PRETREATMENT OF PEANUT SHELLS FOR CO – PRODUCTION OF GLUCOSE AND CONCRETE ADMIXTURE

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PRETREATMENT OF PEANUT SHELLS FOR CO – PRODUCTION OF GLUCOSE AND CONCRETE ADMIXTURE

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ABSTRACT

PRETREATMENT OF PEANUT SHELLS FOR CO – PRODUCTION OF GLUCOSE AND CONCRETE ADMIXTURE

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This thesis work aims the ionic liquid pretreatment of peanut shells for co-production of glucose as fermentable sugar and lignin, considering a multi product perspective. The effects of ionic liquid type and pretreatment time period on the sugar and lignin yields were investigated, as the particle size and temperature parameters were determined in the preliminary studies. Peanut shells were pretreated at constant temperature, 150 ^{o}C, for 5, 15 and 30 minutes with 1-ethyl-3-methylimidazolium acetate and for 15, 30 and 60 minutes with 1-ethyl-3-methylimidazolium chloride. The pretreated peanut shells were then subjected to enzymatic hydrolysis in order to produce fermentable sugars, mostly, glucose. The solid residue obtained upon enzymatic hydrolysis was analyzed in terms of lignin quantity. 1-ethyl-3-methylimidazolium acetate pretreatment for 15 minutes resulted in the maximum reducing sugar and lignin yields; 28 g of reducing sugar and 20 g of solid residue with 70% lignin were obtained per 100 g of peanut shells. Higher pretreatment time resulted in lower yields. Moreover, no optimal time period for 1-ethyl-3-methylimidazolium chloride pretreatment was obtained, since reducing sugar and lignin yields increased as the time period increased. Also all reducing sugar and lignin yields were lower than that obtained with 1-ethyl-3-methylimidazolium acetate. Lignin obtained upon enzymatic hydrolysis of 1-ethyl-3-methylimidazolium acetate pretreated peanut shells were characterized by SEM, FTIR, TGA and XRD analyses, which also showed the morphological and structural effects of pretreatment and enzymatic hydrolysis on peanut shells; and used as concrete admixture, which increased the flow of the concrete by 6%.

Keywords: Biomass, peanut shells, pretreatment, ionic liquid, cellulose, lignin, enzymatic hydrolysis, cellulases, concrete admixture

ÖZ

YER FISTIĞI KABUĞUNDAN ÖN İŞLEM İLE EŞ ZAMANLI GLİKOZ VE BETON KATKI MALZEMESİ ÜRETİMİ

Tatlı, Emre
Yüksek Lisans, Kimya Mühendisliği Bölümü
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Şubat 2013, 89 sayfa

Bu çalışmada, yer fıstığı kabuğunun, çoklu ürün perspektifi ile, iyonik sıvılarla ön işlemden geçirilmesi çalışılmıştır. Sıcaklık ve tane boyu parametreleri önceden belirlenmiş ve sabitlenmiş olmakla birlikte, ön işlem süresi ve iyonik sıvı türünün indirgen şeker ve lignin üretimi üzerindeki etkisi incelenmiştir. Yer fıstığı kabuğu, sabit sıcaklıkta, 150 ^{o}C'de 1-etil-3-metilimidazolyum asetat ile 5, 15 ve 30 dakika boyunca, 1-etil-3-metilimidazolyum klorür ile 15, 30 ve 60 dakika boyunca ön işlemden geçirildi.

Ön işlemden geçirilmiş yer fıstığı kabuğu daha sonra indirgen şeker eldesi için enzimatik hidrolize maruz bırakıldı. Hidroliz sonrası kalan katı atık malzeme, lignin miktarı bakımından incelendi. Sonuç olarak, 1-etil-3-metilimidazolyum asetat ile 150 ^{o}C'de 15 dakikalık ön işlemin 100 gram hammadde için 28 gram indirgen şeker ve %70 saflıkta 20 gram lignin ile en yüksek değerleri verdiği görüldü. Daha uzun ön işlemde değerlerin düştüğü gözlemlendi. Bunun yanında, 1-etil-3-metilimidazolyum klorür ön işlemlerinde optimum bir zaman görülmedi. Ön işlem süresinin artmasıyla ürün verimlerinin de arttığı görüldü. Yine de bu değerlerin 1-etil-3-metilimidazolyum asetat ile alınan sonuçlardan düşük olduğu görüldü.

1-etil-3-metilimidazolyum asetat ön işlemi ile elde edilen ligninin X – ışınımı kırınımı, FTIR, termogravimetrik analiz ve SEM analizleri yapıldı. Bu analizlerle ön işlemin yer fıstığı kabuğu üzerindeki etkileri gözlendi. Ayrıca üretilen lignin malzemesi beton katkı malzemesi olarak test edildi ve malzemenin betonun akışını %6 artırdığı görüldü.

Anahtar kelimeler: Biyokütle, yer fıstığı kabuğu, ön işlem , iyonik sıvı, selüloz, lignin, enzimatik hidroliz, selülaz, çimento katkı malzemeleri

To my family,

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NOMENCLATURE

ABBREVATIONS:

[AMIM][Cl] : 1-allyl-3-methylimidazolium chloride
[EMIM][Ac] : 1-ethyl-3-methylimidazolium acetate
[EMIM][Cl] : 1-ethyl-3-methylimidazolium chloride
FTIR : Fourier transform infrared spectropy
HMF : Hydroxymethylfurfural
HPLC : High – performance liquid chromatography
LRR : Lignin – rich residue
NREL : National Renewable Energy Laboratory
SEM : Scanning electron microscope
TGA : Thermogravimetric Analysis
XRD: X-Ray diffraction

CHAPTER 1

INTRODUCTION

1.1 BIOMASS ECONOMY and BIOREFINERY

Depletion of current fossil fuel resources has been a main concern from both energy and materials point of view. As petroleum resources are depleted, higher price of petroleum and derived fuels' cost is estimated in the 21st century. Moreover, many materials such as plastics are used today are derived from petroleum resources (Mosier et al., 2005).

On the other hand, biomass economy, on the other hand, which is defined to be based on converting biomass in biorefineries into value added products such as fuels, chemicals and materials, gained importance due to depending on renewable sources and being an alternative to petroleum based counterparts. Biorefinery is at the center of the biomass economy concept, and defined as integrated biomass conversion processes, where a variety of products such as fuels, power, chemicals and materials are produced throgh combination of technologies (FitzPatrick et al., 2010).

One good example of biorefinery concept isfirst generation bioethanol which has been used as a gasoline additive for more than three decades. In this concept, ethanol production depends mostly on sugar derived from sugarcane, sugar beet and grain based starch like corn starch. Starch is hydrolyzed to its glucose units, which are later fermented to ethanol. However, the feedstocks used in these processes are also food materials, and using food materials as raw materials for ethanol production will ultimately create a debate over food vs. fuel contradiction (Mabee et al., 2011; Agbor et al., 2011; Vancov et al., 2012).

Hence, another biorefinery concept and processes are developed. In this concept, sugar for fermentation is obtained from the cellulose in lignocellulosic biomass, such as cotton stalk, switch grass, wood chips, corn stover, paper waste etc., as cellulose is the most abundant biopolymer on the earth. However, this concept is still under development, since isolating the cellulose part and converting it into glucose is costly with today's technology. In this concept, the lignocellulosic biomass is firstly treated via physical, chemical, biological methods in order to fractionate and isolate cellulose. After isolating the cellulosic part, it is exposed to either acidic or enzymatic hydrolysis to digest the cellulose and produce glucose monomers, of which the cellulose is made up. Finally, produced fermentable sugar is converted into variety of products via fermentation.

Lignocellulosic ethanol concept has gained importance since the feedstock is inexpensive and renewable, furthermore an estimated amount of over one billion tons of biomass is available as a biofuel raw material (Li et al., 2010). The main drawbacks of the ethanol production using lignocellulosic feedstock are the expensive pretreatment and hydrolysis steps. Normally, lignocellulose has a recalcitrant structure. It is resistant to chemical and biological attacks. Pretreatment is a necessary step to fractionate the lignocellulosic biomass into its main components, isolating cellulose, removing lignin, and altering the cellulosic structure to make it be more prone to enzymatic hydrolysisstep, in which enzymes degrade and digest the cellulose and monomers that make up the cellulose are released.

Another advantage of using lignocelloluse as raw material is that the lignin and hemicellulose parts of the material can be exploited. A biorefinery process framework will be much more effective and economically feasable if there are multiple products in one process line (Bahgecul et al., 2012). In

the case of cellulosic ethanol production, for instance, today many current cellulosic ethanol production pilot plants produce large amounts of residual lignin, when there is large amount of biomass to be processed. Hence, a value – added lignin product along with the fermentable sugars, from the same biomass conversion process, will improve the feasability and economy of the biomass conversion refineries and processes and lessen the disposal rate of a refinery(Doherty et al., 2011). In Figure 1.1, a biorefinery and bioprocess flowchart concept is shown from lignocellulosic biomass to the final products.

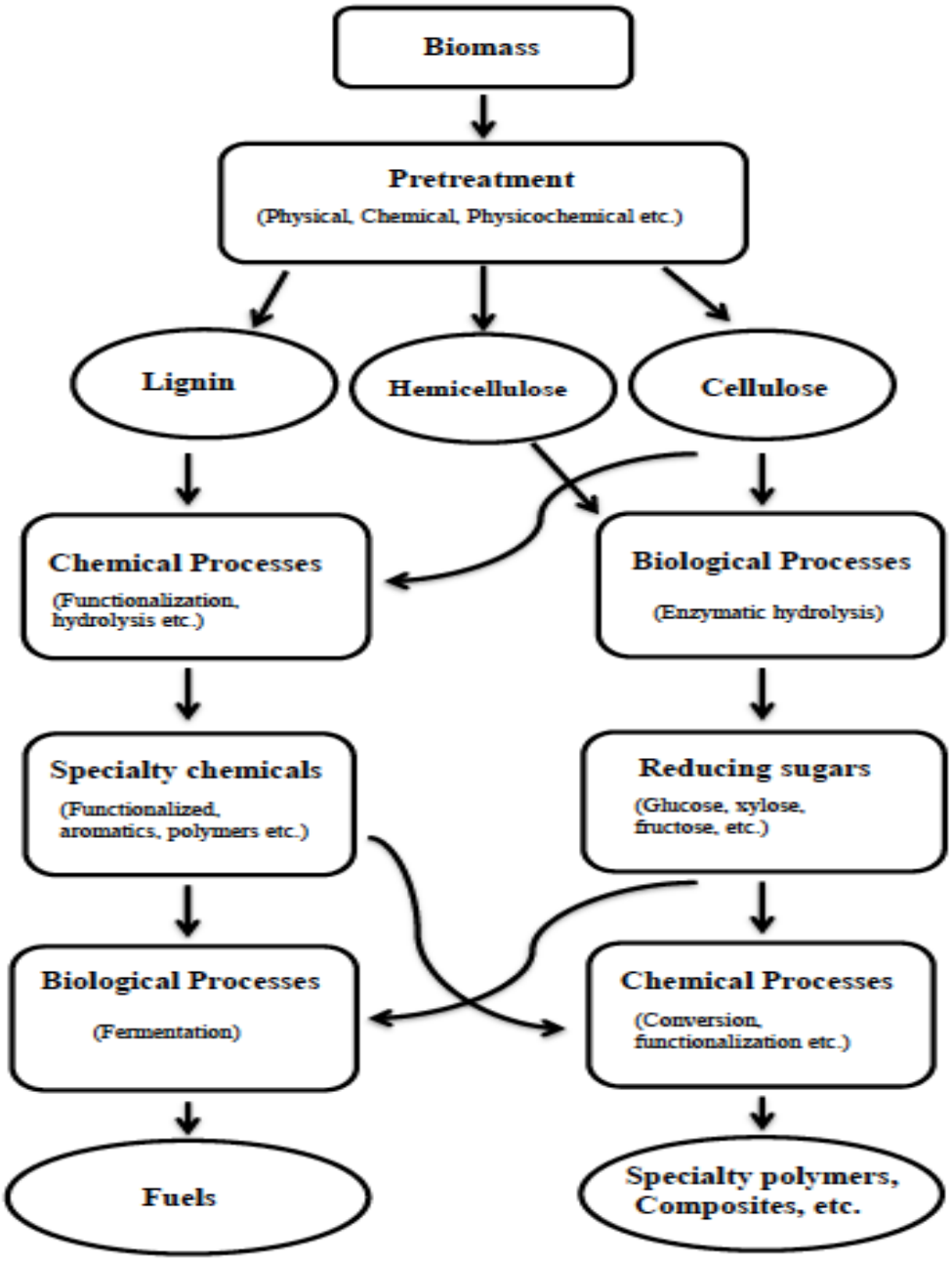


Figure 1.1 Schematic of biorefinery concept (FitzPatrick et al., 2010).

1.2 LIGNOCELLULOSIC BIOMASS

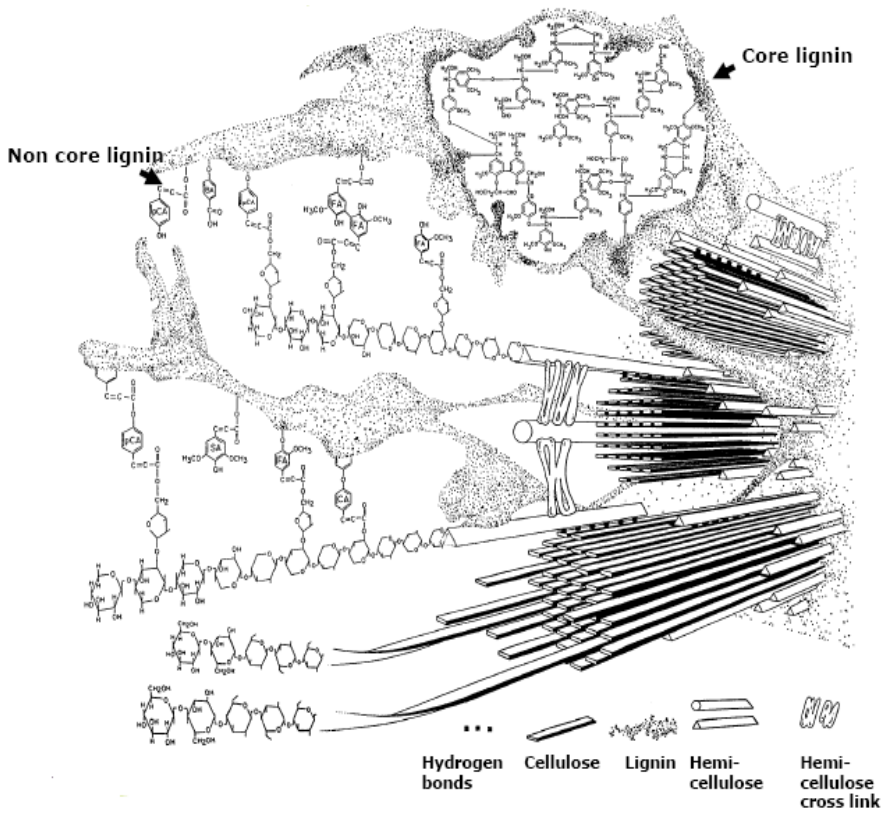


Figure 1.2 Cell wall model (J. Bidlack, M. Malone and R. Benson. Okla. Acad. Sci., 1992, Vol. 72.).

Plant cell wall is what makes the lignocellulosic biomass.It is composed of roughly 40 – 50% cellulose, 25 – 35% hemicellulose and 15 – 20% lignin (Wyman et al., 2005).The amounts of the components, the structure and the complexity of cell wall will depend on the type of the lignocellulosic biomass.

Agricultural residues like corn stover, cotton stalk, corncob, woody materials and energy corps; and also waste paper and textile products such as cotton and linen fabrics are examples for sources of lignocellulosic biomass (Liu et al., 2012).In Table 1.1, compositions of potential lignocellulosic biomass sources are given (Limayem and Ricke, 2012).

