685 nm.[42] Therefore, it is confirmed that the principal trivalent iron species is $FeCl_4^-$ with a coordination number of four for iron exist dominantly in the prepared samples (Eq. 4.1).

$$FeCl_3 + Cl^- \rightleftharpoons FeCl_4^- \qquad (4.1)$$

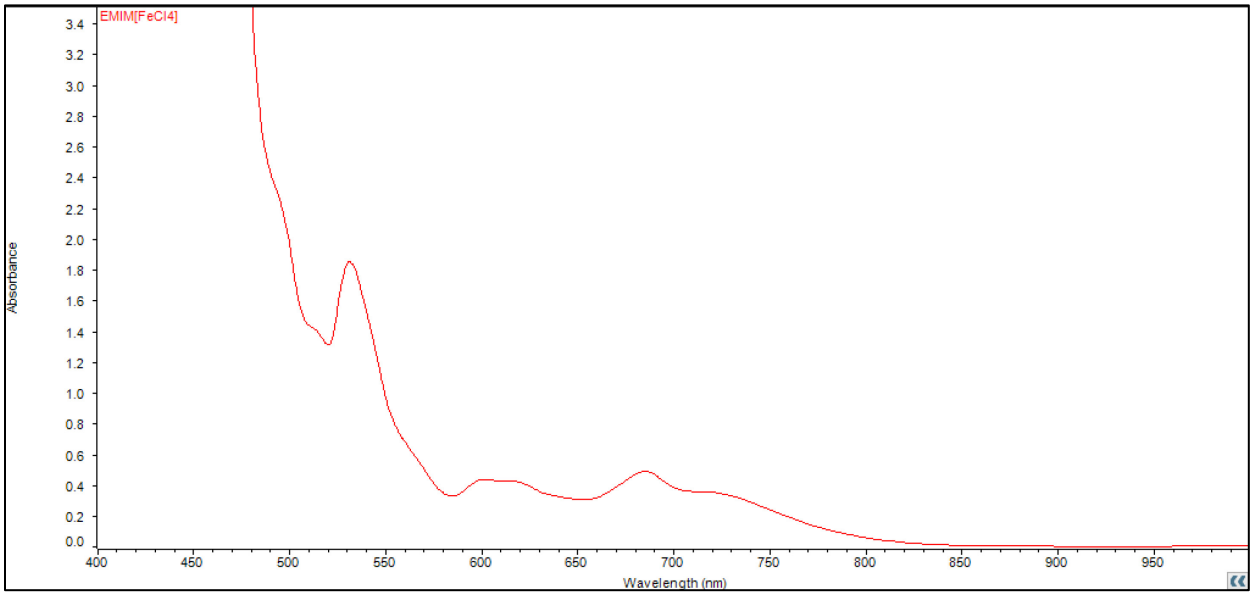

Figure 15: Visible absorption spectra of Emim[$FeCl_4$]

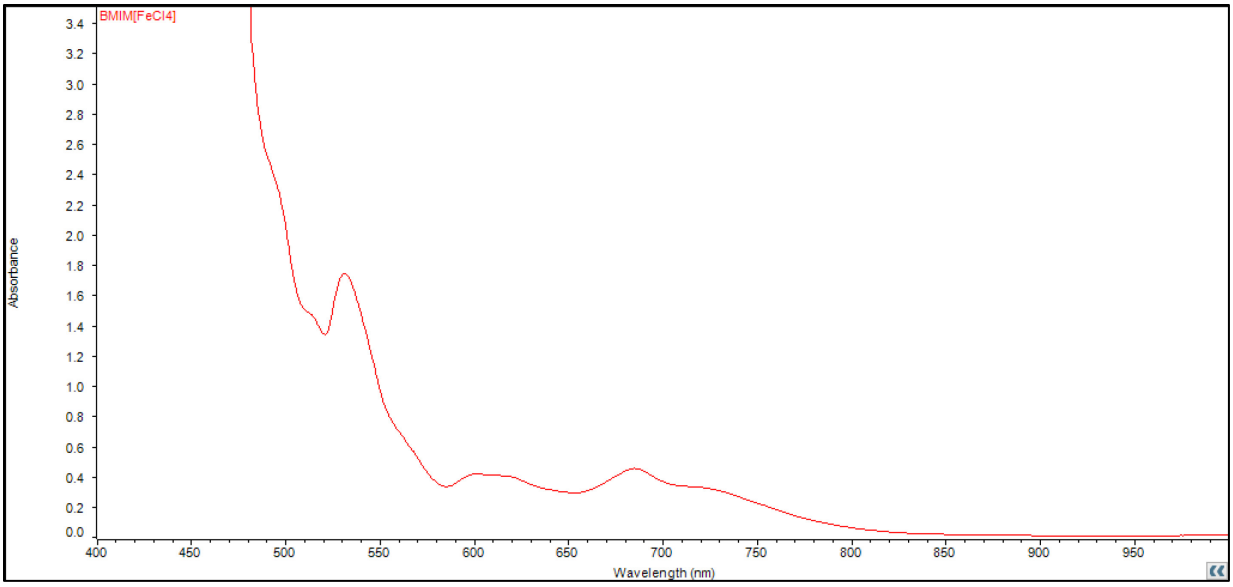

Figure 16: Visible absorption spectra of Bmim[$FeCl_4$]

The ATR-FTIR spectra for the samples were also compared to researchers Yao and Chu. These spectrums display the detection of the characteristic vibration of the imidazole cation in both Liquid 1 and 2. The infrared spectra for the liquids are assigned to the in-plane and out-of-plane flexural vibration mode of the imidazolium ring at the vibrational bands of 832 and 742 cm^{-1} in, respectively (see Figures 17, 18, 19, and 20).[41]