Table 1.1 Compositions of potential lignocellulosic biomass sources

Lignocellulosic material	**Cellulose (%)**	**Hemicelluloses (%)**	**Lignin (%)**
Agricultural residues	37 – 50	25 – 50	5 – 15
Hardwood	45 – 47	25 – 40	20 – 25
Softwood	40 – 45	25 – 29	30 – 60
Grasses	25 – 40	35 – 50	-
Waste paper from chemical process	50 – 70	12 – 20	6 – 10
Newspaper	40 – 55	25 – 40	18 – 30
Switchgrass	40 – 45	30 – 35	12

In Figure 1.2, cell wall model is shown. It is seen that, the main components of the plant cell wall make up a complex network. Cellulose fibers are bound together with hemicellulose structures and lignin makes the main matrix, surrounding the cellulose – hemicellulose packs. In Table 1.2, recoverable lignocellulosic biomass are given.

Table 1.2 Types of recoverable lignocellulosic biomass (Yousuf, 2012).

Food crops	**Non-food/Energy crops**	**Forest Residues**
Rice straw	Poplar	Tree residues
Sugarcane tops	Eucalyptus	Wood processing residues
Maize stalks millet	Mischantus	Recycled wood
Groundnut stalks	Switchgrass	-
Corn straw	Hemp	-
Soybean residue	Cardoon	-
-	Giant reed	-
-	Salix	-
-	Jute stalks	-
-	Willow	-

Lignocellulosic biomass makes up the largest renewable bioethanol resource. The U.S. production of lignocellulosic materials is estimated as 1.4 billion dry tons annually. 30% of this production is from forest biomass, which is about 370 million tons. (Limayem and Ricke, 2012) Woody biomass can be divided into two groups, as softwoods and hardwoods. Softwoods are defined as biomass originated from conifers and gymnosperm trees. Softwoods are less dense than hardwoods and they grow faster. Pine, cedar, spruce, fir and redwood are examples of softwood trees. On the other hand, hardwoods are defined as biomass originated from angiosperm trees, which are poplar, willow, oak, cottonwood and aspen (Limayem and Ricke, 2012).

127 – 317 million metric tons of agricultural residues are harvested in the U.S. per year. Most of the agricultural residues are rice straws, corn stalks and wheat straw. Swtich grass is another potential herbaceous energy crop (Limayem and Ricke, 2012).

Peanut (*Arachis hypogaea*) is an agricultural plant, whose fruits/seeds are one of the most important raw materials of the food industry. Annual world production of peanut (referring the fruit here and hereafter) in 2009/2010 was 33.36 metric million tons (U.S. Department of Agriculture, fas.usda.gov). The fruits of the peanut are used as food and industrial raw material for other products such as peanut butter, snacks, peanut oil and peanut flour.The pods, or the shells in which the fruits develop, are cellulose containing agricultural and industrial wastes. Domestic production of the peanut in Turkey was about 77000 tons in 2008 (Department of Agriculture, www.tarim.gov.tr) and a significant amount (around 40%) of this production is concentrated especially in Osmaniye and Çukurova region.

As around one third of the peanut fruits' mass is the shell, about 25000 tons of peanut shells can be processed annually according to the statistics given by Department of Agriculture of Turkey. Peanut shell is useful agricultural waste feedstock with not only high contents of cellulose, but also high contents of lignin. It is reported to have 36% cellulose, 19% hemicellulose and 30% lignin by Zhang et al., (2006). Thus there is an opportunity for processing about 7500 tons of lignin,9000 tons of cellulose and 4800 tons of hemicellulose per year.

Figure 1.3 Peanut seeds and the shells in which they grow (Images from www.osmaniye.gov.tr, Last accessed: December 2012).

Today, there are vast amout of research studies in which lignocellulosic biomass is in the center of the attention. Not only for cellulosic ethanol, lignocellulosic biomass is also studied for gasification, conversion into chemicals other than ethanol (e.g. acetone, butanol, organic acids etc.), biodegradable films, antioxidants, lignin polymers and etc.In the following parts, the main components of the plant cell wall are discussed.

1.2.1 CELLULOSE

Cellolulose is the most abundant biopolymer and it is the main constituent of the plant cell wall (Wyman et al., 2005).It is a polysaccharide, a polymer of D-glucose units bonded with β(1-4) glucosidic bonds and it is has a crystalline structure and it is water insoluble (Wyman et al., 2005; Mosier et al., 2005). Cellulose and its derivatives are widely used in paper, textile, fiber, polymer and paint industry. (Swatlowski et. al, 2002).In Figure 1.4 molecular structure of cellulose is shown.

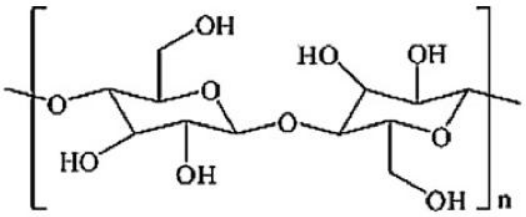


Figure 1.4 Structure of Cellulose (Maki-Arvela et al., 2010; Encyclopedia of Polymer Sciene and Technology).

The β(1-4) glucosidic bonds are different than the α(1-4) glucosidic bonds, which are present in starch and glycogen. Moreover, no branching exists in cellulose molecules opposed to starch. Moreover, cellulose is a heterogeneous porous material with external and internal surfaces. The shape and size of the particles of the cellulose determines the external surface area, as the capillary structure of the cellulose fibers determine the internal surface area (Walker et al., 1991).

There are both crystalline and amorphous regions on cellulose fibers. The fraction of the crystalline parts in the total quantitiy has been considered as an important parameter that affects the hydrolysis of the cellulose, as crystalline cellulose is more recalcitrant to degredation (Walker et al., 1991). Moreover, the cross linkages of hydroxyl groups build the cellulose microfibrils, which makes the molecules strong and compact. Also, extensive hydrogen bondings among the molecules build a strong matrix and gives crystallinity. Starchy materials can have a transition ftom crystalline to amorph structure at 60 – 70 ^{o}C, however cellulose can have this transition at 320 ^{o}C with 25 MPa (Limayem and Ricke, 2012).

Avicel is the commercial name of microcrystalline cellulose. It is also known as cellulose powder or cotton linters. Avicel PH 101 product has a medium particle size about 50 μm. It is used as a food additiveand and it is also used in pharmaceutical industry mainly. Specific surface area of Avicel PH 102 was reported as 1.8 m^2/g and 5.4 m^2/g by Walker et al.(1991).

1.2.2 LIGNIN

Lignin is the second most abundant biopolymer on the earth (Kim et al., 2011).It is one of the three main components of the plant cell wall and it provides strength and rigidity to cell walls. About 15 wt% - 40 wt% of the plant cell wall's dry matter is lignin. It is more resistant to physical, chemical and biological attacks than other plant cell wall constituents (Doherty et al., 2011).

Lignin is a cross – linked, three dimensional amorphous phenolic polymer, composed of monolignols; p-coumaryl, coniferyl and sinapyl alcohols. These monomers are connected heterogeneously by interunit linkages (Kim et al., 2008).Molecular mass of the lignin varies between 1000 - 20000 g/mol. Glass transition temperature of lignin depends on the molecular weight of lignin. It increases when the molecular weight is increased(Doherty et al., 2011).In Figure 1.5, monolignol monomers and in Figure 1.6, schematic representation of lignin molecules from wheat straw are shown.

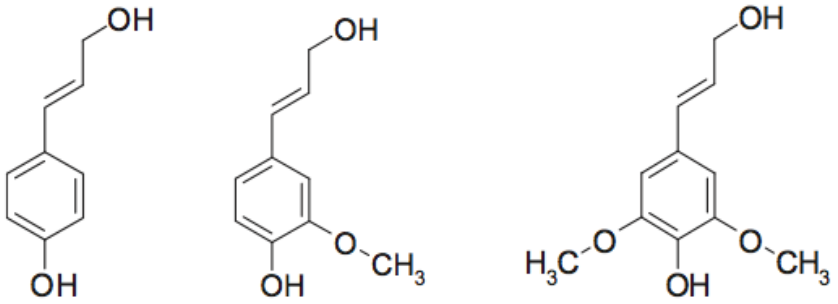


Figure 1.5 Monolignol monomers: From left to right: p-Coumaryl alcohol, Coniferyl alcohol, and Sinapyl alcohol (Doherty et al., 2011)

Carbon – carbon bonds and carbon – oxygend bonds are present between monomers in lignin. Carbon – oxygen bonds exist between p – hydroxy and the b –end of the propenyl groups. The rigidity of the lignin structure depends on the degree of crosslinking and substitution. Conyferil alcohol structure is dominant in softwoods, while sinapyl alcohol is dominant in hardwoods. In grasses, on the other hand, p – coumaryl alcohol is dominant (Doherty el al., 2011).

Being a complex, heterogeneous, three – dimensional polymer, lignin has different intermolecular linkages unlike cellulose. The main intermolecular linkage is arylgycerol-β-O-4-aryl ether linkage. This linkage makes up the 48 – 60% of the total intermolecular linkages in lignin(Braun et al., 2005).

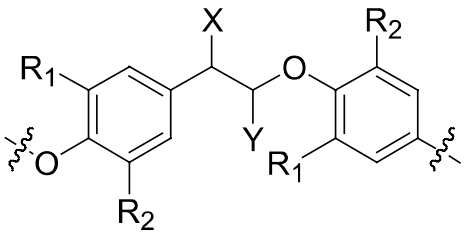


Figure 1.6 Representation of interunit linkages in lignins (Braun et al., 2005).R1 = H, OCH_3 or Ar R2 = H or OCH_3X = OH, OAr, =OY = CH_2OH

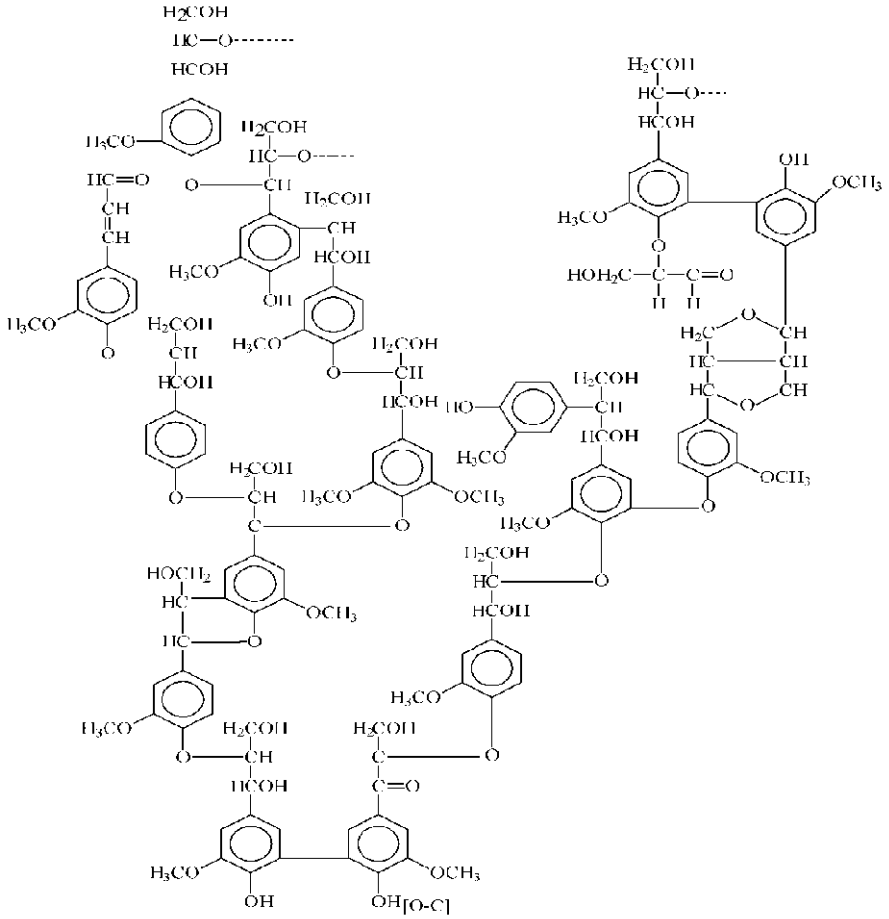


Figure 1.7 Schematic representation of wheat straw lignin molecules. (Pouteau et al., 2003)

In the industry, lignin is used generally as its derivatives and lignin derivatives are mainly by-products of paper pulping industry. Most economically significant lignin derivatives are lignosulfonates. Lignosulfonates have surface – active properties and are used in cement and concrete industry, oil industry and dye industry as water reducers and dispersants; and used as additives in metallic ore processing and dust contol (Kamoun et al., 2003; Doherty et al., 2011).Moreover, lignosulfonates can be used as raw materials in order to produce other value added specialty products such as animal feed pelleting aids, vanilin, pesticides, carbon black dispersant, dyes and pigments, emulsifiers, battery expanders and industrial cleaners (Doherty el al., 2011).

When lignin is blended with natural and synthetic polymers, it generally increases the modulus and cold crystallization temperature, while the melt temperature is decreased. Due to the decrease in the degree of self – association between lignin molecules, mechanical properties of the above mentioned systems, as the lignin – polymer miscibility is improved. As the lignin has hydroxyl and carboxylic acid groups, which are readily functionalisable, the lignin - polymer interaction within different systems are studied (Doherty el al., 2011).On the other hand, lignin is an important key factor in cellulosic ethanol production studies. It is a fact that pilot plants today which produce cellulosic ethanol also produce lignin in large volumes. Hence value adding to this produced lignin became important from the point of cost effectiveness of the plants.

However, today, in most plants, process residue lignin is burned for energy or used as a carbon source for biochar, or even disposed as waste. However, lignin is compatible with most industrial chemicals, it is a source of aromatic groups, which provide mechanical stability. Moreover, it adds good rheological and viscoelastic properties in structural materials (Doherty et al., 2011).

1.2.3 HEMICELLULOSE

Hemicelluloses are heterogeneous branched polymers of xylan, mannan, beta – glucan and xyloglucan polysaccharides (Mosier et al., 2005; Maki-Arvela et al., 2010).β-D-xylose, α-L-arabinose, β-D-mannose, β-D-glucose, α-D-galactose are main pentose and hexose components which make up hemicelluloses along with uronic acids, such as α-D-glucuronic, α-D-4-O-methylgalacturonic and α-D-galacturonic acids) Acetyl groups can substitute the hydroxyl groups of sugars partially. Xylans and glucomannans are the most abundant hemicelluloses (Girio et al., 2010).

Hemicellulose structure can differ according to the biological origin. For example, in the hardwoods, glucuronoxylans and glucuronomannans are the main hemicellulose groups. Around 15 – 30% of the hardwood dry mass is composed of glucuronoxylans, which consist of a linear backbone of β-D-xylopyranosyl units linked with β-(1,4) glycosidic bonds. Acetyl groups can maket he 8% - 17% of total xylan. In softwoods, unlike the glucuronoxylans, galactoglucomannans are dominant, makin up to 25% of the dry biomass. Galactoglucomannans are made up of a linear backbone of β-D-glucopyranosyl and β-D-mannopyranosyl units, again linked with β-(1,4) glycosidic bonds, partially α-D-galactopyranosyl units attached to glucose and mannose by α-(1,6) bonds. On the other hand, arabinoglucuronoxylans are mostly found in non – woody materials like agricultural crops and also in softwoods with minor amounts. They are made up of a linear β-(1,4)-D-xylopyranose backbone containing uronic acid and α-D-arabinofuranosyl linked with α-(1,2) and α-(1,3) glycosidic bonds(Girio et al., 2010).

Xyloglucans and arabinoxylans are the other main major hemicellulose structures. They are found in mostly in hardwoods, grasses and cereal grain cell walls respectively. Xyloglucans are made up of D-glucose backbone and D-xylose, L-arabinose and D-galactose linked with β-(1,4) linkages. Xyloglucans have important role on hydrogen bonds by interacting with cellulose fibrils (Girio et al., 2010).In Table 1.3, main types of hemicellulose polysaccharides are given.In Table 1.4, hemicellulose compositions of different lignocellulosic materials are shown.