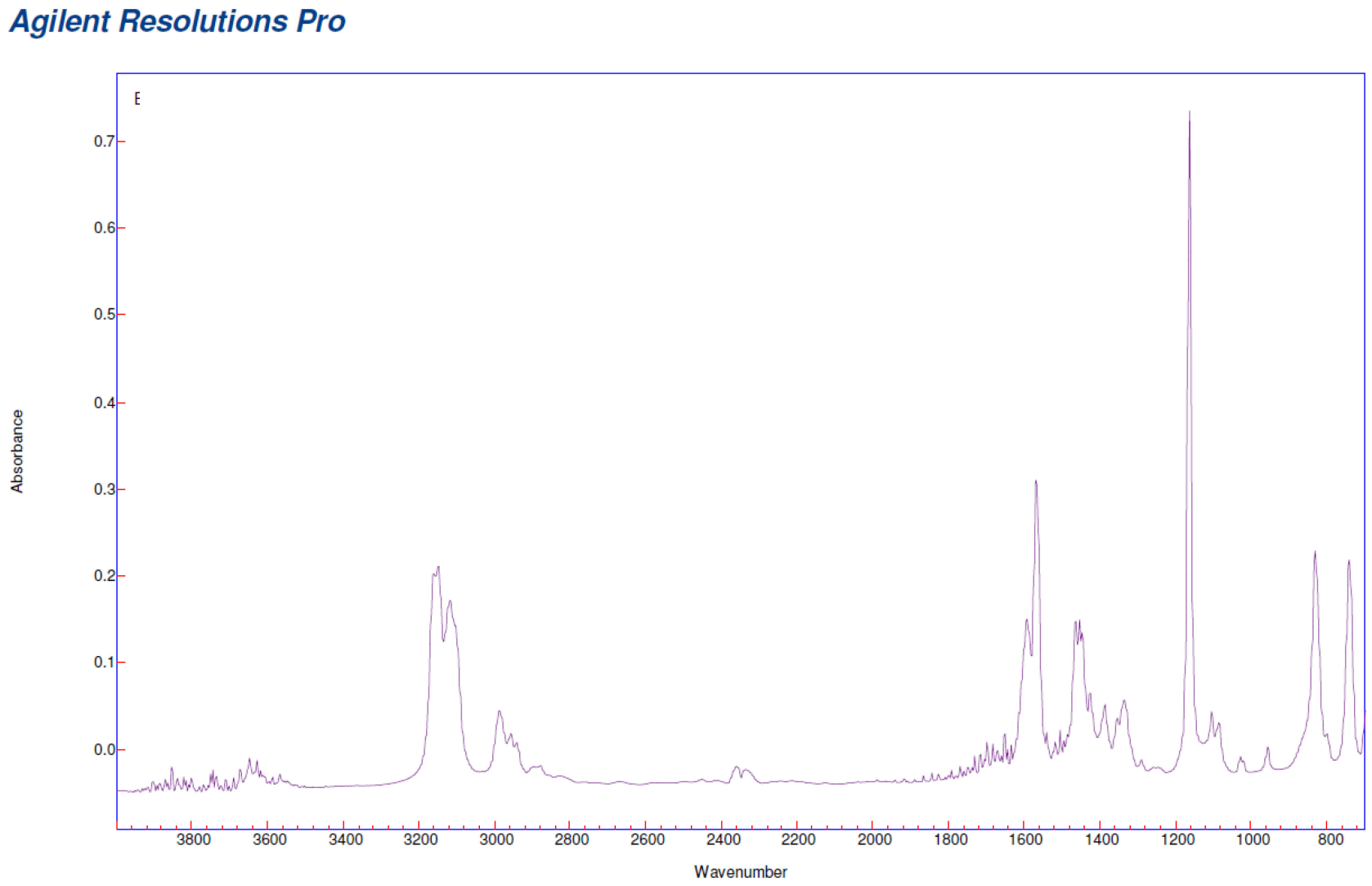

Figure 17: ATR-FTIR spectra of Emim[$FeCl_4$] (4000-700cm^{-1})

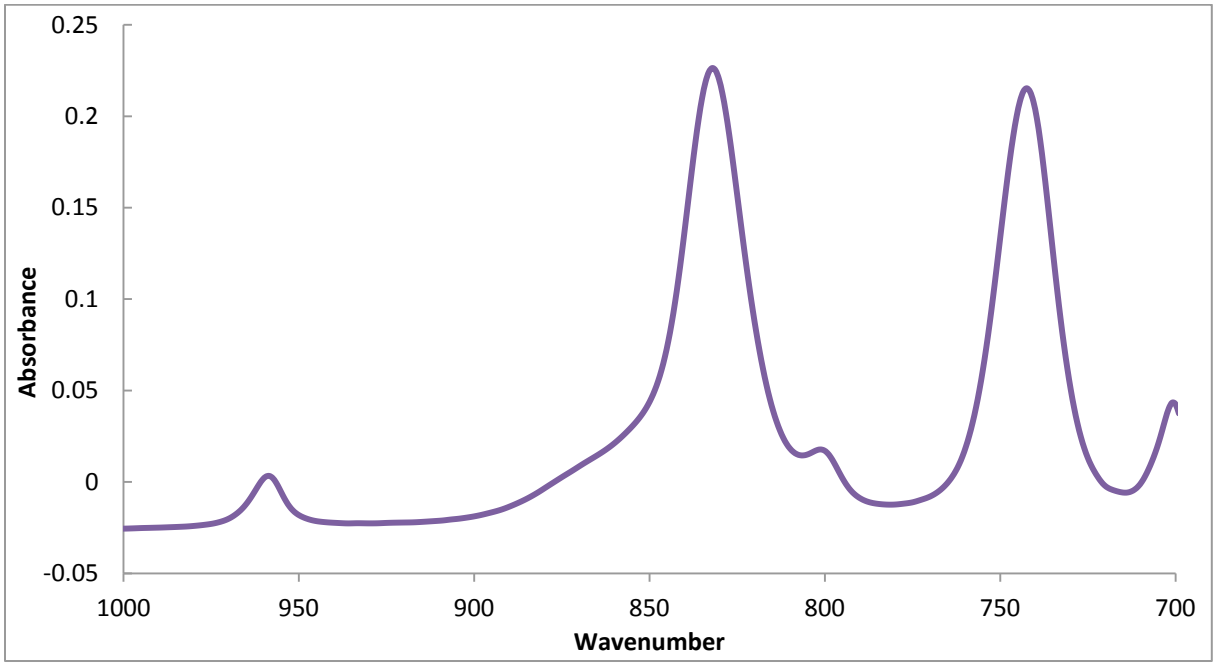

Figure 18: ATR-FTIR spectra of Emim[$FeCl_4$] fingerprint region (1000-700cm^{-1})

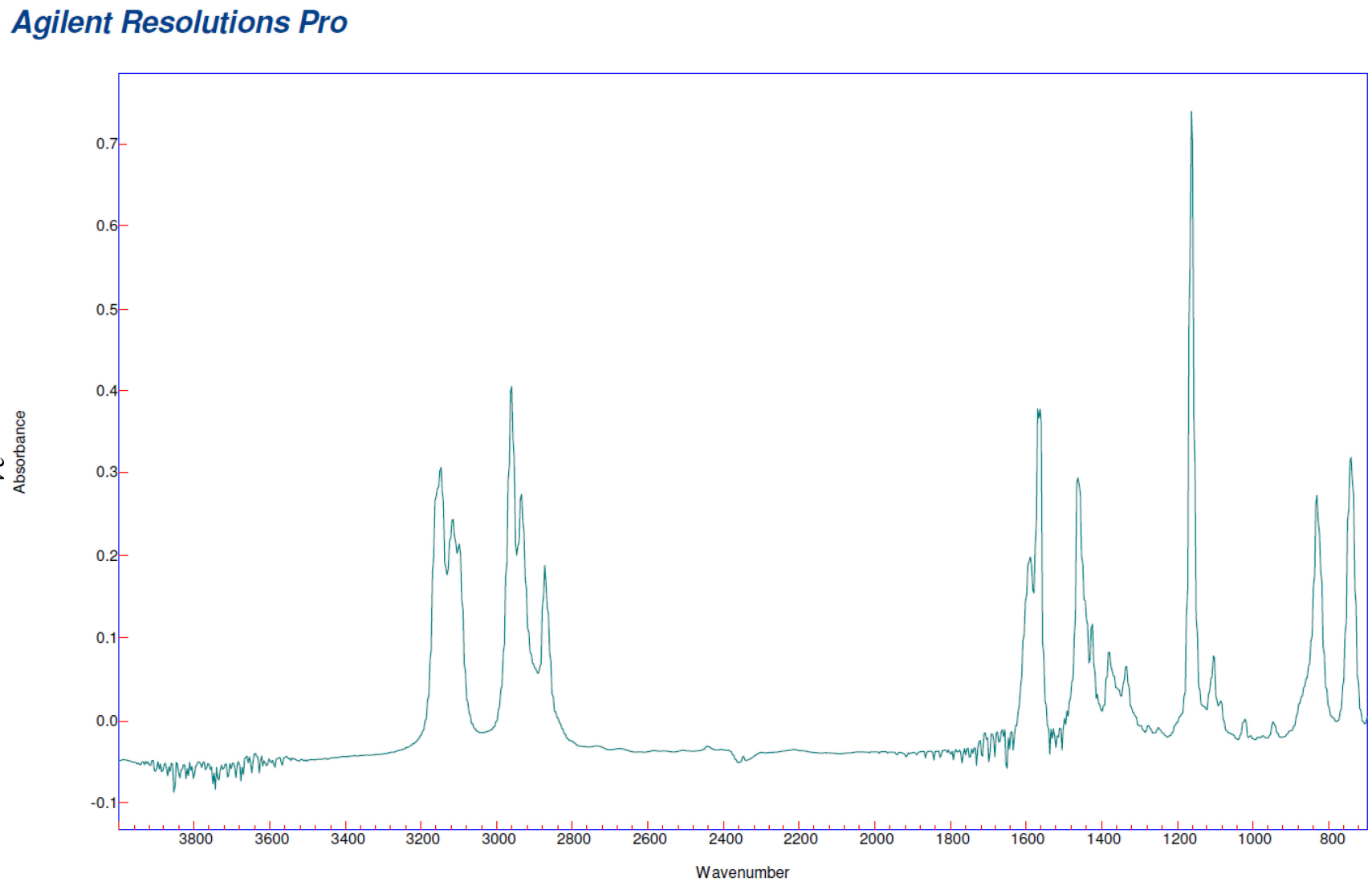

Figure 19: ATR-FTIR spectra of Bmim[$FeCl_4$] (4000-700cm^{-1})

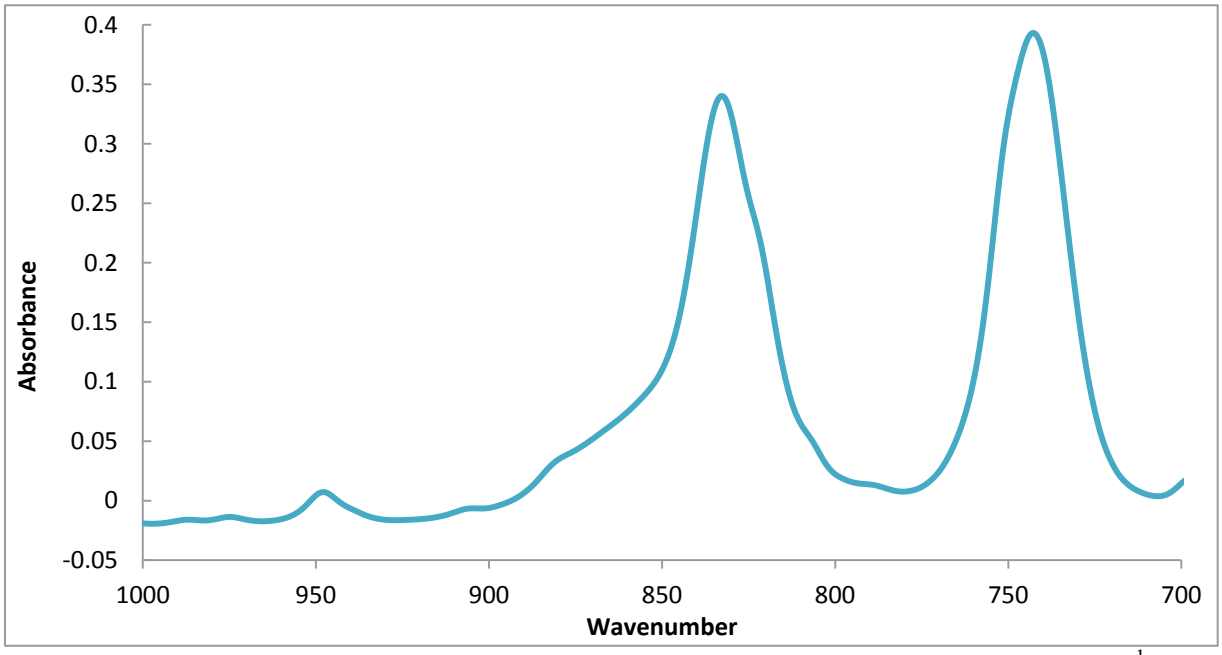

Figure 20: ATR-FTIR spectra of Bmim[$FeCl_4$] fingerprint region (1000-700cm^{-1})

4.4 Conclusion

In conclusion, from the above spectroscopic analysis it suggests that the procedures for the synthesis of magnetic ionic liquids Bmim[$FeCl_4$] and Emim[$FeCl_4$] were successful. Additionally, it is known that with the addition of excess Cl^- anions the forward reaction direction of the equilibrium is favored and that the below reactions (4.2 & 4.3) are valid.

$$\begin{matrix} Bmim^+ + FeCl_4^- \\ Bmim^+ + Fe_3^+ + 4Cl^- \end{matrix} \rightleftharpoons [Bmim]FeCl_4 \qquad (4.2)$$

$$\begin{matrix} Emim^+ + FeCl_4^- \rightleftharpoons \\ Emim^+ + Fe_3^+ + 4Cl^- \rightleftharpoons \end{matrix} [Emim]FeCl_4 \qquad (4.3)$$

CHAPTER V

5.0 DISSOLUTION OF BIOMASS IN MAGNETIC IONIC LIQUIDS

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Abstract

The focus of this project is to determine the effectiveness, in the preprocessing of biomass when magnetic ionic liquids (MIL) (1-butyl-3-methylimidazolium tetrachloroferrate (Bmim[$FeCl_4$]) and 1-ethyl-3-methylimidazolium tetrachloroferrate (Emim[$FeCl_4$])) are used as a green solvent. Lignocellulose is a promising starting material for a plethora of products, ranging from biofuels to custom chemicals; however, lignocellulose is resistant to enzymatic degradation. Various biomass-preprocessing techniques such as microbial, mechanical, and chemical pretreatment are used for enhancing the digestibility of biomass to sugars for ethanol production. Varieties of ionic liquids have demonstrated the ability to fragment lignocellulose. However, after fragmentation, separation of biomass and ionic liquids has proven to present economic challenges for this pretreatment process. Research has proven that the addition of magnetic properties to the ionic liquid can be used to stabilize the ionic liquids and prevent its loss or other detrimental fluid/fluid interactions in the bioreactor. Therefore, this paper presents the outcomes of such MIL dissolution studies.

5.1 Introduction

Some of the smallest known molecules fuel the world that we live in today. Our planet has developed a plethora of materials based upon amino acids, proteins, and polysaccharides. They range from DNA, cellulose, chitin, elastin, silk produced from worms, and more.[1] The converted biomass provides many sources of usable energy such as hydrocarbon fuels and chemical compounds like alcohols, gums, sugars, and lipid-based products.

The objectives stated in the 2006 research roadmap published by the United States Department of Energy were to place biomass energy conversion research on a fast-track, helping make biofuels an everyday resource and economically feasible by 2012, and by 2030 have the potential to offset 30% of the nation's current gasoline consumption.[2] Therefore, in recent years, there has been a renewed interest and increased research devoted to the development of biofuels made from lignocellulose biomass derived from agricultural byproducts, forest residues, and dedicated energy crops.[3–5]

With this new found push for renewable green-energy sources, important processes involved in the biochemical production of biofuels are being optimized. However, some of the difficulties arise from the fact that the conversion to biofuel and type of biofuel produced depends greatly on the source/type of biomass. Consequently, most unit operations and processes for converting biomass to biofuel have four major steps: biomass handling, biomass pretreatment, hydrolysis, and fermentation.[6] Out of these steps, the pretreatment process proves to be the most difficult to optimize because complex structures in the biomass of choice are broken down into oligomeric subunits. These subunits are ultimately converted into monomers

via hydrolysis and fermentation. Therefore, the development of a more universal pretreatment process is the goal of many bioresource researchers today.