Table 1.3 Main types of polysaccharides in hemicelluloses (Girio et al., 2010).

Polysaccharide Type	**Biological Origin**	**Backbone units**
Arabinogalactan	Softwoods	β-D-Galp
Xyloglucan	Hardwoods, grasses	β-D-Glcp β-D-Xylp
Galactoglucomannan	Softwoods	β-D-Manp β-D-Glcp
Glucomannan	Softwoods and hardwoods	β-D-Manp β-D-Glcp
Glucoronoxylan	Hardwoods	β-D-Xylp
Arabinoxylans	Grasses, cereals, softwoods	β-D-Xylp

Table 1.4 Hemicellulose compositions of different lignocellulosic materials(Girio et al., 2010).

Raw Material	**Xyl**	**Ara**	**Man**	**Gal**	**Rha**	**UA**	**AcG**
SOFTWOODS							
Douglas Fir	6.0	3.0	-	3.7	-	-	-
Pine	5.3-10.6	2.0 – 4.2	5.6-13.3	1.9-3.8	-	2.5-6.0	1.2-1.9
Spruce	5.3-10.2	1.0-1.2	9.4-15.0	1.9-4.3	0.3	1.8-5.8	1.2-2.4
HARDWOODS							
Aspen	18-27.3	0.7-4.0	0.9-2.4	0.6-1.5	0.5	4.8-5.9	4.3
Birch	18.5-24.9	0.3-0.5	1.8-3.2	0.7-1.3	0.6	3.6-6.3	3.7-3.9
Black Locust	16.7-18.4	0.4-0.5	1.1-2.2	0.8	-	4.7	2.7-3.8
Eucalypt	14-19.1	0.6-1.0	1-2.0	1.0-1.9	0.3-1	2	3-3.6
Maple	18.1-19.4	0.8-1.0	1.3-3.3	1.0	-	4.9	3.6-3.9
Oak	21.7	1.0	2.3	1.9	-	3	3.5
Poplar	17.7-21.2	0.9-1.4	3.3-3.5	1.1	-	2.3-3.7	0.5-3.9
Sweet Gum	19.9	0.5	0.4	0.3	-	2.6	2.3
Sycamore	18.5	0.7	1.0	-	-	-	3.6
Willow	11.7-17.0	2.1	1.8-3.3	1.6-2.3	-	-	-
AGRICULTUAL AND AGRO-INDUSTRIAL MATERIALS							
Almond shells	34.3	2.5	1.9	0.6	-	-	-
Barley straw	15	4.0	-	-	-	-	-

Table 1.4 (Continued)

Cardoon	26.0	2.5	3.7	1.4	0.9	-	-
Corn cobs	28-35.3	3.2-5.0	-	1.0-1.2	1	3	1.9-3.8
Corn fiber	21.6	11.4	-	4.4	-	-	-
Corn stalks	25.7	4.1	<3.0	<2.5	-	-	-
Corn stover	14.8-25.2	2.0-3.6	0.3-0.4	0.8-2.2	-	-	1.7-1.9
Olive stones	2.0-3.7	1.1-1.2	0.2-0.3	0.5-0.7	0.3-0.5	1.2-2.2	-
Rice husks	17.7	1.9	-	-	-	-	1.62
Rice straw	14.8-23	2.7-4.5	1.8	0.4	-	-	-
Sugar cane bagasse	20.5-25.6	2.3-6.3	0.5-0.6	1.6	-	-	-
Wheat bran	16.0	9.0	0	1.0	0	2.0	0.4
Wheat straw	19.2	2.4-3.8	0.0-0.8	1.7-2.4	-	-	-

Xyl: Xylose, Ara: Arabinose, Man: Mannose, Gal: Galactose, Rha: Rhamnose, UA: Uronic acids, AcG: Acetyl groups

Hemicellulose makes hydrogen bonds to cellulose fibrils and forms a network which makes a structural backbone in the plant cell wall. Up to 20 – 30 % of the plant cell wall is made up of hemicelluloses(Mosier et al., 2005, Girio et al., 2010).In the industry, hemicelluloses are mainly used as sweeteners, thickeners and emulfisiers (Maki-Arvela et al., 2010). D-xylose is an industrial feedstock for xylitol production. Along with glucose, xylose and other pentoses can also be used for fermentation. Xylose is also a feedstock for furfural production. Moreover, acetic acid can be directly obtained from hemicellulose parts that contain acetyl groups (Macrotullio et al., 2011).

In contrast to crystalline structure of the cellulose, hemicelluloses show amorphous structure, with little strength and they tend to hydrolyze easily even in dilute acidic and basic conditions. They can be dissolved and hydrolyzed in water (Macrotullio et al., 2011). In Figure 1.8 and 1.9, structures of glucomannan and xylan are shown.

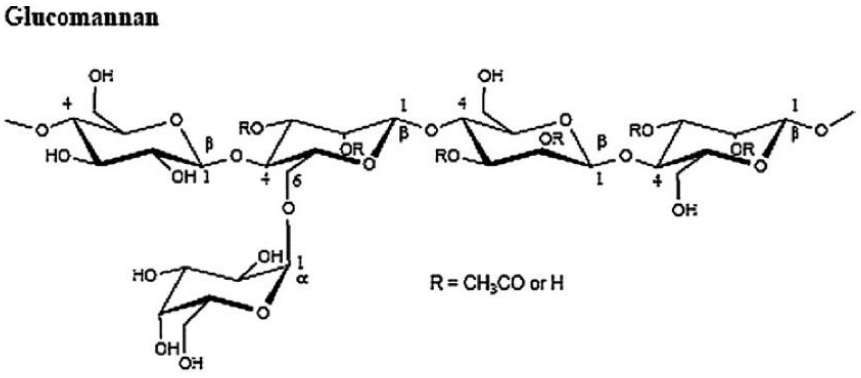


Figure 1.8 Structure of o-acetyl-galactoglucomannan (Maki-Arvela et al., 2010).

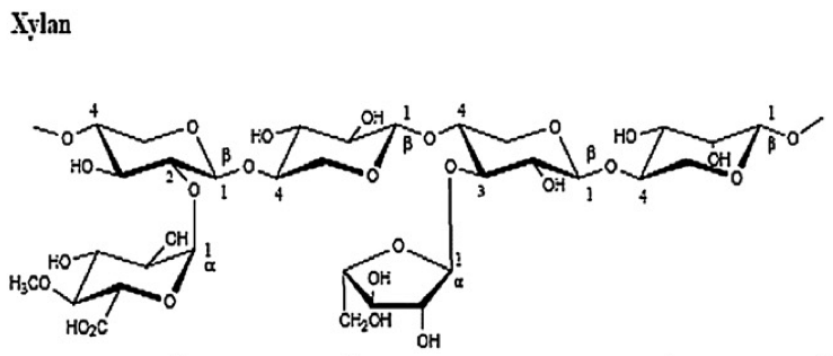


Figure 1.9 Structure of arabino-4-o-methylglucoronoxylan (Maki-Arvela et al., 2010).

1.3 PRETREATMENT METHODS

Plant cell wall is a complex structure as mentioned before. The crystallinity of the cellulose, lignin's and hemicellulolses' being physical barriers to the cellulose, and accessible surface area of the cellulose related to the former and latter, makes the cellulosic biomass resistant to hydrolysis (Mosier et al., 2005). Therefore pretreatment processes should be performed to remove the lignin, decrease the crystallinity of the cellulose; meanwhile not harming the cellulosic structure; in order to make the biomass susceptable for the further hydrolysis of the cellulose and hemicellulose contents (Liu et al., 2012; Vancov et al., 2012).

In this framework, pretreatment has come up as the most important step, determining the total cost of the lignocellulose to fuel/chemicals process. Most of the research has been conducted on developing both effective and inexpensive pretreatment methods. Many of the pretreatment methods focus on disrupting the complex structure of the cellulose-hemicellulose-lignin network, decreasing the crystallinity of the cellulose and removing the lignin and hemicellulose parts in the cell wall (Wyman et al., 2005; FitzPatrick et al., 2010).

Native cellulose structure is disrupted with pretreatment. Crystallinity index and degree of polymerization of the cellulose is decreased. It brigns the cellulose structure to a more amorph state. Increased surface area improves the enzyme attack, hence increasing in the efficiency of the enzymatic hydrolysis. Also, as lignin and the hemicelluloses are removed from the plant cell wall's complex matrix, accessible surface area of the cellulose molecules is increased.

Pretreatment processes are not only performed for destruction of the lignocellulosic structure and ehnancing the hydrolysis, but also fractionation of the cell wall components. Fractionation of the biomass into the three main components is an important point in developing an efficient biorefinery technology (FitzPatrick et al., 2010). An efficient fractionation process could make it possible to utilize the cellulose, hemicellulose and lignin components all together efficiently.
On the other hand, along with the cellulosic ethanol, a value added lignin or lignin – derived product can make the pretreatment step more feasable and economical (Doherty et al., 2011).

To sum up, as being more amorph and disruption of the crystal structure, and removal of the hemicellulose and lignins, accessible area of cellulose increases, which results in more cellulose becoming prone to the enzyme attack during hydrolysis, and as a result more cellulose is digested. This shows the physical importance of the pretreatment process from the point of view of enzymatic hyrolysis efficiency.

An effective and economical pretreatment method should be covering following considerations:

- An effective fractionation should be performed. Lignin removal should be in sufficient amount.
- During the pretreatment, existing cellulosic structure should not be harmed, modified or degraded.
- Pretreatment process should be cost effective and economically feasable.
- If it is a chemical process, chemicals used during the pretreatment should be enviromentally friendly. Organic solvents, corrosive chemicals should be avoided.
- There should not be much chemical or physical residue to be handled carefully.
- Pretreatment process should not employ harsh environments like very high temperature or high pressure. This will also increse the equipment and energy costs.
- Possible inhibitors like HMF should not be released during the pretreatment methods for the following fermentation steps.

As there is no one single pretreatment method satisfying all requirements above, different methods are used for different purposes. Mainly, pretreatment methods can be classified as physical, chemical, physicochemical and biological pretreatment methods.In the following parts, several pretreatment methods are discussed.

1.3.1 PHYSICAL PRETREATMENT METHODS

Physical pretreatment methods aim the increase the accessible surface area and size of pores, and decrease the cristallinity and degrees of polymerization of cellulose. Physical pretreatment methods are mostly employed before other chemical or physicochemical pretreatments (Taherzadeh et al.,2008).

1.3.1.1 MILLING

Milling of the lignocellulosic biomass is one of the mostly used pretreatment methods and it aims the size reduction of the lignocellulosic biomass. It also changes the degree of crystallinity of the cellulosic structure (Taherzadeh et al., 2008). Milling is either performed before directly enzymatic hydrolysis or before other chemical of physicochemical pretreatments.

However, with milling, only physical size reduction and increase in the surface area of the biomass is obtained. Lignin or hemicelluloses are not removed, which again restricts the enzymatic interaction.

1.3.1.2 IRRADIATION AND ULTRASOUND

Irradiation and ultrasound processes can be performed by gamma rays, electron beam, microwaves and ultrasound (Taherzadeh et al., 2008). Cellulose components in the ligninocellulosic materials can be degraded to fibers and low molecular weight oligosaccharides (Taherzadeh et al., 2008). Again with these methods, lignin and hemicellulose removal cannot be achieved, and irradiation methods are expensive and it is difficult to apply in industrial processes.

1.3.2 BIOLOGICAL PRETREATMENT METHODS

Biological pretreatment methods involve the use of microorganisms which degrade the lignin and hemicellulose in the ligninocellulosic materials. Several fungi, e.g. brown-, white- and soft-rot fungi, have been used for this purpose (Taherzadeh et al., 2008). Biological pretreatment methods require less energy than any other pretreatment methods and require mild conditions. No corrosive and toxic chemicals are used. However, the main drawback of the process is that, it is very slow (Taherzadeh et al., 2008).

1.3.3 PHYSICOCHEMICAL PRETREATMENT METHODS

Physicochemical pretreatment methods involve the methods that have both physical and chemical effects on pretreatment manners.

1.3.3.1 STEAM EXPLOSION

In steam explosion method, formerly physically chipped or ground biomass is exposed to high temperature (160 – 260 ^{o}C) and high pressure steam. Hemicellulose removal is provided with high temperature and pressure effect, afterwards presssure is released suddenly. With pressure release, the cellulose containing solid fraction experiences an explosive decomposition, which is believed to increase the digestibility of cellulose by making it more accessible to enzymes (Taherzadeh et al., 2008; Agbor et al., 2011).

Steam explosion method has advantages with no chemicals usage, hence no recycling or neutralization processes are required. However, there is a risk of condensation of soluble lignin on cellulosic parts, which makes the biomass less accessible to enzymes, and possiblility of formation inhibitor byproducts with degredation of hemicelluloses (Agbor et al., 2011).

Steam explosion is in near commercialization step, with pilot scale plants in Golden, Sweden, SEKAB plant; Iogen, Ottowa, Canada and The National Renewable Research Laboratory (NREL) in the USA (Agbor et al., 2011).

1.3.3.2 AMMONIA FIBER EXPLOSION (AFEX)

AFEX is one of the physicochemical pretreatment methods. In this method, highly concentrated ammonia is combined with biomass material and heated to moderate temperatures and high pressures. (70-100 ^{o}C) (Kim et al., 2008) Afterwards, pressure is released suddenly.

Under the treatment conditions, ammonia causes depolymerization of cellulose and partial dissolution and hydrolyzation of hemicellulose. Also, lignin is removed to the surface of the biomass (Kim et al., 2008).
One advantage of AFEX is that ammonia can be recycyled and there is no formation of some types of inhibitory materials that are produced in other types of (e.g. acid added steam explosion) pretreatments (Kim et al., 2008; Taherzadeh et al., 2008). However, one disadvantage is that, not all amount of lignin is removed and some phenolic fragments of lignin still remains in on the cellulosic surfaces, which requires extra washing with water, which also brings extra waste water and cost.

1.3.4 CHEMICAL PRETREATMENT METHODS

In chemical pretreatment methods, chemicals are used in order to disrupt the lignocellulosic biomass, either removing lignin and hemicellulose, or dissolving and regeneration of the biomass in solvents.

1.3.4.1 ALKALI PRETREATMENT

Alkali pretreatment is one of the pretreatment methods, in which alkali solutionssuch as NaOH, $Ca(OH)_2$ or ammonia are used in lower temperatures and pressures compared to other methods (Mosier et al., 2005; Taherzadeh et al., 2008). Actually, alkali pretreatment method is similarly used in paper industry as Kraft pulping process. Main action of the alkali pretreatment is lignin removal and also removal of the acetyl and uronic acid substitutions on hemicellulose, and breaking the ester bonds between lignin and the other components (Mosier et al., 2005; Taherzadeh et al., 2008).Main drawback of alkali pretreament method is the cost and difficulty in recovering and recycling of the chemicals (Wyman et al., 2005). Again, corrosion – resistant materials for reaction media is another consideration, which increases the equipment cost.

1.3.4.2 ACID PRETREATMENT

Sulfuric acid pretreatment is a leading method in chemical pretreament methods, which was under commercial development as of 2010 (Li et al., 2010). Acid pretreatment methods employ either high acid concentrations and low temperature or dilute acid concentrations with high temperature (Taherzadeh et al., 2008). Other than sulfuric acid, hydrochloric acid, nitric acid, phosphoric acid have been studied in biomass pretreatment (Agbor et al., 2011).

In sulfuric acid pretreatment, hemiellulose is solubilized and the hydrogen bonds in the lignocellulosic structure is disrupted. However, by products, like HMF can be produced, which have inhibitory effects on the microorganisms that are used in fermentation steps. Moreover, lignin can be condensed on the cellulosic parts, which may block the enzymes in hydrolysis (Li et al., 2010).

Another disadvantage of acid pretreatment is that, corrosion – resistant materials should be used as pretreatment medium materials, which may increase the equipment cost. Also, acid recovery and neutralization are the other main concerns (Mosier et al., 2005; Taherzadeh et al., 2008).

1.3.4.3 IONIC LIQUID PRETREATMENT

Ionic liquids are organic salts with melting point below 100 ^{o}C (Feng et al., 2008; FitzPatrick et al., 2010; Li et al., 2010). They have low vapor pressure, they are thermally and chemically stable, non - flammable and they can be tuned in order to have desired physicochemical properties with different combinations of anions and cations (FitzPatrick et al., 2010; Li et al., 2010).Due to the low vaporpressure and non – flammablility, they are considered to be good candidates as green solvents asreplacement to the conventional organic solvents, which, on the contary, are environmentally harmful and relatively volatile (Vancov et al., 2012). Ionic liquids are also used in catalysis, electrochemistry, separation and materials studies besides the lignocellulosic biomass pretreatment (Liu et al., 2012).

Ionic liquids have organic cations (e.g., pyridinium, imidazolium, isoquinolium) and either organic or inorganic anions (e.g. Cl^-, NO_3^-, acetate, phosphate) (Liu et al., 2012). Below, structures of some common imidazolium and pyridinium ionic liquids are shown.

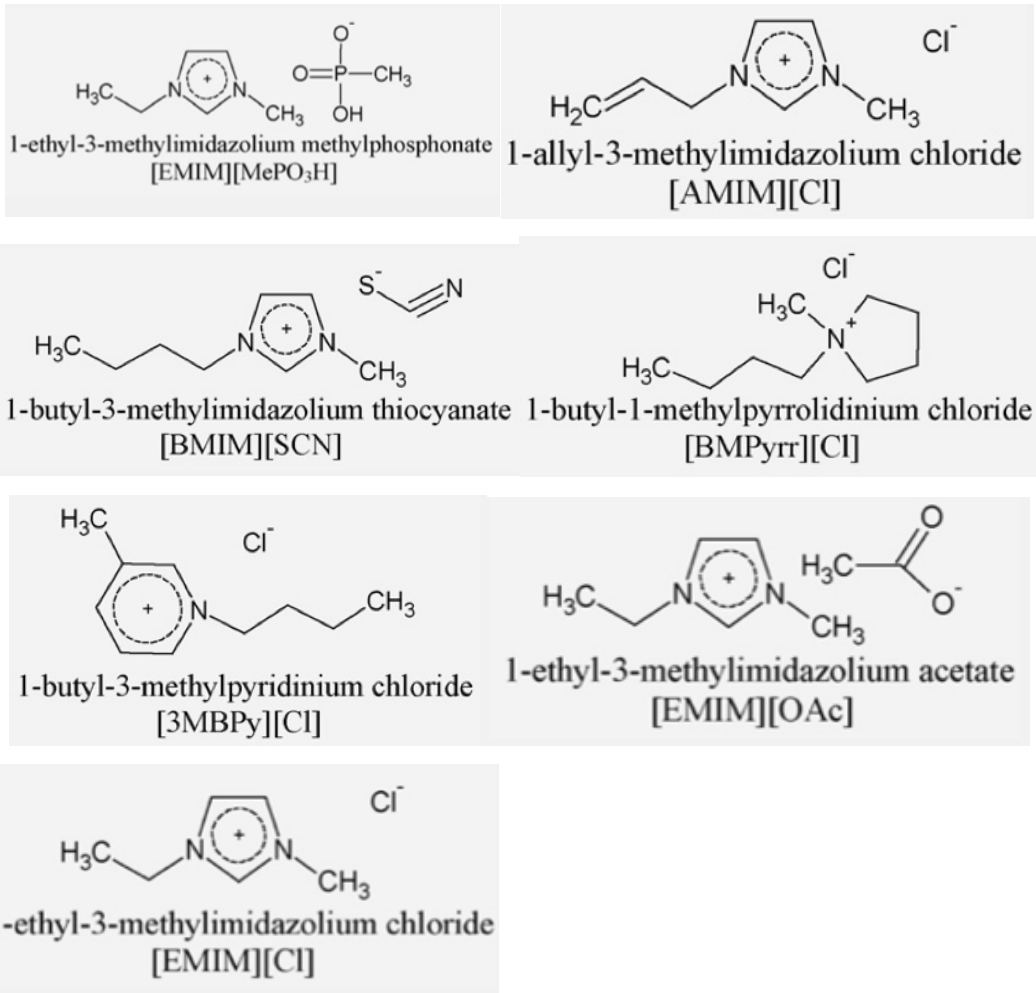


Figure 1.10 Molecular structures of some common ionic liquids.

Ionic liquds are shown to be capable of dissolving cellulose. Moreover, dissolved cellulose is precipitated with an anti-solvent addition such as water, methanol, ethanol, acetone (Swatloski et al., 2002; Kilpelainen et al., 2007; Maki-Arvela et al., 2010). Furthermore, lignocellulosic biomass is also dissolved in ionic liquids (Kilpelainen et al., 2007; Wang et al., 2011).1-butyl-3-methylimidazolium chloride and 1-allyl-3-methylimidazolium chloride have shown good solvation capability of wood of Norway spruce (Kilpelainen et al., 2007). Also, lignin can be dissolved in ionic liquids, as below in Table 1.5, solubilities of lignin in different ionic liquids is given (Lee et al., 2008). Given solubility values are obtined by incubation of Indulin AT, a Kraft lignin, at 90 ^{o}C for 24 hours.

Table 1.5 Solubilities of lignin in different ionic liquid types.

Ionic Liquid Type	**Lignin Solubility (g/kg)**
[MMIM][$MeSO_4$]	> 500
[EMIM][Ac]	> 300
[AMIM][Cl]	> 300
[BMIM][Cl]	> 100
[BMIM][BF_4]	> 500

Ionic liquid anions and cations interact with the intermolecular and intramolecular hydrogen bonds in the cellulose structure thus solubility of the cellulose is provided. Feng et al. and Zhang et al. proposed cellulose dissolution mechanisms in ionic liquids.Figure 1.12 shows the proposed a mechanism for lignin extraction with [EMIM][Ac] by Kim et al.

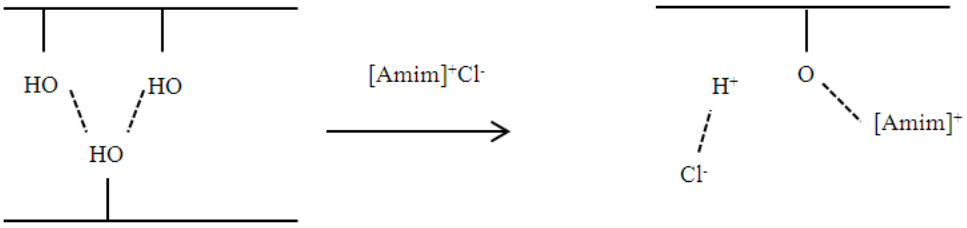


Figure 1.11 Cellulose dissolution mechanism proposal by Zhang et al.

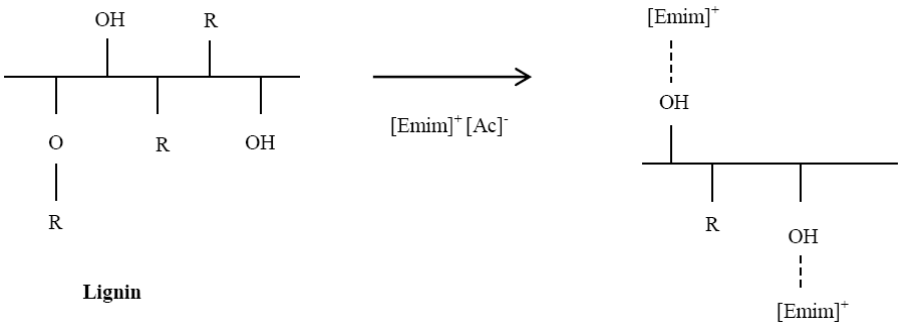


Figure 1.12 Lignin extraction mechanism with [EMIM][Ac] (Kim et al., 2012).

When an anti – solvent, e.g. water, is added into a ionic liquid – biomass solution, the water molecules surround the ionic liquid's ions and the ionic liquid molecules are extracted into aqueous phase. Due to the breaking of the interaction between ionic liquid ions and cellulose, precipitation of the cellulose occurs (Maki-Arvela et al., 2010).

In Table 1.6, dissolution of lignocellulosic biomass, wood, is shown with ionic liquid types, wood types and dissolution conditions are given.

Table 1.6 Dissolution of wood in ionic liquids with different conditions (Maki – Arvela et al., 2010).

Ionic liquid	**Wood**	**Dissolution**	**Conditions**
[MMIM][$MeSO_4$]	Maple wood flour	Very low	80 ^oC, 24 h
[MMIM][$(MeO)_2PO_2$]	Spruce chips	5 wt% partial	90 ^oC
[EMIM][Cl]	Spruce chips	5 wt% partial	90 ^oC
[EMIM][Ac]	Spruce chips	5 wt% complete	90 ^oC
	Southern yellow pine chips, 0.25 – 0.5 mm	92.6%	110 ^oC, 16h
	Poplar chips	92% conversion to glucan after enz. Hydrolysis	120 ^oC, 24 h
[AMIM][Cl]	Maple wood flour	>30 g/kg	90 ^oC, 24 h
	Norway spruce saw dust	8 wt%	80 ^oC, 8 h
	Norway spruce TMP	7 wt%	130 ^oC, 8 h

Table 1.6 (Continued)

[BMIM][Cl]	Pine	67 wt%	100 ^{o}C, 24 h, cosolvent DMSO-d_6
	Poplar	68 wt%	100 ^{o}C, 24 h, cosolvent DMSO-d_6
	Oak	56 wt%	100 ^{o}C, 24 h, cosolvent DMSO-d_6
	Eucalyptus	64 wt%	100 ^{o}C, 24 h, cosolvent DMSO-d_6
[BMIM][Cl]	Wood chips	Partially soluble	130 ^{o}C, 15 h
	Norway spruce TMP	7 wt%	130 ^{o}C, 8 h
	Spruce chips	5 wt% partial	90 ^{o}C
	Southern yellow pine, chips 0.35 – 0.5 mm	26%	110 ^{o}C, 16 h
	Maple wood flour	<0.1g/kg	90 ^{o}C, 24 h
[BMIM][BF_4]	Maple wood flour	<0.1g/kg	90 ^{o}C, 24 h
[BMIM][PF_6]	Maple wood flour	>30g/kg	90 ^{o}C, 24 h
[BMIM][OTf]	Maple wood flour	>10g/kg	90 ^{o}C, 24 h
[BnMIM][Cl]	Southern pine TMP	2 wt%	130 ^{o}C, 8 h
	Southern pine TMP	5 wt%	130 ^{o}C, 8 h
[BnMIM][DCA]	Aspen wood chips or powder	Dissolved	150 ^{o}C, 24 h
[3MeOBnMIM][Cl]	Aspen wood chips or powder	Almost completely dissolved	100 ^{o}C, 24 h
[HDBU][Cl]	Aspen wood chips or powder	Partly dissolved	100 ^{o}C, 24 h
[HDBU][HCOO]	Aspen wood chips or powder	Partly dissolved	100 ^{o}C, 48 h
[HDBU][Ac]	Aspen wood chips or powder	Partly dissolved	100 ^{o}C, 48 h
[BDBU][Cl]	Aspen wood chips or powder	Partly dissolved	100 ^{o}C, 48 h
[C_8DBU][Cl]	Aspen wood chips or powder	Partly dissolved	100 ^{o}C, 48 h

Ionic liquid pretreatment also has effect on the biomass composition. In the study of Lee et al., 2008; effect of ionic liquid pretreatment on the composition of the wood flour has been given, along with the effect of temperature. The ionic liquid pretreatment is conducted with [EMIM][Ac] at different temperatures for 90 minutes at 5% (w/w) biomass loading.

Table 1.7 Effect of ionic liquid pretreatment on the composition of the wood flour.

Pretreatment			**Composition of Pretreated Biomass**			**Enzymatic Hydrolysis of Pretreated Biomass**
Temperature	Extracted Lignin	Recovered Biomass	Cellulose	Xylan	Lignin	Digestibility
Untreated	0%	100%	52%	28%	17%	46%
50 ^{o}C	19%	84%	52%	31%	17%	50%
70 ^{o}C	21%	85%	53%	30%	16%	58%
90 ^{o}C	28%	83%	55%	30%	15%	80%
110 ^{o}C	44%	80%	55%	30%	12%	90%
130 ^{o}C	63%	73%	60%	29%	8.8%	95%

[EMIM][Ac] pretreatment decreased the lignin amount in the biomass, with increasing pretreatment temperature. As the pretreatment temperature increase, recovered biomass also decreased and digestibility of the pretreated biomass increased. Altough ionic liquids are expensive materials today, they can be recycled by evaporating the anti – solvents and used again for 4 – 5 times for pretreatment purposes (Maki-Arvela et al., 2010; Vancov et al., 2012). Moreover, aqueous ionic liquids are studied for pretreatment of biomass (Fu and Mazza, 2011).Thus, reducing the cost for ionic liquid pretreatment can be achieved with these methods. Ionic liquid pretreatment of lignocellulosic biomass is a relatively new research area, which has gained attention after Swatloski reported that ionic liquids dissolved cellulose (Swatloski et al., 2002). In this study, pulp cellulose is dissolved in various ionic liquids and solubilities are reported. It is also reported that the water presence in ionic liquids decreased the cellulose solubility, which is probably related to the competetive hydrogen bonding of water to cellulose microfibrils, instead of ionic liquid. Solubilities of pulp cellulose in various ionic liquids are given by Swatloski is given in Table 1.8 (Swatloski et al., 2002).

Table 1.8 Solubility of pulp cellulose in various ionic liquid types.

Ionic Liquid Type	**Dissolving Method**	**Solubility (wt %)**
$[C_4MIM][Cl]$	Heat (100 ^oC)	10%
$[C_4MIM][Cl]$	Heat (70 ^oC)	3%
$[C_4MIM][Cl]$	Heat (80 ^oC) + Sonication	5%
$[C_4MIM][Cl]$	Microwave heating	25%
$[C_4MIM][Br]$	Microwave	5 – 7%
$[C_4MIM][SCN]$	Microwave	5 – 7%
$[C_4MIM][BF_4]$	Microwave	Insoluble
$[C_4MIM][PF_6]$	Microwave	Insoluble
$[C_6MIM][Cl]$	Heat (100 ^oC)	5%
$[C_8MIM][Cl]$	Heat (100 ^oC)	Slightly soluble

In 2007, Kilpelainen obtained transparent solutions of wood, Norway spruce sawdust and Southern pine pulp. The transparent solutions were prepared by dissolving the wood biomass in 1-benzyl-3-methylimidazolium chloride. Furthermore, Li, Asikkala and Fliponen studied the factors affecting wood dissolution and regeneration of ionic liquids. In this study, wood is regenerated from [AMIM][Cl]. Increase in the digestibility of the recovered residue is observed. Moreover, recycling of the ionic liquids is realized. However, recycyled ionic liquids showed decreased efficiencies with resuing.Lateef and coworkers (2009) dissolved cellulose and lignin from paper – based waste, in cyanoMIMBr, propylIMIMbr and butylIMIMCl. Cellulose recovery yield was 98 – 99%. This study showed that ionic liquids can solubilize cellulose and lignin, and these components can be recovered from mixed systems.

Zhao et al. (2009) showed that crystallinity of regenerated cellulose is 58 – 75% lower than the original native cellulose in ionic liquid pretreatments. Avicel, filter paper and cotton were the cellulose sources and these materials were hydrolyzed 2 – 10 times faster than untreated counterparts.

Lee and Doherty (2009) also studied the crystallinity of cellulose which is subjected to ionic liquid pretreatment. They reported that cellulose in the pretreated wood flour became far less crystalline without undergoing solubilization. When the lignin in the wood flour is removed by 40%, more than 90% of the cellulose in the maple wood flour is hydrolyzed.