There have been many papers published about different pretreatment methods that can be applied to enhance the digestibility of lignocellulosic material. Biomass is either grown or acquired from various sources; then is transported to the production sites for biochemical conversion to fuels. Until the 1970s, the idea of agricultural residues such as straw grass or corn byproducts being potential sources of lignocellulosic biofuels was not well recognized. The fuel emergencies during the 1970-1980s was a significant reason for breakthroughs in alternative fuels and engines.[12] Biofuels are some of the most efficient alternatives thus far despite existing criticism, often incorrect, for an unfavorable net energy balance and significant arable land and water requirements.[13]

Biomass is categorized as all materials derived from plant, animal, and microbial origins (see Figure 21). The classification of biomasses used today in conversion to biofuels, are usually based on the animal or plant origin, woody or herbaceous carbon source and physical and chemical characteristics.[12] The plants are the preferred choice of biomass because they are abundant and have high potential to mitigate the emission of greenhouse gases.

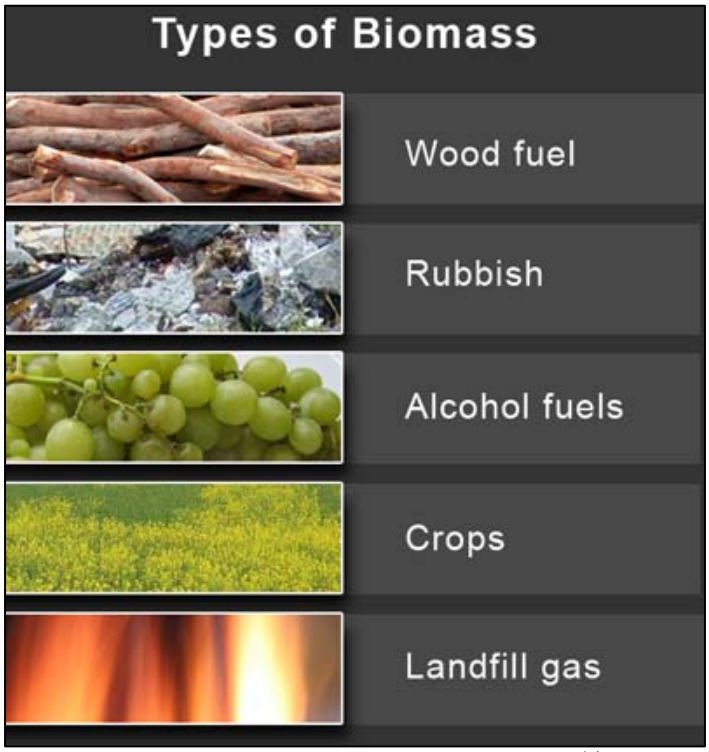

Figure 21: Types of biomass[14]

Ionic liquids (ILs) are a class of organic salts that exist as liquids at temperatures below 100^{o}C. There are a plethora of different ILs, though, they all have common characteristics of being composed of an inorganic anion and organic cation making a very heterogeneous molecular structure. Most common liquids (water, oil, etc.) are predominantly composed of electrically neutral molecules. However, ions, ionic bonds, and Van der Waals dispersion forces help to create ILs unique properties. The difference between the anion and cation molecular structure makes the bonding of the ions weak enough for the salt to act as a liquid at moderate temperatures.[24]

ILs have a wide variety of applications from being electrical conducting fluids to being deemed as powerful green solvents. Most of the current use for ILs are kept in a laboratory

setting due to several uncertainties; lack of experience, ability to recover the ILs, the toxicity of the compounds, and the combination of water with the ILs. Since this is a growing area of research relatively little information is known about lignocellulosic interaction with ILs.

In 2004, the Hayashi group noted that a new class of magnetic fluids were discovered: magnetic ionic liquids (MIL). This new class has the same physical properties as non-magnetic ILs, but with the added characteristics of being paramagnetic.[25] Thus, here we synthesize two magnetic ionic liquids, 1-butyl-3-methylimidazolium tetrachloroferrate (Bmim[$FeCl_4$]) and 1-ethyl-3-methylimidazolium tetrachloroferrate (Emim[$FeCl_4$]). We use both liquids in various pretreatment conditions and perform dissolution studies using microcrystalline cellulose, fibrous cellulose, and a biomass of choice- switch grass.

5.2 Experimental Procedure

5.2.1 Materials

Commercial chemicals were of reagent or analytical grade and were used without further purification. The ionic liquids 1-butyl-3-methylimidazolium chloride [Bmim]Cl (CAS#: 79917-90-1), 1-ethyl-3-methylimidazolium chloride [Emim]Cl (CAS#: 65039-09-0), acetone, silicon oil (AP100), methanol, Iron(III) chloride hexahydrate (CAS#: 10025-77-1), microcrystalline cellulose (CAS#: 9004-34-6), BCA Protein Assay Kit (reagent A & B), Cellulase from *Trichoderma reesei* ATCC 26921 (CAS#: 9012-54-8), and Whatman paper (grade #1) were obtained from Sigma Aldrich (sigmaaldrich.com). Molecular structures and properties of the

ionic liquids are shown in Appendix B. Switch grass of 1'' grind size was obtained from BioDimensions, Memphis, TN. The switch grass was dried for 12 hours at 100^{o}C before use.

5.2.2 Synthesis of Magnetic Ionic Liquids (MILs)

In the present study, Bmim[$FeCl_4$] was prepared via a similar method previously described by Hayashi and et al.[25] Equimolar amounts of crystal powder [Bmim]Cl and $FeCl_3$**.**$6H_2O$ were weighed out in a N_2 enriched glove box. The two compounds are mixed to produce a dark brown two layer liquid from an endothermic solid-state reaction. The lower layer, hydrophobic MIL, was purified by repeated washing with deionized water. Acetone was added to the washed MIL and was dried in a rotary evaporator system at 18 torr, and 80^{o}C for 6 hours. The same procedure was performed for the synthesis of Emim[$FeCl_4$].

5.2.3 Characterization of Magnetic Ionic Liquids (MILs)

In order to spectroscopically characterize the liquids, first the visible absorption (VIS) spectrum was recorded on an Evolution 201Thermo Scientific spectrometer. Fourier transform infrared spectroscopy (FTIR) were measured using an Agilent Tech Cary 660 Series FTIR Spectrometer with a potassium bromide Attenuated Transmitted Reflectance (ATR) crystal. IR spectra over a 4000-700 cm^{-1} range were collected at 40 scans, 2 cm^{-1} resolution using the normal Happ-Genzel function.

5.2.4 Size separation and preparation of biomass

Switch grass of 1" grind size was obtained from BioDimensions, Memphis, Tennessee. A similar procedure performed by Dr. Swetha Mahalaxmi was followed for the biomass size separation and preparation.[12] A stacked sieve system, comprising of U.S.A Standard Testing Sieves (organized in a top to bottom sequence) #10 (2 mm), #18 (1 mm) and a collection pan, was used for separating the 1'' ground switch grass into three fractions, >2 mm (material remained above the #10 pan), 1-2 mm (material remained below the #10 and above the #18) and <1 mm (material remained in the collection pan). A known amount of un-partitioned switch grass (UP) is taken in the pan #10, of the staked sieve system, and subjected to manual shaking for a minute. This procedure was repeated five times, weighed, collected, subjected to milling in an IKA MF 10.1 impact mill with an internal 1 mm circular screen, and saved separately for further experiments.