Tan et al. (2009) studied lignin extraction using alkylbenzenesulfonate ionic liquids. Lignin extraction was achieved by ionic liquid mixture of [EMIM] cation and mixtures of alkylbenzenesulfonates with xylenesulfonate. An extraction yield of 93% is achieved.
Zhao and Baker (2010) studied pretreatment of switchgrass. Enzymatic saccharification of cellulose up to 96% in 24 hours is obtained. Moreover, decrease in crystallinity is also observed. Fu and Mazza (2010) also studied lignin extraction from straw by ionic liquid. Triticale straw is pretreated with [EMIM][Ac] for 90 min. at 150 ^oC. Regenerated residue is subjected to enzymatic hydrolysis and more than 95% cellulose digestibility is achieved. This study made a parameter narrowing at temperature scale for the current thesis study.

1.4 CELLULASES and ENZYMATIC HYDROLYSIS of CELLULOSE

Cellulose can be degraded to its monomers by chemical methods, as well as it can be hydrolyzed by enzymatic reactions. Enzymatic hydrolysis refers to the breakdown of the cellulose polymer to its sugar units via cellulolytic enzymes (Taherzadeh et al., 2007).

Due to the complex and rigid structure of the plant cell wall, without pretreatment, enzymatic conversion of the cellulose to glucose monomer is very low. Hence, a pretreatment process is required before the hydrolysis step.

Enzymatic hydrolysis has advantages over chemical hydrolysis methods such as acid hydrolysis. Relatively mild conditions are employed. pH 4.5 – 5.0 and 40 – 50 ^{o}C is the conditions. No corrosive or toxic chemicals such as HCl, H_2SO_4, NaOH, acetone are used, hence no any neutralization or post – treatment process is required. Also, there is no risk of formation of HMF or any other fermentation inhibitors like in acid hydrolysis. However, there are some disadvantages as well. Firstly, enzymes are costly. Moreover, during the hydrolysis, glucose release has an inhibitory role on cellulases. Another drawback is that enzymatic hydrolysis is a slower process relative to the rapid acid hydrolysis (Taherzadeh et al., 2007).

Enzymatic hydrolysis is performed with the help of cellulase enzymes. Cellulases are defined as a class of enzymes, glycosyl hydrolases, which catalyze the hydrolysis of 1,4-β-D-glycosidic bonds. Also, enzymes that hydrolyze hemicellulose are referred as hemicellulases and they are generally clasified under cellulases (Hall et al., 2011; Taherzadeh et al., 2007).Cellulases are categorized into three classes: Endo – glucanases, exo – glucanases and β- glucosidases. The endo - glucanases act mainly on the low – crystalline parts of the cellulose, attacking the internal bonds; where the exo – gluconases attack on the ends of the cellulose chains and produce cellobioses. β- glucosidases further convert the produced cellobioses into glucose units (Taherzadeh et al., 2007; Yeh et al., 2010).

Hemicellulases are also a class of enzymes that hydrolyze hemicelluloses. However, since hemicelluloses are made up of different sugar units and have different structure than cellulose, there are more complex and more enzyme classes than cellulases in hemicellulases. endo-1,4-β-D-xylanases, exo-1,4-β-D-xylosidases, endo-1,4-β-D-mannases, β-D-mannosidases, acetyl xylan esterases, α-galactosidases are the main groups of hemicellulases (Taherzadeh et al., 2007).

Many microorganisms produce cellulase enzymes. Many species of *Clostridium, Thermomonospora, Bacillus, Acetovibrio, Tricoderma, Fusarium* are able to produce cellulase and hemicellulase enzymes. *Trichoderma reesei* cellulases are the most studied and known among the cellulases produced by different microorganisms. Most commercial cellulase enzymes are obtained from *T. reesei* and *Aspergillus niger*(Taherzadeh et al., 2007).

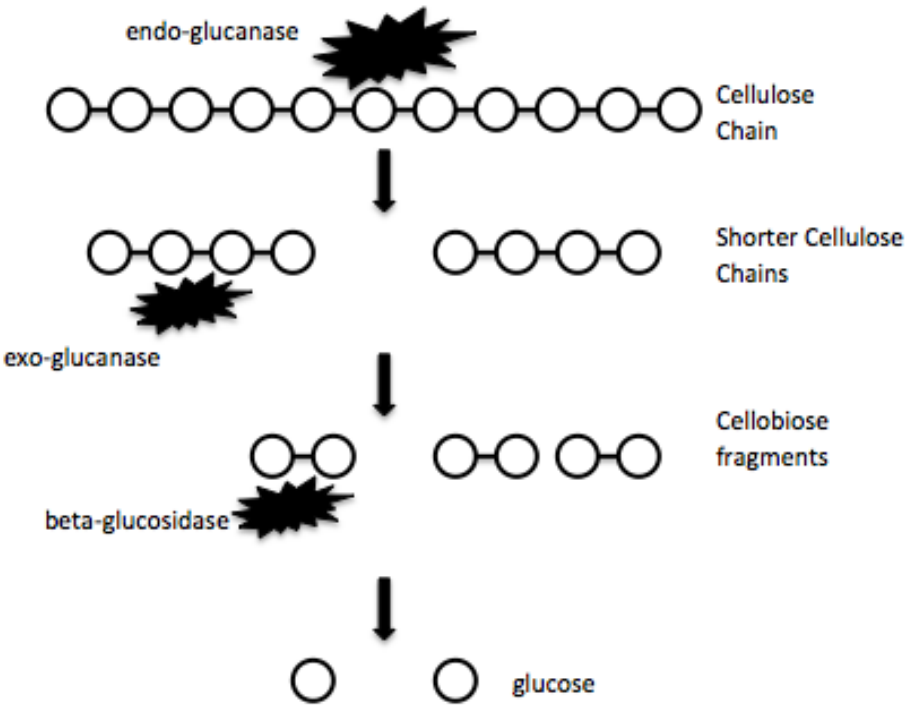


Figure 1.13 Schematic representation of cellulase activity (Wyman, 1994).

Hydrolysis of cellulose is studied mostly with the crude cellulases from *Trichoderma reesei*, which constitutes the most of the kinetic study knowledge. There are multiple cellulase enzymes, hence adsorption of enzymes and structural features of the enzymes differ. There are many mathematical models in order to predict the enzymatic hydrolysis reactions' rates. Much of these models are empirical (Ghose, 1969; Van Dyke, 1972, Lee et. al, 1980).Some of these models include structural features (Van Dyke, 1972; Brandt et al, 1973) (Walker and Wilson, 1991).

Rate of extent of the cellulose hydrolysis depend on the amount of enzyme adsorbed on the cellulose. Hence kinetics of adsorbtion also plays role on extent of hydrolysis. Enzyme adsorption is a function of enzyme concentration, surface area of the cellulosic particles, physical properties of the cellulases and hydrolysis medium, which is pH, salt concentration and temperature. (Walker and Wilson, 1991) Langmuir adsorption kinetics was applied to adsorption of cellulase on cellulose by Peitersen, Lee et al., Ooshima et al. and Beldman et al. Langmuir adsorption model relates the rate of adsorption proportionally to the number of free sites on cellulose, i.e, available surface area and the enzymequantity in the supernatant.

1.5 CONCRETE ADMIXTURES

Chemical admixtures are several chemicals which are used in concrete in order to give properties to it. Chemical admixtures can be simply classified as air – entraining admixtures, water – reducing admixtures and plasticizers, accelerating admixtures, retarding admixtures, corrosion inhibitors, hydration – controlling admixtures, shrinkage reducers and coloring agents. Chemicals are used in concretes as admixtures in order to have benetifs such as cost reduction, establishing certain properties in concrete, making the concrete mixture more effective in different stages of mixing, transporting and curing. (Jolicoeur et al., 1998; Design and Control of Concrete Mixtures, EB001 notes, Chapter 6, www.ce.memphis.edu)

In Table 1.9, types of concrete admixtures, effects and materials are shown.

Table 1.9 Types of concrete admixtures and their effects (Design and Control of Concrete Mixtures, EB001 notes, Chapter 6, www.ce.memphis.edu).

Chemical Admixture Type	**Effetcs**	**Material**
Accelerators	Accelerating setting	Calcium chloride, sodium thiocyanate, calcium formate
Air detrainers	Decreasing the air content	Tributyl phosphate, octyl alcohol, silicones
Air entrainers	Improving the durability in freeze – thaw, deicer, sulfate and alkali – reactive environments	Salts of wood resins, salts of sulfonated lignin, alkylbenzene sulfonates
Antiwashout admixtures	Reducing alkali – aggregate reactivity expansion	Cellulose, acrylic polymer
Coloring admixtures	Coloring concrete	Modified carbon black, iron oxide, chromium oxide, titanium oxide
Corrsion inhibitors	Reducing steel corrosion activity	Calcium nitrite, sodium nitrite, sodium benzoate, ester amines
Foaming agents	Producing lightweight, foamed concrete with low density	Cationic and anionic surfactants

Table 1.9 (Continued)

Gas formers	Causing expansion before setting	Aluminum powder
Retarders	Retarding the setting time	Lignin, borax, sugars, tartaric acid
Superplasticizers, plasticizers, water reducers	Increasing the flowability of concrete, reducing water – cement ratio	Sulfonated melamine formaldehyde, carbohydrates, lignosulfonates, polycarboxylates

The effects of the chemical admixtures occur due to altering the cement hydration reactions, which are mostly described with interactions between admixture and cement particles. Surface adsorption occurs when the admixture is mixed with the cement solution, as the admixture particles show affinity to the cement particles. Electrostatic forces will be effective between charged admixture molecules with SO_3^- and COO^- groups and cement molecules. Moreover, polar groups of organic molecules have also affinity to the polar hydrated groups. In Figure 1.14, adsorption of the charged organic molecules on the cement particles are shown (Jolicoeur et al., 1998).

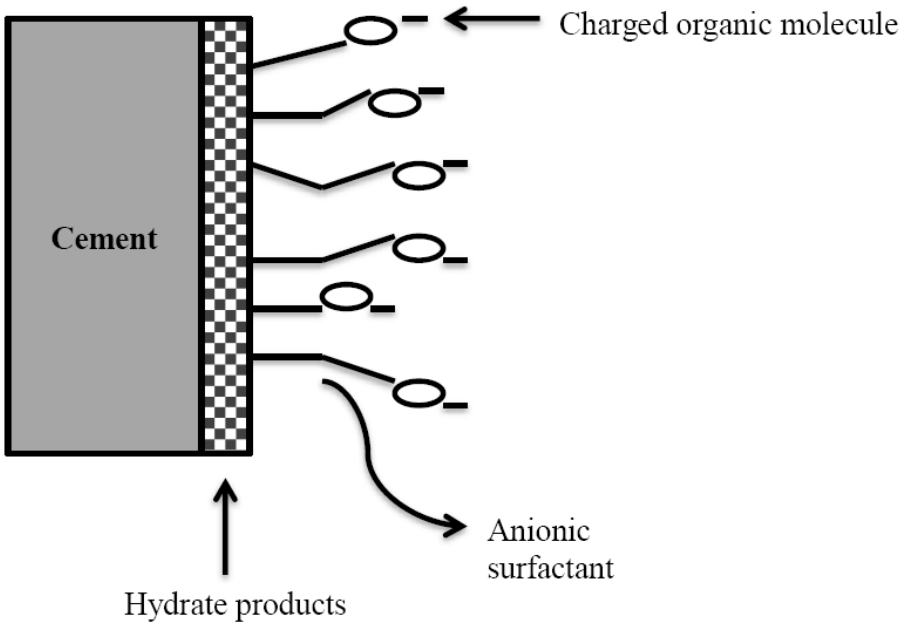


Figure 1.14 Adsorption of the charged organic molecules on the cement particles.

Sodium gluconate and lignosulfonates are examples for admixtures having charged ionic groups. Sugars have polar groups. Surfactants have hydrophobic groups either aliphatic or aromatic. Hydrophobic groups have interaction with the hydrating surfaces of the cement particles. .
The adsorbed molecules on the cement particles will have effect on the properties of cement mixture as the surface properties of the particles change. Generally, anionic surfactants and polymers will bring a negative charge on the particle surface, which will result in particle – particle repulsion due to the same negative charges on particles. This particle – particle repulsion will result in dispersion and fluidification effect (Jolicoeur et al., 1998). In Figure 1.15, repulsion of the molecules due to the electrostatic forces are shown (Jolicoeur et al., 1998).

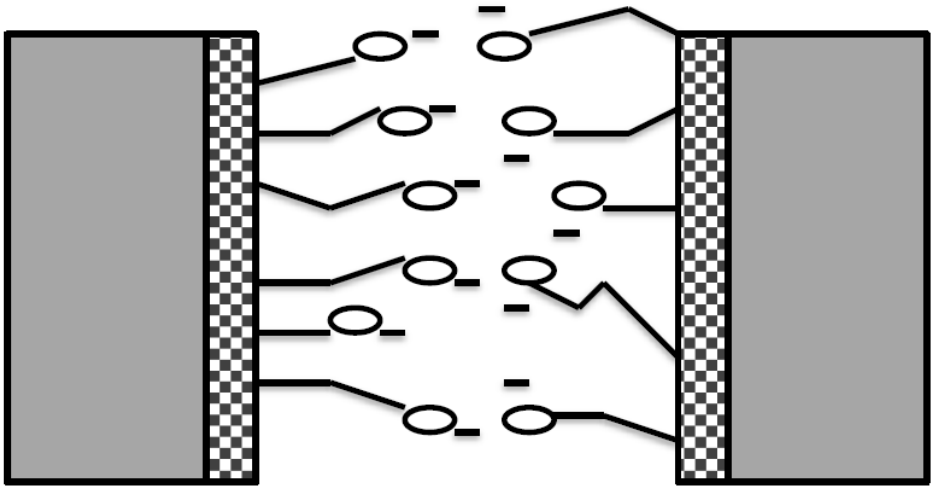


Figure 1.15 Steric and electrostatic particle – particle repulsion.

Air entratining admixtures adsorb on the cement particles and at the cement – solution interface and may make up film – like structures as found in lipid layers. In Figure 1.16, film formation on cement particles are shown (Jolicoeur et al., 1998).

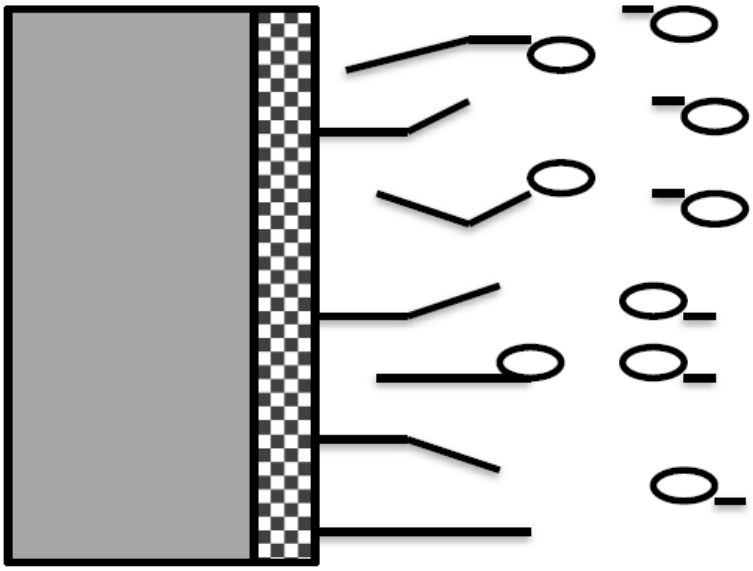


Figure 1.16 Film formation at cement particle – solution interface.

Moreover, hydroxy carboxylic acids and sugars can improve the solubilities of the ionic species such as $Ca2^{+}$, SiO_x^{n-}, $Al(OH)_4^{-1.31}$ via association and complexation. (Jolicoeur et al., 1998).