5.2.5 Dissolving of Cellulose in Magnetic Ionic Liquids (MILs)

1 mL of the MIL was filled into a small test tube (~16mL), weighed on a microbalance and preheated to 100^{o}C. The biomass sample (particle size= 0.1-2mm) of choice was quickly added into the ionic liquid. The temperature of the dissolution process was controlled in an oil bath at different predetermined temperatures ranging from 100^{o}C to 160^{o}C. This mixture was allowed to react under atmospheric pressure for a minimum of 1 hour, and allowed to proceed at specified time intervals. All experiments were performed in triplicates. The solubility of the organic material in the MIL was checked visually. If the organic sample appeared to dissolve,

biomass was added in portions of only 1 wt% of the magnetic ionic liquid each time with mechanical stirring.

5.2.6 Recovery of MIL and Regeneration of Biomass

Method I- After a set reaction time, deionized water was added to the biomass mixture where a precipitate and two layers of liquid quickly form. The slurry is placed in a centrifuge at 10,000 rpm for 10 minutes. The top layer containing the biomass is poured off and was washed three times with additions of deionized water in order to remove excess ionic liquid.

Method II- After a set reaction time, 10 mL deionized water was added to the biomass mixture where a precipitate and two layers of liquid immediately form. A magnet (N35 – N40) is placed at the bottom of the test tube to induce a magnetic field for a minimum of 2 hours. After which visual inspection of the solution was recorded for separation of the biomass and the MIL.

Method III- After a set reaction time, 10mL of methanol was added to the biomass mixture where a homogenous mixture formed.[11] This solution was filtered using a glass-fiber filter, separating solids from MIL. The filtrate is then heated to boil off the methanol.

For all of the above three methods, the presence of the magnetic ionic liquid was verified by measuring absorbance on the Evolution 201 Thermo Scientific spectrometer of the recovered liquid. The remaining biomass solids were dried and frozen for further analysis.

5.2.7 Enzymatic Hydrolysis & Glucose Determination

The frozen regenerated biomass samples were treated with cellulase from *Trichoderma reesei* (filter paper activity-132 FPU/mL determined by Cellulase Assay, See Appendix C). The enzymatic hydrolyses were carried out at 50± 1 °C for 24 h using 50 mM citrate buffer (pH 4.8± 0.3) in a New Brunswick Scientific (model C24) incubator shaker at 120 rpm. The solutions were filtered, and the filtrate from the enzymatic hydrolysis was then diluted to a volume of 250 mL using a volumetric flask. A series of 100 mL of distilled water dilutions, containing 100-1000 μL (in 100 μL intervals) of the above filtrate were prepared. Four milliliters of each dilution were then added into a test tube. A series of glucose standards solutions (GSS) were made (and verified by a standard curve). The coloring reagent is prepared by mixing 1 part of reagent B with 50 parts of reagent A of BCA test kit (protein assay kit). Reagent B is a copper solution, and reagent A is a BCA solution. The resulting solution had a green color, which is prepared for every analysis. The color is developed by adding (to each of the 4 mL of GSS and diluted filtrates) 1 mL of coloring reagent. The samples were then mixed using a Vortex mixer, reacted at 60 °C and incubated for 2 hours. Each vial is protected from light by covering with aluminum foil. Samples containing glucose turned purple and the concentration is determined at 562 nm against a blank via UV-VIS spectroscopy.

5.3 Results and Discussions

For ionic liquid pretreatment of biomass, multiple studies have found that 1.[Bmim]Cl^- is an effective solvent to solubilize the plant cell wall at mild temperatures[43], 2. subsequent

cellulose precipitation and regeneration via addition of an anti-solvent could reject lignin in the solution[43] and 3. optimal reaction temperature and time for switch grass are 160°C and 3 hours.

The first step to the screening of a solvent for delignification and pretreatment of lignocellulosic biomass was to develop and verify reproducible methods to synthesize Bmim[$FeCl_4$] and Emim[$FeCl_4$]. After which several simple 8-hour screening studies were conducted. Table 4 summarizes the various pretreatment conditions and solvents used throughout the dissolution studies. Preliminary results show that it is possible to achieve dissolution of biomass in a magnetic ionic liquid. It must be noted however, that the studies were not conducted at temperatures below 100°C.[10]

Table 4: Dissolution conditions and preliminary results *Reaction conditions: 1 atm, 8 hours, ~2 grams of MIL and 1 wt% biomass

Temperature (°C)	**Bmim[$FeCl_4$]**	**Emim[$FeCl_4$]**	**[Bmim]Cl**	**$FeCl_3$**
100	No	No	Yes	No
140	Yes	No	Yes	No
160	Yes	Yes	Yes	No

The main goals of this study are to disrupt the hydrogen bonding and increase the separation factor between the ionic liquids and the biomass of choice; thereby, creating a more universal pretreatment process. Partial sample dissolution [up to 2% (w/w)] occurred by simply mixing a dried sample with the magnetic ionic liquid and mechanical stirring at temperatures greater than 140°C (see Table 5). There was an apparent trend that higher temperatures increased

solubility in the two magnetic ionic liquids. The temperature accelerated the diffusion of the MIL into the lignocellulose resulting in possible more lignin being dissolved into the MIL. Further research must be conducted to confirm the dissolution of lignin.

Table 5: Dissolution behavior of lignocellulosic materials in different magnetic Imidazolium-Based ionic liquids (BC: Biomass-Switch grass, CF: Cellulose fiber, and MC: Microcrystalline cellulose)

Magnetic Ionic Liquid	Sample	Solubilization conditions	Solubility (wt%)
Bmim[$FeCl_4$]	MC, BM, CF	100^oC, 8 hours	0
Emim[$FeCl_4$]	MC, BM, CF	100^oC, 8 hours	0
Bmim[$FeCl_4$]	MC	140^oC, 8 hours	1
Emim[$FeCl_4$]	MC	140^oC, 8 hours	Partially soluble
Emim[$FeCl_4$]	BC	160^oC, 8 hours	Partially soluble
Bmim[$FeCl_4$]	MC, CF	160^oC, 8 hours	2

However, through-out the studies it was discovered that the recovery of the magnetic ionic liquids from the biomass is a significant issue. Thus, three different methods for separation were used. Method I employed the use of a solid Neodymium magnet to exploit the Fe-magnetism property. We believe that if a stronger magnetic field, perhaps an electromagnetic induced field, is introduced to the MIL it might be able to increase the separation factor between biomass and MIL. Method II used a centrifuge to exploit the density of the MIL. Because of the hydrophobic nature of the magnetic ionic liquid when water is introduced into the solution, the centrifuge had little to no affect on the separation. Lastly, Method III used the liquid state of the MIL, which allowed it to pass through a glass-fiber filter and separate it from the solid biomass.

As a result, gravimetric analysis proved that out of all three methods used, Method III worked best for MIL recovery and was performed thought-out the remainder of the study.

Enzymatic saccharification was performed on the processed samples to better understand some of the impacts that the magnetic ionic liquid had as a pretreatment method. The biomass samples of switchgrass were compared to untreated and 10% H_2SO_4 pretreatment process (see Appendix D for method) for glucose release during hydrolysis. The data suggests that both untreated and H_2SO_4 pretreatment methods release more glucose than those treated with Bmim[$FeCl_4$] and Emim[$FeCl_4$] (see Table 6).

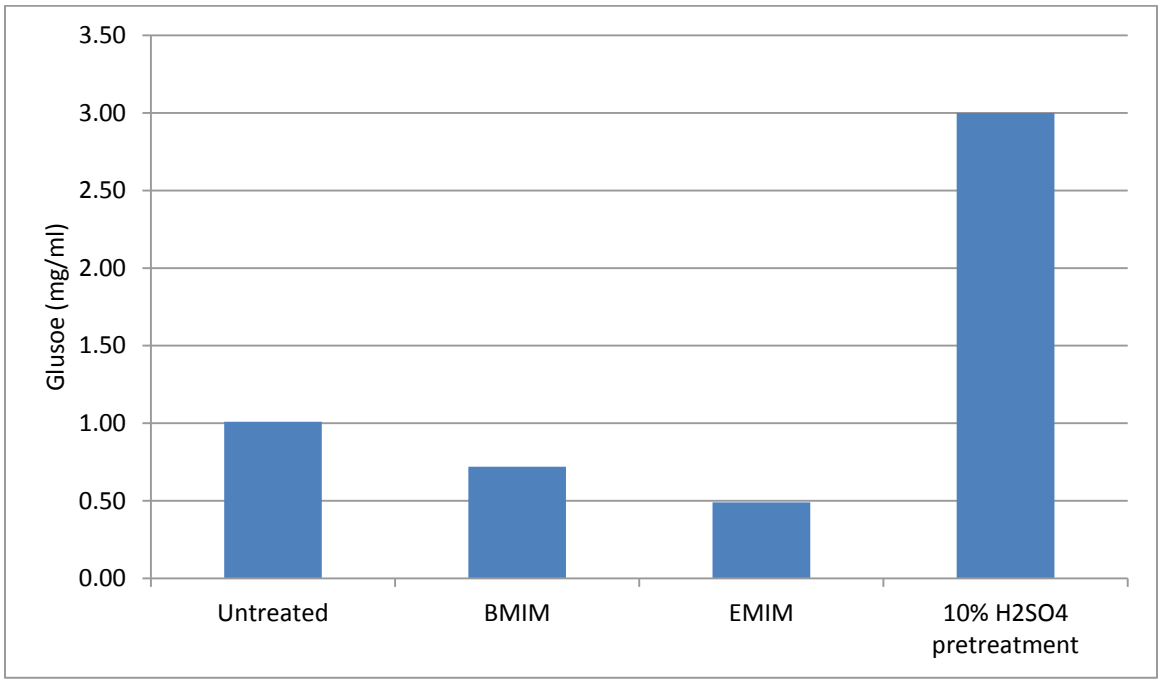

Figure 22: Amount of glucose released during enzymatic hydrolysis with the MIL (in Bmim[$FeCl_4$] or Emim[$FeCl_4$] compared to controls of 10% H_2SO_4 and untreated samples

Therefore, only placing lignocellulose in a magnetic ionic liquid does not have sufficient capability to produce readily biodegradable cellulose. When compared to other IL-H_2O

mixtures the [H+] concentration is an important player for the rate of dissolution and sugar degradation[29]. Therefore, the [H^+] of the MIL must not be interacting with the biomass structure, and thereby, cannot catalyze the depolymerization-hydrolysis of cellulose into water-soluble reducing sugars under suitable conditions.

5.4 Concluding remarks and future perspectives

This research has developed reproducible methods for the synthesis of Bmim[$FeCl_4$], Emim[$FeCl_4$], completed the screening for dissolution of switch grass, microcrystalline cellulose, and fibrous cellulose. It was demonstrated that Bmim[$FeCl_4$] and Emim[$FeCl_4$] can both facilitate the depolymerization of select biomass. A comparison of the dissolution effects of Bmim[$FeCl_4$], Emim[$FeCl_4$], and $FeCl_3$ indicates that the activity by Bmim[$FeCl_4$] is the highest. However, it must be noted that the solubility of the magnetic iron complex of [Bmim]Cl^- is significantly less than that of the non-magnetic complex which have up to 25 w/w%[10]. Investigation also shows that a biomass/magnetic ionic liquid reaction needs sufficiently higher temperatures when compared to [Bmim]Cl^- to achieve slight dissolution.

To further this research, results indicate that it might be of worthy investigation to perform many other studies. Determine the water content of Bmim[$FeCl_4$] and Emim[$FeCl_4$] to see if moisture is preventing greater dissolution. To test other ionic liquids with paramagnetic properties and adjust the pH to see if dissolution rates and solubility would increase. To develop inexpensive methods of separation intensification for the recovery of the magnetic ionic liquids (i.e. increasing the magnetic field strength induced on the MIL/biomass slurry during separation). In addition, it might of interest to explore other reaction conditions employing the

use of ultrasound or microwave technology or varying the pressures and temperatures. Lastly, to understand the interaction mechanism between magnetic ionic liquids and cellulose, hemicellulose, or lignin further through macroscopic and microcosmic methods.

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APPENDIX

APPENDIX A

Table A.1: Effect of various pretreatment methods on the chemical composition and chemical/physical structure of lignocellulosic biomass. H: high effect, L: low effect, ND: not determined, *Depends on the chemical nature of the solvent.[7]

Pretreatment method	Increases accessible surface area	Decrystalizes cellulose	Removes hemicellulose	Removes lignin	Alters lignin structure
Alkaline	H	ND	L	H	H
Acid	H		H		H
Green Solvent	H	H	L	H or L*	L
Steam Explosion	H		H		L
LHW	H	ND	H		L
AFEX	H	H	L	H	H
ARP	H	H	L	H	H
SCF	H	H	H		L

Table A.2 Advantages and disadvantages of different pretreatment methods of lignocellulosic biomass.[7]

Pretreatment method	Advantages	Disadvantages
Alkali	(i) Efficient removal of lignin	(i) High cost of alkaline catalyst
	(ii) Low inhibitor formation	(ii) Alteration of lignin structure
Acid	(i) High glucose yield	(i) High costs of acids and need for recovery
	(ii) Solubilizes hemicellulose	(ii) High costs of corrosive resistant equipment
		(iii) Formation of inhibitors
Green solvents	(i) Lignin and hemicellulose hydrolysis	(i) High solvent costs
	(ii) Ability to dissolve high loadings of different biomass types	(ii) Need for solvent recovery and recycle
	(iii) Mild processing conditions (low temperatures)	
Steam	(i) Cost effective	(i) Partial hemicellulose degradation
	(ii) Lignin transformation and hemicellulose solubilization	(ii) Acid catalyst needed to make process efficient with high lignin content material
	(iii) High yield of glucose and hemicellulose in two-step process	(iii) Toxic compound generation
LHW	(i) Separation of nearly pure hemicellulose from rest of feedstock	(i) High energy/water input
	(ii) No need for catalyst	(ii) Solid mass left over will need to be dealt with (cellulose/lignin)
	(iii) Hydrolysis of hemicellulose	
AFEX	(i) High effectiveness for herbaceous material and low lignin content biomass	(i) Recycling of ammonia is needed
	(ii) Cellulose becomes more accessible	(ii) Less effective process with increasing lignin content
	(iii) Causes inactivity between lignin and enzymes	(iii) Alters lignin structure
	(iv) Low formation of inhibitors	(iv) High cost of ammonia
ARP	(i) Removes majority of lignin	(i) High energy costs and liquid loading
	(ii) High cellulose content after pretreatment	
	(iii) Herbaceous materials are most affected	
Supercritical fluid	(i) Low degradation of sugars	(i) High pressure requirements
	(ii) Cost effective	(ii) Lignin and hemicelluloses unaffected
	(iii) Increases cellulose accessible area	