1.5.1 PLASTICIZERS/WATER REDUCERS

Lignosulfonates, melamine formaldehyde polymers and sulfonated alkali salts of naphthalene are examples for plasticizer admixtures (Kamoun et al., 2003). Plasticizers or water reducers, and superplasticizers or high range water reducers are used as admixtures in concrete in order to improve the workability and flow of the concrete. Water reducers effect and improve the workability and the final strength of the concrete. They are usually added to the concrete mixture with 1 – 2 % w/w. Water reducers effect the ionic interaction between cement particles, giving them negative charges and make them repulse each other, so that a dispersion occurs as described and depicted in previous part.

Lignosulfonates are sodium and calcium salts of lignosulfonic acids and by products of the sulfite pulping process. They are mostly used as concrete admixtures as dispersing agents. They are also used in drilling muds, organic dyes and emulsifiers (Kamoun et al, 2003).Sulfonated naphthalene and sulphonated melamine formaldehyde are other two examples for superplasticizers. Moreover in the study of Nadif et al., (2002), sulfur free lignins which are obtained from different sources have been tested as mortar additives.

The plasticizer effect takes place by modifying the inter particle forces, as the cement particles tend to agglomerate in the water as in the concrete mixture. There are basically four mechanisms in the dispersion effect of the plasticizers according to Lazinewska – Piekarczyk.

- Plasticizers create a grease layer on cement particles and fillers, which decreases the internal friction of the concrete suspension mixture. Sulfone melamine – formaldehydehygenic resins act in this way.

- Plasticizers surround the cement particles and give negative charge to them, which causes the repelsion of the particles from each other. In this case, the repelsion and dispersion is affected by the type of the plasticizer. Sulfonenaphthalene – formaldehygenic resins act in this way.

- Plasticizers can also decrease the water's surface tension. Modified lime or sodium lignosulfonates, copolymers of formic acid with naphthylic – sulfone acid, copolymers of methacrylate acid with sodium salts have this effect on concrete mixture.

- Plasticizers have polymeric effects and they create long chains of polymers, which makes the cement particles approach each other. Polycarboxylants, copolymers of acrylic acid with acrylate have this effect on concrete mixtures (Lazinewska – Piekarczyk, 2012).

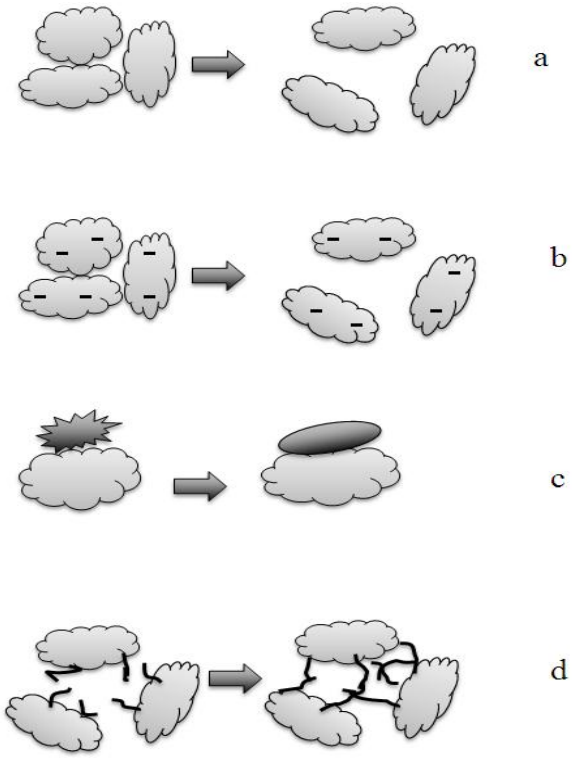


Figure 1.17 Effects of plasticizers on concrete and cement particles. a) creating a grease layer, b) surrounding the particles and giving negative charges, c) decreasing water's surface tension d) creating long polymer chains (Lazinewska – Piekarczyk, 2012).

1.6 AIM of THE STUDY

Ionic liquid pretreatment of lignocellulosic biomass has been one of the major research area for renewable and sustainable fuels, chemicals and materials. Having no vapor pressure, being non – toxic, non – flammable,thermally and chemically stable, and easily recycled, ionic liquids are considered as green solvents for replacement of the traditional toxic organic solvents, which is at the heart of the sustainable and renewable production concept.With ionic liquid pretreatment, effective fractionation of the biomass can bring a multi - product perspectiveand may add value to the lignocellulosic biomass processing and make the biorefineries in which lignocellulose is processed more viable (Doherty et al., 2011).However, ionic liquid pretreatment is not economically feasibletoday due to high costs of ionic liquids.Furthermore, utilization of different antisolvents which are required for selective precipitation of biomass components makes the process costly and sophisticated.

The framework of this study was based on ionic liquid pretreatment of peanut shells, a high cellulose and lignin containing agricultural by product as the lignocellulosic resource. The pretreated biomass was obtained upon single step precipitation via water, which was afterwards hydrolyzed enzymatically. During the enzymatic hydrolysis, cellulose content of the biomass was digested and fermentable sugars were obtained. The solid residue after enzymatic hydrolysis was the second product with high lignin content. Hence, two products were obtained in one process without the requirement of other antisolvents.

The aim of this study was to investigate the effect of ionic liquid type and pretreatment time period on reducing sugar and lignin yields.In the preliminary studies, [AMIM][Cl], [EMIM][Cl], [EMIM][Ac] were used in pretreatments and, [EMIM][Cl] and [EMIM][Ac] were found to be the most effective ones. A short time period of pretreatment at high temperature was aimed, i.e., shorter than 60 minutes. Time periods of 5, 15, 30 were conducted for [EMIM][Ac] pretreatments and time periods of 15, 30 and 60 minutes wereconducted for [EMIM][Cl]. Temperature was set to 150 ^{o}C, which was expected to be effective with short time periods. Instead of low temperature – long time pretreatments, experiments were set up on high temperature - short time concept, which would again conceptually considered as a way to a high throughput bioprocess and biorefinery facility. A short contact time may indicate more biomass can be pretreatedwithin a specific time period, which also means a more economical process design, as more output can be obtained in the same time base. Lignin obtained upon the optimum pretreatment conditions with highest yield was characterized by means of FTIR, XRD, SEM and TGA analyses, and tested as a concrete admixture for flow properties of the concrete.

CHAPTER 2

EXPERIMENTAL

2.1 MATERIALS

Peanut (*Arachis hypogaea*) shells obtained from Osmaniye, Turkey, harvest of 2010, were milled (Arthur Thomas Co., Philadelphia, USA) and screened to achieve a size of between 0.300 and 0.180 mm. Such a narrow particle size distribution was selected in order to minimize the particle size effects during experiments. The sample prepared was stored in a dessicator for use in all experiments.

Corncobs (Adıyaman, Turkey) were milled (Arthur Thomas Co., Philadelphia, USA) and screened to achieve a size of smaller than 0.100 mm. Such a small particle size distribution was chosen since this material was used in preliminary biomass dissolving experiments and using smaller particle size would be easier to observe the dissolution. The sample prepared was stored in a dessicator for use in all experiments. This sample was used in the preliminary [AMIM][Cl] and [EMIM][Cl] studies.

Corncobs (Adıyaman, Turkey) were milled (Arthur Thomas Co., Philadelphia, USA) and screened to achieve a size of between 0.300 and 0.180 mm and smaller than 0.150 mm. The samples prepared were stored in a dessicator for use in all experiments. This sample was used in the preliminary [EMIM][Ac] studies.

2.2 CHEMICALS

3,5-dinitro salicylic acid, potassium sodium tartarate, sodium sulfate, phenol and avicel were purchased from Sigma – Aldrich (St. Louis, Mo, USA). D-glucose, tri-sodium citrate dehydrate, sodium azide and citric acid monohydrate were purchased from Merck (Darmstadt, Germany). 1-ethyl-3-methylimidazolium chloride (BASF, $\geq$ 95% purity) and 1-ethyl-3-methylimidazolium acetate (BASF, $\geq$ 90% purity) were purchased from Sigma - Aldrich. 1-ethyl-3-methylimidazolium chloride and 1-allyl-3-methylimidazolium chloride were obtained from Solvionic (Toulouse, France). Cellulase preparation from *Trichoderma reesei*, (Celluclast 1.5L), and β-1,4-glucosidase preparation from *Aspergillus niger,* (Novozym 188)were obtained from Novozymes (Bagsvaerd, Denmark).

2.3 METHODS OF EXPERIMENTS

2.3.1 COMPOSITIONAL ANALYSIS OF BIOMASS

Compositional analysis of the raw peanut shells was conducted according to NREL procedures (Sluiter el al., 2008). The prepared material described in section 2.1 was first subjected to water and ethanol extraction with Soxhlet extractor. Peanut shells, 6 g, were extracted with firstly 120 g of deionized water. Water extraction was performed for 16 hours. The extract part was taken into a pre – dried flask and dried at 105 ^{o}C until constant weight was achieved. The weight increase of the pre – dried flask was counted as the water soluble parts of the biomass. Afterwards, the water – extracted peanut shells were subjected to ethanol extraction. Ethanol extraction was performed for 8 hours. The same weight calculation was done and counted as the ethanol soluble parts.

After the water and ethanol extraction, remaining solvent was evaporated at 60 ^{o}C for overnight and dried biomass was analyzed for compositional analysis. The extraction setup is shown in Figure 2.1.

Figure 2.1 The extraction setup.

For the compositional analysis, firstly, porcelain crucibles were placed in a furnace and heated to 105 ^{o}C. After at least for 4 hours, weight of the crucibles were determined. This procedure was repeated until a constant weight was achieved.

Then, 300 mg of biomass samples were taken into test tubes. Afterwards, 72% (wt/wt) H_2SO_4 solution was added into the test tubes. Tubes were placed in a 30 ^{o}C water bath and stirred with glass spoons every 5 – 10 minutes for 60 minutes. After 60 minutes, the samples in the test tubes were diluted to 4% H_2SO_4 by adding deionized water. Diluted samples were then placed into glass vials and heated to 121 ^{o}C for 60 minutes. After this procedure, samples in the glass vials were filtered with vacuum pump. Filtered samples and the crucible were placed in a 105 ^{o}C furnace. After for 4 hours, weight of the crucibles and the filtered samples were determined. This procedure was repeated until a constant weight was achieved. The dry acid – insoluble part on the crucibles are the acid insoluble lignin, according to NREL procedure, and the acid insoluble lignin quantity in the biomass was determined as follows:

Weight of the acid insoluble lignin: (Dry weight of the crucible and the filtered sample) – (Dry weight of the crucible)

Acid insoluble lignin percent (LP) (%):

$$\frac{\text{Weight of the acid insoluble lignin (mg)}}{\text{Weight of the original biomass sample (mg)}} \text{ x100} \qquad (2.1)$$

The filtrate part contained hydrolyzed sugars. After filtration, 5 – 10 mL of samples from the liquid part was taken and neutralized with $CaCO_3$ until pH reached 5 – 6. The $CaCO_3$ added liquid part was then filtered with filter paper and the filtratewas analyzed for glucose, xylose and arabinose contents in order to determine the sugar content of the biomass by HPLC. The HPLC analysis of the samples were performed with a Shimadzu LC-20A HPLC system (Kyoto, Japan, using a BIORAD Aminex HPX-87H column (Hercules, CA, USA) at 55°C with flow rate of 0.6 ml/min.of 5 mM H_2SO_4 as the mobile phase. The concentrations found by the HPLC were used to calculate the total sugars originated from cellulose, xylose and arabinose parts, thus the quantities of cellulose, xylose and arabinose in the 300 mg sample was calculated, along with the percent values.

The ash content of the biomass was determined as follows. Firstly, porcelain crucibles were dried to constant weight at 105 ^{o}C. Weight of the dried crucibles were determined. Afterwards, ground peanut shells were taken into crucibles and the crucibles were placed into a furnace. According to NREL Laboratory Analytical Procedure, a technical report of "Determination of Ash in Biomass" (Sluiter et al., 2008), crucibles were held at 105 ^{o}C for 12 minutes. Then temperature was ramped to 250 ^{o}C with 10 ^{o}C/min. Temperature was hold at 250 ^{o}C for 30 minutes. Afterwards, temperature was ramped to 575 ^{o}C with 20 ^{o}C/min. Temperature was hold at 575 ^{o}C for 180 minutes. Then, temperature was allowed to drop to 105 ^{o}C. Afterwards, weight of the crucibles and the ash was determined.

The ash quantity was determined as follows:

Weight of the ash: (Dry weight of the crucible and the ash (mg)) – (Dry weight of the crucible (mg))

Ash Quantitiy (%):

$$\frac{\text{Weight of the ash (mg)}}{\text{Weight of the original biomass sample (mg)}} \text{ x100} \qquad (2.2)$$

After the compositional analysis, the ionic liquid experiments resumed.

2.3.2 IONIC LIQUID PRETREATMENTS

In peanut shell pretreatment studies, 20 g of ionic liquid was taken into 50 mL flasks and heated in oil bath to 150 ^{o}C at open atmosphere. Afterwards, 1.0 g of peanut shell was incubated in ionic liquid at 150 ^{o}C, with magnetic stirring at 600 rpm, for a specific time period. This procedure was performed for 15, 30 and 60 minutes for [EMIM][Cl] and for 5, 15 and 30 minutes for [EMIM][Ac]. [EMIM][Cl] from BASF was used in the peanut shell pretreatments.

The pretreatment times differed for [EMIM][Cl] and [EMIM][Ac] pretreatments due to the different solubility effects of the ionic liquid types on the biomass which was found during preliminary studies. Since the [EMIM][Ac] had better solubility of the biomass, the biomass could dissolve in it much easier and the effect of time could be seen with even for 5 – 15 minutes.

However, unlike the [EMIM][Ac], the biomass did not dissolve in [EMIM][Cl] as easily as in [EMIM][Ac], longer pretreatment times were necessary in order to be able to see the effect of time.

Pretreatment was immediately terminated by adding 200 mL of deonized water as the anti solvent. Regenerated solid content was filtered on filter paper and washed three times with 100 mL deionized water. Recovered solid was dried overnight at 60 ^{o}C before exposing to enzymatic hydrolysis.

In the preliminary studies, [EMIM][Ac], [EMIM][Cl] and [AMIM][Cl] pretreatments were conducted using corncob. [EMIM][Cl] from Solvionic was used in the preliminary pretreatment studies.

In the preliminary [AMIM][Cl] pretreatments, 15 g of ionic liquid was taken into 50 mL flasks and heated in oil bath to 130 ^{o}C and 100 ^{o}C at open atmosphere. Afterwards, 0.15 g of ground corncob was combined with ionic liquid, with magnetic stirring with 800 rpm, for 90 minutes at 130 ^{o}C and for 4 hours at 100 ^{o}C. The pretreatment time period was this long since the experiment's aim was to observe the biomass dissolution in the ionic liquid. Pretreatment was immediately terminated by adding 100 mL deonized water as the anti solvent. Regenerated solid content was filtered on filter paper and washed three times with 100 mL deionized water. Recovered solid was dried overnight at 60 ^{o}C before exposing to enzymatic hydrolysis.

In the preliminary [EMIM][Cl] pretreatments, 15 g of ionic liquid was taken into 50 mL flasks and heated in oil bath to 130 ^{o}C at open atmosphere. Afterwards, 0.45 g of ground corncob was combined with ionic liquid, with magnetic stirring with 800 rpm, for 30 minutes at 130 ^{o}C. Pretreatment was immediately terminated by adding 100 mL deonized water as the anti solvent. Regenerated solid content was filtered on filter paper and washed three times with 100 mL deionized water. Recovered solid was dried overnight at 60 ^{o}C before exposing to enzymatic hydrolysis.

In the preliminary [EMIM][Ac] pretreatments, two types of corncob were used. One part was with particle size of between 0.300 mm and 0.180 mm, another part was with parcile size of smaller than 0.150 mm. 15 g ionic liquid was taken into 50 mL flasks and heated in oil bath to 130 ^{o}C at open atmosphere. Afterwards, 0.75 g of ground corncob was combined with ionic liquid, with magnetic stirring with 600 rpm, for 30 minutes at 130 ^{o}C. Pretreatment was immediately terminated by adding 100 mL deonized water as the anti solvent. Regenerated solid content was filtered on filter paper and washed three times with 100 mL deionized water. Recovered solid was dried overnight at 60 ^{o}C before exposing to enzymatic hydrolysis.

Pretreatment Solid Recovery (%): It is the percent of amount of solid material recovered after the pretreatment of peanut shells to the amount of untreated peanut shells which is subjected to pretreatment.

PSR (%)(w/w) :

$$\frac{\text{Dry weight of the solid materials recovered after ionic liquid pretreatment (mg)}}{\text{Dry weight of the untreated material subjected to ionic liquid pretreatment (mg)}} \text{ x100} \qquad (2.3)$$

Figure 2.2 Experimental setup for the ionic liquid pretreatments.

2.3.3 ENZYMATIC HYDROLYSIS

Recovered and dried pretreated samples were exposed to enzymatic hydrolysis in 0.05 M, pH 4.8 citrate buffer, at 50 ^{o}C, with 3% (w/v) biomass loading and 2% (v/v) (50 FPU/g biomass) Celluclast 1.5L, and 0.5% (v/v) (60 cellobiose units/g biomass) Novozyme 188. Samples were taken from hydrolysis batch at zeroth, first, third and twenty fourth hours. Taken samples were immersed immediately in boiling water for five minutes to stop enzymatic reaction and kept at 4 ^{o}C until analysis. After the hydrolysis, hydrolysis medium was also immersed in boiling water for five minutes to stop enzymatic reaction and the hydrolysis residue is filtered on filter paper. Recovered filtrate was then washed with deionized water and dried overnight at 60 ^{o}C. This residue was named as lignin – rich residue (LRR) herafter.

The lignin quantitiy in the LRR was determined with the same procedure discussed in part 2.3.1, as determined for the compositional analysis of the biomass.

Avicel and untreated peanut shells were exposed to enzymatic hydrolysis at the same operation conditions as control groups and observe the effect of ionic liquid pretreatment on enzymatic hydrolysis.

Hydrolysis Solid Recovery (%): It is the percent ratio of amount of solid material recovered after the enzymatic hydrolysis to the amount of pretreated peanut shell which is subjected to enzymatic hydrolysis.
HSR (%) :

$$\frac{\text{Dry weight of recovered solid materials after enzymatic hydrolysis}}{\text{Dry weight of pretreated material subjected to enzymatic hydrolysis}} \text{ x100} \qquad (2.4)$$

Lignin Purity (%): It is the percent ratio of amount of acid insoluble lignin in the recovered solid material after enzymatic hydrolysis, which is determined by NREL procedure (Sluiter et al., 2008).

$$\frac{\text{Weight of acid insoluble lignin in recovered material after hydrolysis}}{\text{Weight of recovered solid material after hydrolysis}} \text{ x100} \qquad (2.5)$$

Overall Lignin Yield (%): It is the percent of amount of acid insoluble lignin in the recovered solid material after enzymatic hydrolysis to the amount of total original untreated peanut shell.

$$\frac{\text{Weight of acid insoluble lignin in recovered material after hydrolysis}}{\text{Weight of untreated biomass subjected to ionic liquid pretreatment}} \text{ x100} \qquad (2.6)$$

2.3.4 FLOW TESTS

The LRR was used as a concrete admixture in flow tests, as a possible water reducer, along with cotton stalk lignin and alkali lignin.

After the enzymatic hydrolysis process, 1.16 g of lignin rich residue was mixed with 11.6 g of deionized water. Afterwards, 5 mL of 4 M NaOH solution was added to the lignin – water mixture. Finally, 27 mL of deionized water was added to the solution. pH of the solution was determined as 13.04. Lignin – water mixture was left for stirring for 13 hours. pH of the solution was adjusted to 10 by adding 2.5% (w/w) HCl. At the end, a mixture of 116 grams of 0.01 % w/w lignin – water was obtained. This mixture was afterwards used making a concrete mixture. The mixture was then diluted to 225 g and used in the concrete mixture. The lignin – water mixtures were prepared with the same proportion for cotton stalk lignin and alkali lignin.

Concrete mixtures were prepared. A control mixture without an admixture was prepared. In addition, a concrete mixture prepared with LRR, a mixture prepared with lignin obtained from cotton stalk and another concrete mixture prepared with alkali lignin (Sigma – Aldrich) were prepared according to the proportions which are based on TS EN – 196 standard given in Table 2.1. The proportions are given in grams.

Table 2.1 Mix proportions of the concrete mixtures.

Mix ID	Cement	Water	Lignin Admixture	Fine Aggregate
Control	450	225.0	0.0	1350
Alkali Lignin	450	221.5	4.5	1350
Cotton Stalk Lignin	450	221.5	4.5	1350
LRR	450	221.5	4.5	1350

The flow tests were performed according to ASTM C 1437. The concrete was placed on the horizontally moving table in a mold. Afterwards, mold was removed and initial diameter of the concrete block was recorded. Then, the horizontally moving table was moved horizontally and, then the table falls hardly back. This procedure was repeated several times. During the hard fell, the concrete block flows. The final diameter of the concrete block was recorded. The flow of the concrete was calculated by the following equation:

Flow (%) :

$$\frac{\text{Final diameter of concrete} - \text{Initial diameter of concrete}}{\text{Initial diameter of concrete}} \text{ x100} \qquad (2.7)$$

Figure 2.3 Flow table with the concrete mold.

Figure 2.4 Flow table with the concrete mold removed.

Figure 2.5 Control mix after flow.

Figure 2.6 Concrete mixture with alkali lignin after flow, diameter measured.

Figure 2.7 Concrete mixture with cotton stalk lignin after flow.

2.4 METHODS OF ANALYSES

2.4.1 REDUCING SUGAR ANALYSIS

Reducing sugar analysis was performed according to DNS method (Miller, 1959). 10 µL of sample from enzymatic hydrolysis medium was taken into a test tube, and was diluted to 1000 µL by adding 990 µL of deionized water. Afterwards, 1.5 mL of DNS reagent was added, vortexed and immersed in boiling water for 5 – 10 minutes. During the hot water immersion, colour change can be observed in the test tubes. After immersion, test tubes were taken out of the boiling water and cooled to room temperature. Tubes were vortexed again and read against a blank sample with UV – Visible spectrophotometer (Nicolet Evolution 100, Thermo Fisher Scientific Inc., USA)at 540 nm.

In each sample preparation for the analysis, two blank tubes and standard samples were prepared. Blank tubes were prepared via mixing only deionized water and DNS reagent. Standard D-Glucose solutions were prepared with concentrations in the range of 0.03 mg/mL to 0.15 mg/mL. These solutions were then used to prepare the standard calibration curve.

Enzymatic Hydrolysis Percentage of Pretreated Solid (%): It is the percent ratio of obtained quantitiy of reducing sugar at the 24th hour to the quantitiy of pretreated peanut shells subjected to enzymatic hydrolysis.

EH (w/w) (%) :

$$\frac{\text{Reducing sugar concentration obtained upon enzymatic hydrolysis at the 24th hour } (g/L)}{\text{Pretreated peanut shell concentration subjected to ezymatic hydrolysis} (g/L)} \text{ x100} \qquad (2.8)$$

Overall Reducing Sugar Yield (%): It is the percent of obtained quantitiy of reducing sugar to the quantity of the original untreated peanut shell.

2.4.2 FTIR ANALYSIS

FTIR analysis of the untreated, pretreated samples and the lignin – rich residue were conducted at Metu Central Laboratory. The analysis was performed within the bands of 400 – 4000 cm^{-1} on the powdered samples by pelleting with KBr.

2.4.3 XRD ANALYSIS

X – ray diffraction analysis of the untreated, pretreated samples and the lignin – rich residue were conducted at Metu Central Laboratory. Cu / 40 kV / 30 mA X – ray source was utilized. Goniometer was Ultima IV In – plane. No filter was used. Scan mode was continuous, sampling width was 0.0200 degrees. Scan range was 5.0000 to 40.0000 degrees. Scan speed was 0.500 deg./min., scan axis was 2θ/θ.

X – Ray : Cu / 40 kV / 30 mA
Goniometer : Ultima IV in-plane
Attachment : Sample rotation (with Z) Att.

Scan mode : Continuous
Scan speed : 0.500 deg./min.
Sampling width : 0.0200 deg.
Scan range : 5.000 → 40.000 deg.

2.4.4 SEM ANALYSIS

Scanning electron microscopy analysis of the untreated, pretreated samples and the lignin – rich residue were conducted at Metu Central Laboratory. The samples were coated with Au/Pd before the analysis. Magnifications of 800, 1600, 3000 and 4000 times were performed.

2.4.5. THERMOGRAVIMETRIC ANALYSIS

The thermogravimetric analysis was performed with a Perkin – Elmer 100 series, with 10 ^{o}C/min. ramp from 50 ^{o}C to 800 ^{o}C in nitrogen atmosphere, with 50 mL/min flow rate. The analysis was performed at TUBITAK SAGE.

CHAPTER 3

RESULTS AND DISCUSSION

The aim of this study was to investigate the effect of pretreatment time on reducing sugar and lignin yields after enzymatic hydrolysis, following the ionic liquid pretreament. Two ionic liquids, [EMIM][Ac] and [EMIM][Cl] were used for pretreatment experiments. Pretreatments with [EMIM][Ac] were conducted for 5, 15 and 30 minutes. Pretreatments with [EMIM][Cl] were conducted for 15, 30 and 60 minutes. Longer pretreatment times were chosen for [EMIM][Cl] since shorter times would not result in distinctive results to be able to compare the time effect. A high temperature as 150 ^{o}C was chosen, with short time periods would be effective at the temperature. Instead of low temperature – long time pretreatments, experiments were built on short time process concept, which would again conceptually considered as a way to a high throughput bioprocess and biorefinery facility. Compositional analysis of the raw peanut shells was conducted according to NREL procedures (Sluiter el al., 2008). Results are given below in Table 3.1.

Table 3.1 Compositional analysis of dry peanut shells

Component	**Percentage (%)**
Cellulose	32.0 ± 0.9
Xylan	12.6 ± 0.8
Arabinan	5.9 ± 0.6
Acid Insoluble Lignin	34.1 ± 0.3
Ash	5.6 ± 0.1

The compositional analysis showed consistent and very close results with the reported data given in section 1.2. Peanut shells contain 32% cellulose, 34% acid insoluble lignin and 18% hemicelluloses (xylan and arabinan). Approximately, 84% of the peanut shell's mass was composed of the parts that can be fractionated and utilized as a raw material in a biomass fractionation process.

3.1 PRELIMINARY STUDIES

In the early phases of the study, a know - how was tried to be built. Literature was investigated, along with the experiments conducted. Methods of ionic liquid pretreatments, anti solvent adding and regeneration, solid - liquid separation of the regenerated samples were studied extensively while effects of biomass loading, particle size, temperature and time periods were observed.
The first experiments were performed with [AMIM][Cl] on corncob.

3.1.1 PRETREATMENT of CORNCOB with [AMIM][Cl]

In the preliminary experiments of pretreatment of corncob with [AMIM][Cl], the main aim was to see if the corncob was to be dissolved in the ionic liquid. For this purpose, high temperatures of pretreatment and long time periods were chosen and the biomass loading was kept at 1%, with biomass of a small particle size. (Less than 0.100 mm). Pretreatment time periods were 90 minutes for 130 ^{o}C and 4 hours for 100 ^{o}C. In Figure 3.1 appearance of [AMIM][Cl] pretreated corncob and in Figure 3.2, precipitated and recovered biomass upon [AMIM][Cl] pretreatment is shown.

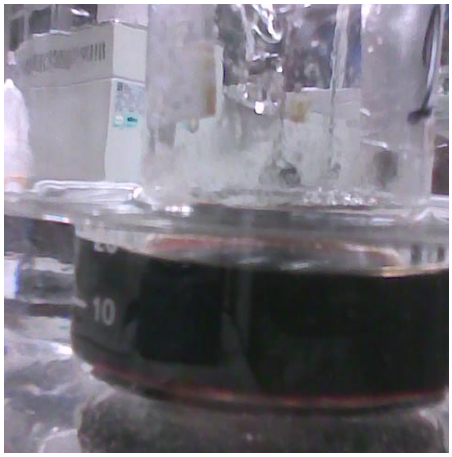


Figure 3.1 – Appearance of [AMIM][Cl] and corncob mixture. The dissolved biomass and the ionic liquid mixture turned a dark – colored appearance.

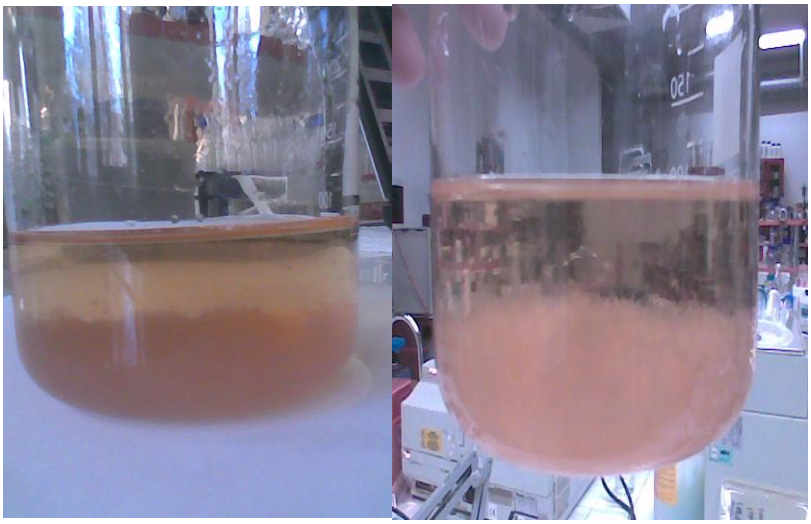


Figure 3.2 Appearance of [AMIM][Cl] and corncob mixture after the pretreatment is terminated via addition of deionized water as the anti solvent. The precipitated biomass and the ionic liquid mixture took a light – yellowish appearance and the biomass took a white – like appearance.

In Table 3.2, effect of time period and temperature of [AMIM][Cl] pretreatments of corncob on solid recovery is shown. The pretreatment process was performed at constant temperature, 130 ^{o}C for 90 minutes, and 100 ^{o}C for 4 hours. with 0.15 g of corncob per 15 g of [AMIM][Cl].

Table 3.2 Effect of time period and temperature of [AMIM][Cl] pretreatments on solid recovery.

Property	**90 minutes of [AMIM][Cl] pretreatment at 130 ^{o}C**	**4 hours of [AMIM][Cl] pretreatment at 100 ^{o}C**
Pretreatment solid recovery (%)	< 10%	55.0 ± 5.0 %

Pretreatment of corncob at 130 ^{o}C resulted in very low solid recovery. This was attributed to the probable degradation of the cellulosic and hemicellulosic parts of the corncob in the [AMIM][Cl] at such a high temperature for 90 minutes time period. The corncob is reported to have high values of cellulosic and hemicellulosic parts in the literature (about 70 – 80% total), which explains the degradation and very low solid recovery value. Moreover, the low initial biomass loading could have facilitated the degradation.

The recovered solid parts were exposed to enzymatic hydrolysis. In Table 3.3, effect of [AMIM][Cl] pretreatment of corncob on enzymatic hydrolysis of recovered solid after pretreatment is shown.

Table 3.3 Effect of [AMIM][Cl] pretreatment on the enzymatic hydrolysis of recovered solid after pretreatment.