APPENDIX B

B1: Physical and Chemical properties of 1-ethyl-3-methylimidazolium chloride

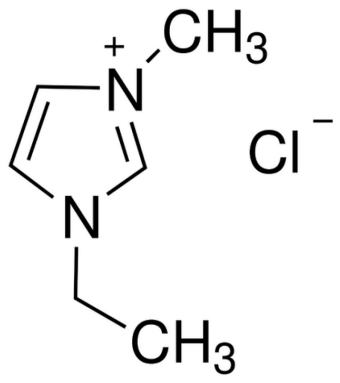

Figure B.1: [EMIM]Cl^-

Name: 1-ethyl-3-methylimidazolium chloride

CAS: 65039-09-0

Empirical Formula: $C_6H_{11}ClN_2$

Molecular Weight: 146.62 g/mol

pH: 7.7 at 100 g/l

Melting point: 77-79^oC

Flash Point: 186.00 ^oC

Relative Density: 1.112 g/cm^3 at 80^oC

B2: Physical and Chemical properties of 1-butyl-3-methylimidazolium chloride

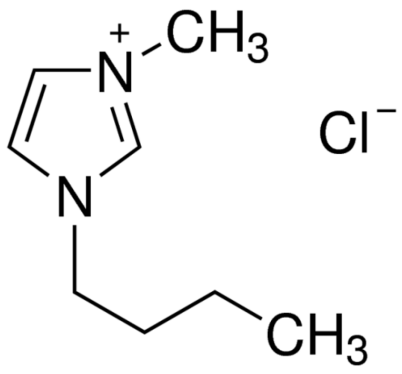

Figure B.2: [BMIM]Cl^-

Name: 1-butyl-3-methylimidazolium chloride

CAS: 79917-90-1

Empirical Formula: $C_8H_{15}ClN_2$

Molecular Weight: 174.67 g/mol

pH: 7.9 at 100 g/l

Melting point: 70^oC

Flash Point: 192 ^oC

Relative Density: 1.086 g/cm^3 at 20^oC

APPENDIX C

C: Procedure for the Filter Paper Assay for Saccharifying Cellulase

Authors: *Y.H. Percival Zhang, Jiong Hong, and Xinhao Ye, Cellulase Assays, Biofuels: Methods and Protocols, Methods in Molecular Biology, Jonathan R. Mielenz (ed.), vol. 581; 2009 Chapter 14*

C1: Assay description

FPA is the most common total cellulase activity assay recommended by the International Union of Pure and Applied Chemistry (IUPAC)[1]. IUPAC recommends a filter paper activity (FPA) assay that differs from most enzyme assays based on soluble substrate for initial reaction rates. This assay is based on a fixed degree of conversion of substrate, i.e. a fixed amount (2 mg) of glucose (based on reducing sugars measured by the DNS assay) released from 50 mg of filter paper (i.e., both amorphous and crystalline fractions of the substrate are hydrolyzed) within a fixed time (i.e., 60 min). In part due to the solid heterogeneous substrate, reducing sugar yield during hydrolysis is not a linear function of the quantity of cellulase enzyme in the assay mixture. That is, twice the amount of enzyme does not yield two times the reducing sugar within equal time. Total cellulase activity is described in terms of "filter-paper units" (FPU) per milliliter of original (undiluted) enzyme solution. The strengths of this assay are that (1) the substrate is widely available and (2) the substrate is reasonably susceptible to cellulase activity. However, the FPA has long been recognized for its complexity and susceptibility to operator errors.[2]

[1] Ghose TK (1987) Measurement of cellulase activities. *Pure Appl. Chem.* 59:257–268.
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C2: Reagents and Materials

Supplies:

13x100 mm test tubes/caps	Citric acid monohydrate
30 x 115 mm conical tubes	Distilled Water
Whatman No. 1 paper strips	Sodium Hydroxide
Phenol	3,5 Dinitrosalicylic acid
Sodium metabisulfite	Sodium Potassium Tartrate

Dilute Enzyme Solution (DES)-

Dilute commercial cellulase solution 20 fold using 0.05M Na-citrate buffer (1 part solute, 19 part solvent, CV=CV). Use conical tube.

Enzyme Stock (ES)-

Use conical tube.
ES1: 0.10 ml of DES + 1.90 ml of citrate buffer (dilute rate = 0.0250).
ES2: 0.15 ml of DES + 1.85 ml of citrate buffer (dilute rate = 0.0375).
ES3: 0.20 ml of DES + 1.80 ml of citrate buffer (dilute rate= 0.0500).
ES4: 0.30 ml of DES + 1.70 ml of citrate buffer (dilute rate = 0.0750).
ES5: 0.35 ml of DES + 1.65 ml of citrate buffer (dilute rate = 0.0850).

Glucose Standards Stocks (GSS)-

A working stock solution of anhydrous glucose (10 mg/mL) should be made up. Use conical tube
GSS1: 1.0 ml of glucose stock + 4.0 ml buffer = 2 mg/ml (1.0 mg/0.5 ml).
GSS2: 1.0 ml of glucose stock + 2.0 ml buffer = 3.3 mg/ml (1.65 mg/0.5 ml).
GSS3: 1.0 ml of glucose stock + 1.0 ml buffer = 5 mg/ml (2.5 mg/0.5 ml).
GSS4: 1.0 ml of glucose stock + 0.5 ml buffer = 6.7 mg/ml (3.35 mg/0.5 ml).

Glucose Standards Tubes (GSs)-

Use test tube
GS1: 0.5 ml of GSS1 + 1.0 ml of .05M citrate buffer
GS2: 0.5 ml of GSS2 + 1.0 ml of .05M citrate buffer
GS3: 0.5 ml of GSS3 + 1.0 ml of .05M citrate buffer
GS4: 0.5 ml of GSS4 + 1.0 ml of .05M citrate buffer

Enzyme Controls Tubes (EC)-

Use test tube
EC1: 1.0 ml of .05M citrate buffer + 0.5 ml ES1
EC2: 1.0 ml of .05M citrate buffer + 0.5 ml ES2
EC3: 1.0 ml of .05M citrate buffer + 0.5 ml ES3
EC4: 1.0 ml of .05M citrate buffer + 0.5 ml ES4
EC5: 1.0 ml of .05M citrate buffer + 0.5 ml ES5

Substrate Control Tube (SC)-

Use test tube
SC: 1.5 ml of 0.05M citrate buffer + filter paper strip

Reagent Blank Tube (RB)-

Use test tube
RB: 1.5 ml of 0.05M citrate buffer

Enzyme Assay Tubes (E)-

Use test tube
E1: 0.50 ml ES1 + filter paper strip + 1.0 ml buffer
E2: 0.50 ml ES2 + filter paper strip + 1.0 ml buffer
E3: 0.50 ml ES3 + filter paper strip + 1.0 ml buffer
E4: 0.50 ml ES4 + filter paper strip + 1.0 ml buffer
E5: 0.50 ml ES5 + filter paper strip + 1.0 ml buffer

A. Citrate Buffer: For *this procedure,* cellulase assays are carried out in 0.05 M citrate buffer pH 4.8. The assay conditions must be defined when reporting results.

1. Mix and dissolve:

Citric acid monohydrate	210 g
Distilled Water	750 mL
NaOH - add until pH equals 4.3	50 to 60 g

2. Dilute to 1 L and check pH. If necessary, add NaOH until the pH is 4.5. This is 1M stock citrate buffer

3. Citrate buffer (50 mM, pH 4.8):

a. Dilute 1M stock citrate buffer solution 20 fold using distilled water (1 part solute, 19 part solvent, $C_1V_1=C_2V_2$)

b. After diluting the citrate buffer stock check and adjust the pH if necessary to pH 4.8.

4. *Store at room temperature (up to 3 months) or at 4^oC for longer storage.*

B. DNS Reagent

1. Mix and Dissolve on stir plate:

Distilled water	1416 mL
3,5 Dinitrosalicylic acid	10.6 g
Sodium hydroxide	19.8 g

2. Add:

Rochelle salts (sodium potassium tartrate)	306 g
Phenol (melt at 50oC)	7.6 mL
Sodium metabisulfite	8.3 g

3. Titrate 3 ml of the DNS reagent using 0.1 M HCl using the phenolphthalein endpoint pH check. It should take 5–6 ml of HCl for a transition from red to colorless. Add NaOH if required (2 g of NaOH added = 1 ml of 0.1 M HCl used for 3 ml of the DNS reagent)

4. *Store in darkness at 4°C for at least 1 month. It could lose its reducing ability after long storage.*

C3: Procedure

1. Place a rolled filter paper strip into each 13 × 100 test tube.

2. Add 1.0 ml of 50 mM citrate buffer (pH 4.8) to the tubes; the paper strip should be submerged in the buffer.[3]

3. Prepare the enzyme dilution series, of which at least two dilutions must be made of each enzyme sample, with one dilution releasing slightly more than 2.0 mg of glucose (~2.1 mg) and one slightly less than 2.0 mg of glucose (1.9 mg)[3].

4. Prepare the dilute glucose standards (GSs).

5. Prepare the blank and controls.

6. Pre-warm the enzyme solutions, blank, and controls until equilibrium.

7. Add 0.5 ml of the enzyme dilution series to the tubes with filter paper substrate (E1–5); add 0.5 ml of the enzyme dilution series to the tubes without filter paper substrate (EC1–5).

8. Incubate the tubes of E1–5, GSs, RB, EC1–5, and SC in a 50°C water bath (or shaker incubator) for exactly 60 min.

9. Add 3.0 ml of the DNS reagent to stop the reaction, and mix well.[3]

10. Boil all tubes for exactly 5.0 min.[4]

11. Transfer the tubes to an ice-cold water bath.

12. Withdraw ~0.5 ml of the colored solutions into 1.5-ml microcentrifuge tubes and centrifuge at ~10,000 g for 3 min.

13. Add 0.200 ml of the supernatant into 3-ml spectrophotometer cuvette tubes, add 2.5 ml of water, and mix well by using a pipette or by inversion several times.

14. Measure absorbance at 540 nm, where the absorbance of RB is used as the blank.

[3] See Reagents and Materials sheet for procedure.
[4] The boiling condition should be severe, and the volume of the boiling water bath should be maintained above the level of the total liquid volume of the test tubes to promote full color development.

C4: Calculations

1. Draw a standard sugar curve (sugar along the x-axis vs. absorbance at 540 along the y-axis), as shown in Fig. 1.

2. Calculate the delta absorbance of dilute enzyme solutions (DE1–4) for E1–5 by subtraction of the sum of the absorbance of EC1–5 and SC.

3. Calculate the real glucose concentrations released by E1–5 according to a standard sugar curve.

4. Draw the relationship between the real glucose concentrations and their respective enzyme dilution rates (EDRs) (Fig. 1).

5. Link the points less than 2 mg and greater than 2 mg by a line, and identify the EDR by using the point for 2-mg glucose based on the line (Fig. 1).

6. Calculate the FPA of the original concentrated enzyme solution in terms of FPU/ml: 0.37 FPA= 0.37/EDR where 2 mg glucose = 2 mg/(0.18 mg/mmol) × 0.5 ml × 60 min = 0.37 mmol/min/ml.

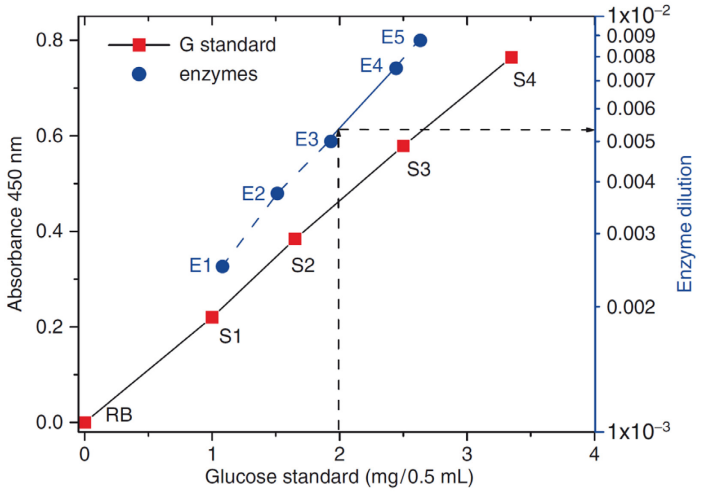

Fig. 1 The relationship of absorbance at 540 nm for the DNS assay and EDRs in terms of glucose concentration.

APPENDIX D

D1: Sulfuric Acid Pretreatment

Author: *Mahalaxmi, Swetha PREPROCESSING OF BIOMASS USING MECHANICAL CHEMICAL AND MICROBIAL TECHNIQUES. (2012).*

In a set of experiments, >2 mm, 1-2 mm, <1 mm and Un-partitioned samples were subjected to 0.69 %, 2 %, 5 % and 10 % concentrations of sulfuric acid pretreatment for 30 minutes in 20 mL tightly capped hungate tubes at 260 °F. The reaction mixture comprised 1 g biomass and 10 mL of H_2SO_4 (0.097 mL, 0.28 mL, 0.69 mL and 1.39 mL of 72 % H_2SO_4 made up to 10 mL with water, to make 0.69 %, 2 %, 5 % and 10 % H_2SO_4 respectively). The tubes were allowed to cool to room temperature; the reaction mixture was filtered and washed to obtain filtrate and residue. The filtrate was analyzed for sugars, furfural, hydroxyl methyl furfural (HMF) and polyphenols using HPLC.

VITA

I, Christopher R. Riley, was born in a small southern town- Cordele, Georgia. He spent the early part of his academic career as a homeschool student. He attended such universities as the University of Virginia, Harvard University, and Darton College to obtain college credit before his graduation in May 2005 with his high school diploma with an emphasis in pre-medicine. In the summer of 2005, he attended an intensive pre-medicine summer program at the Medical College of Georgia where he was awarded first place in Medical Library Research. Christopher attended Georgia Southern University where he graduated with Honors in Analytical Chemistry with a minor in Biology, in 2009. His honors research was published in a journal and presented at several conferences.

In 2011, Christopher started the masters program in Chemical Engineering at the University of Mississippi. During his tenure at the university, he was an active member of several campus organizations and gave back to his community via a variety of volunteering activities. Additionally, he continued his relationship within the field of Chemistry and is an active member of the American Chemical Society. During his spare time, he is an avid cyclist, gym enthusiast and thoroughly enjoys cooking, playing video games, and reading about the latest advancements in technology and science. After graduation, he plans to develop his engineering career via a full-time position as a process engineer and looks forward to traveling the world.