Property	**90 minutes of [AMIM][Cl] pretreatment at 130 ^{o}C**	**4 hours of [AMIM][Cl] pretreatment at 100 ^{o}C**
Enzymatic hydrolysis of pretreated solid (%)	-	27.5 ± 3.5 %
Overall reducing sugar yield (%)	-	15.0 ± 0.5 %

Since the recovered solid was less than 10%, the 90 minutes pretreatment's enzymatic hydrolysis values were not obtained as the recovered solid was too small to be hydrolyzed. However, the recovered solid of the 4 hours pretreatment at 100 ^{o}C was resulted in biomass hydrolysis of 27.5 ± 3.5 % at the end of 24th hour. The untreated corncob was digested at 15% at the end of 24th hour. Hence, the [AMIM][Cl] pretreated corncob were enzymatically hydrolyzed about two times more than the untreated corncob, which indicates that [AMIM][Cl] pretreatment of corncob increased the enzymatic digestibility.Another important knowledge that was gained from [AMIM][Cl] pretreatments of the corncob was that complete biomass dissolution is not essential. Partial dissolution of the biomass had also effect on the enzymatic digestibility of the biomass.

3.1.2 PRETREATMENT of CORNCOB with [EMIM][Cl]

After the preliminary studies of pretreatment of corncob with [AMIM][Cl], [EMIM][Cl] was chosen as the ionic liquid type to pretreat the corncob. In the [EMIM][Cl] pretreatments, particle size of the biomass was set to less than 0.150 mm. The particle size was widened and made larger in order to avoid the degradation of the biomass at high temperatures. It was thought that larger particles sizes would be more recalcitrant to polymeric degredation at high temperatures. Biomass loading was increased to 3% in order to make the pretreatment study more efficient.

The pretreatment solid recovery in the preliminary [EMIM][Cl] pretreatment was 10%. The recovered solid parts were exposed to enzymatic hydrolysis. Recovered solid could be digested at 54.0 ± 0.0 % at the end of 24th hour. Untreated corncob was digested at 15% at the end of 24th hour. Hence, the [EMIM][Cl] pretreatment of corncob resulted in almost three times higher biomass digestibility compared to the untreated corncob, which indicates that [EMIM][Cl] pretreatment of corncob increased the enzymatic digestibility. Moreover, the digestibility was about 30% with [AMIM][Cl] pretreatment, as it was 54% with [EMIM][Cl], even the pretreatment time period is 4 hours for [AMIM][Cl] and 30 minutes for [EMIM][Cl]. However, the pretreatment temperature is 100 ^{o}C for [AMIM][Cl] and 130 ^{o}C for [EMIM][Cl]. It was observed that [EMIM][Cl] pretreatment was much more efficient than the [AMIM][Cl], with higher temperature, shorter time period. An additional [AMIM][Cl] pretreatment at 130 ^{o}C for 30 minutes was necessary to be able to compare the ionic liquid type, however it could not be performed due to ionic liquid shortage. However, [EMIM][Cl] preliminary experiments showed that high temperatures with shorter time periods can be effective. Also biomass loading was decided to be increased to 3%.

3.1.3 PRETREATMENT of CORNCOB with [EMIM][Ac]

In the preliminary [EMIM][Ac] pretreatments, two types of corncob were used. One part was with particle size of between 0.300 mm and 0.180 mm, another part was with partilce size of smaller than 0.150 mm. The effect of the particle size was observed.

Figure 3.3 Apperance of corncob subjected to [EMIM][Ac] pretreatment.

The first noticable difference during [EMIM][Ac] pretreatments was the appearance of the pretreated and precipitated biomass. In [AMIM][Cl] and [EMIM][Cl] pretreatments, the precipitated corncob showed an appearance like in Figure 3.2, however, [EMIM][Ac] pretreated corncob showed a clustered, single part appearance as shown in Figure 3.3. It was necessary to break up the precipitated structure with glass rods and spoons.

In Table 3.4, effect of particle size of corncob on [EMIM][Ac] pretreatments on solid recovery is given.

Table 3.4 Effect of particle size on the solid recovery upon [EMIM][Ac] pretreatment of corncob.

Property	**Particle size <0.150 mm**	**Particle size 0.300 – 0.180 mm**
Pretreatment solid recovery (%)	69.2 ± 0.0 %	64.5 ± 0.1 %

[EMIM][Ac] pretreatment was conducted at 130 ^{o}C for 30 minutes with 5% (w/w) biomass loading.

An interesting observation in the pretreatment solid recovery values was that the smaller particle sized biomass gave higher values than the higher particle sized biomass, normally the opposite to be expected. However, the difference was only about 5%, which may be in the normal variation range.

The recovered solid parts were exposed to enzymatic hydrolysis. In Table 3.5, effect of [EMIM][Ac] pretreatment of corncob on enzymatic hydrolysis of recovered solid after pretreatment is shown.

Table 3.5 Effect of time period of [EMIM][Ac] pretreatments on the enzymatic hydrolysis of recovered solid after pretreatment and lignin – rich solid.

Property	**Particle size <0.150 mm**	**Particle size 0.300 – 0.180 mm**
Enzymatic hydrolysis of pretreated solid (%)	54.4 ± 0.0 %	59.8 ± 1.1 %
Overall reducing sugar yield (%)	37.6 ± 0.0 %	38.6 ± 0.7 %

According to data in Tables 3.4 and 3.5, the particle size did not have a major effect on pretreatment solid recovery and enzymatic digestibility of the recovered solid, along with overall reducing sugar yields. It brought an advantage that extra grinding was not necessary in order to have better results, which could also bring an advantage to the process economy.

The pretreatment of corncob with [EMIM][Ac] at 130 ^{o}C for 30 minutes resulted in about 56% enzymatic digestibility, while it was 54% with [EMIM][Cl] at the same temperature and time

period conditions. However, the initial biomass loading was 3% with [EMIM][Cl] and 5% with [EMIM][Ac]. Giving the similar results for enzymatic digestilibility for higher initial biomass loading, it was observed that [EMIM][Ac] pretreatment was more efficient than the [EMIM][Cl], which was already more efficient than the [AMIM][Cl] pretreatment. Although the three ionic liquid types were not compared at the same conditions and parameters such as biomass loading, it was concluded that [EMIM][Ac] had the best pretreatment effect on corncob.

With the experience gained during the preliminary studies, pretreatment of peanut shells with [EMIM][Ac] and [EMIM][Cl] with different time periods was aimed. These two ionic liquid types were chosen based on preliminary experimental experiences. Moreover, it would be much easier to compare the experimental results with literature, since the two ionic liquid types were the most studied ones.

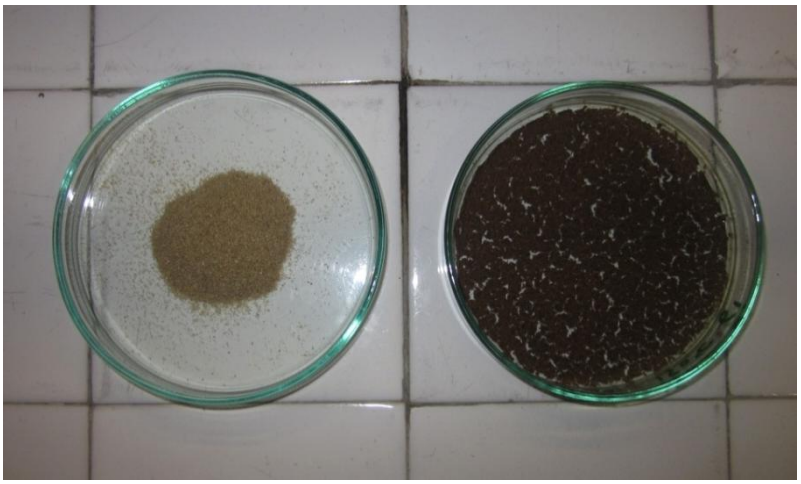
3.2 EFFECT of TIME PERIOD ON IONIC LIQUID PRETREATMENT of PEANUT SHELLS USING [EMIM][Ac]

In Figure 3.4, untreated and [EMIM][Ac] pretreated peanut shell samples are presented. [EMIM][Ac] pretreated samples (right) have different appearances compared to the untreated peanut shell samples (left). The top and middle images, which were the 5 minutes and 15 minutes of [EMIM][Ac] pretreatments, show similar appearances. They formed a film - like layer on drying. However, at the bottom image, which belongs to the the 30 minutes of [EMIM][Ac] pretreatment, had a different appearane than the top two images. The pretreated biomass was tough and aggregated when precipitated and dried, which required grinding before the enzymatic hydrolysis. It was much easier to grind the recovered solids of the 5 minutes and 15 minutes of [EMIM][Ac] pretreatments.In Table 3.6, effect of time period of [EMIM][Ac] pretreatments of peanut shells on solid recovery is shown.

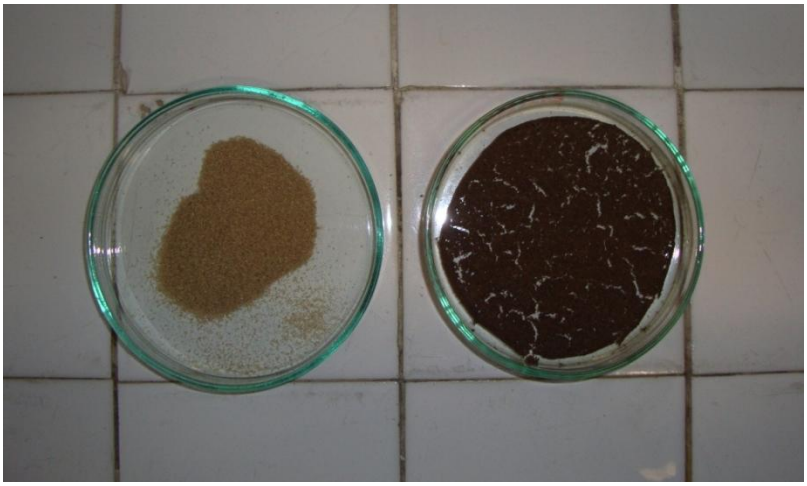
Table 3.6 Effect of time period of [EMIM][Ac] pretreatments on solid recovery.

Property	**5 minutes of [EMIM][Ac] pretreatment**	**15 minutes of [EMIM][Ac] pretreatment**	**30 minutes of [EMIM][Ac] pretreatment**
Pretreatment solid recovery (%)	64.1 ± 2.9 %	59.8 ± 2.2 %	55.5 ± 1.6 %

The pretreatment process was performed at 150 ^{o}C, 600 rpm of stirring rate and 5% (w/w) biomass loading.



a



b

c

Figure 3.4 Appearances of 5 min. (a), 15 min. (b) and 30 min. (c) [EMIM][Ac] pretreated peanut shells (left) with untreated peanut shells (right).

Solid recovery decreased as the pretreatment time period of the [EMIM][Ac] pretreatement increased, which is probably due to the degradation of cellulose and hemicellulose contents of the biomass at high temperatures.

Pretreated peanut shell contents were subjected to to enzymatic hydrolysis. In Table 3.7, effect of time period of [EMIM][Ac] pretreatments of peanut shells on enzymatic hydrolysis of recovered solid after pretreatment and lignin – rich solid is shown.

Table 3.7 Effect of time period of [EMIM][Ac] pretreatments on the enzymatic hydrolysis of recovered solid after pretreatment and lignin – rich solid.

Property	**5 minutes of [EMIM][Ac] pretreatment**	**15 minutes of [EMIM][Ac] pretreatment**	**30 minutes of [EMIM][Ac] pretreatment**
Enzymatic hydrolysis of pretreated solid (%)	33.2 ± 5.2 %	47.4 ± 0.8 %	37.5 ± 2.0 %
Overall reducing sugar yield (%)	21.3 ± 2.3 %	28.3 ± 0.5 %	20.8 ± 1.7 %
Solid recovery after enzymatic hydrolysis (%)	35.8 ± 6.8 %	29.0 ± 0.6 %	27.5 ± 0.9 %
Purity of lignin in the recovered hydrolysis residue (%)	41.8 ± 0 %	67.6 ± 0.6 %	69.8 ± 1.0 %
Overall lignin yield (%)	17.2 ± 3.3 %	19.5 ± 0.2 %	19.2 ± 0.1 %

The pretreatment process was performed at 150 ^{o}C, 600 rpm of stirring rate and 5% (w/w) of biomass loading.

The reducing sugar concentrations obtained upon enzymatic hydrolysis of Avicel (control), untreated peanut shells (control) and pretreated peanut shells are given in figures 3.5, 3.6 and 3.7.

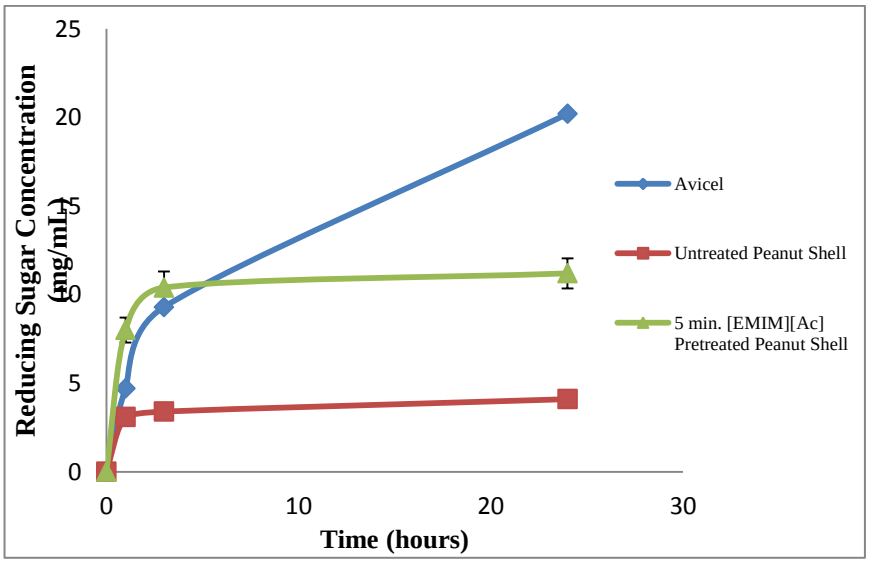


Figure 3.5 Reducing sugar concentrations of untreated avicel, untreated peanut shell and 5 min. [EMIM][Ac] pretreated peanut shell samples during enzymatic hydrolysis.

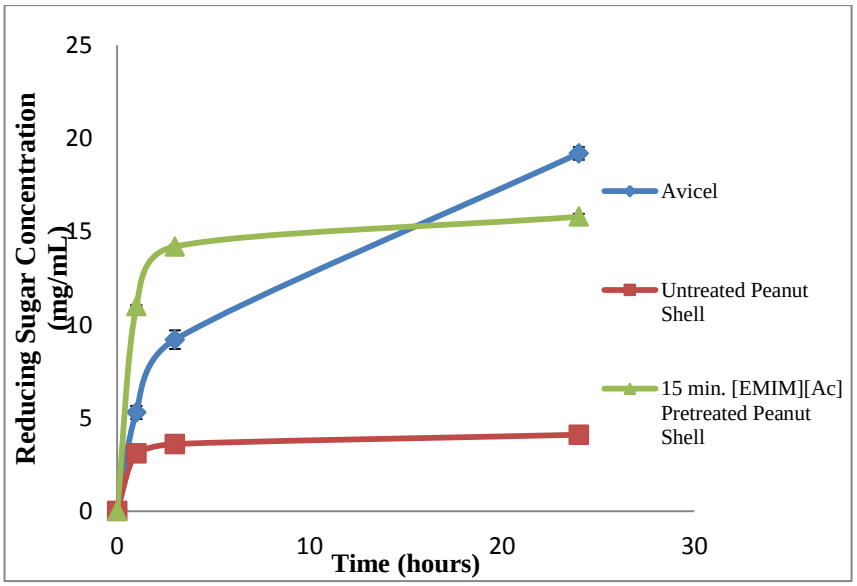


Figure 3.6 Reducing sugar concentrations of untreated avicel, untreated peanut shell and 15 min. [EMIM][Ac] pretreated peanut shell samples during enzymatic hydrolysis.

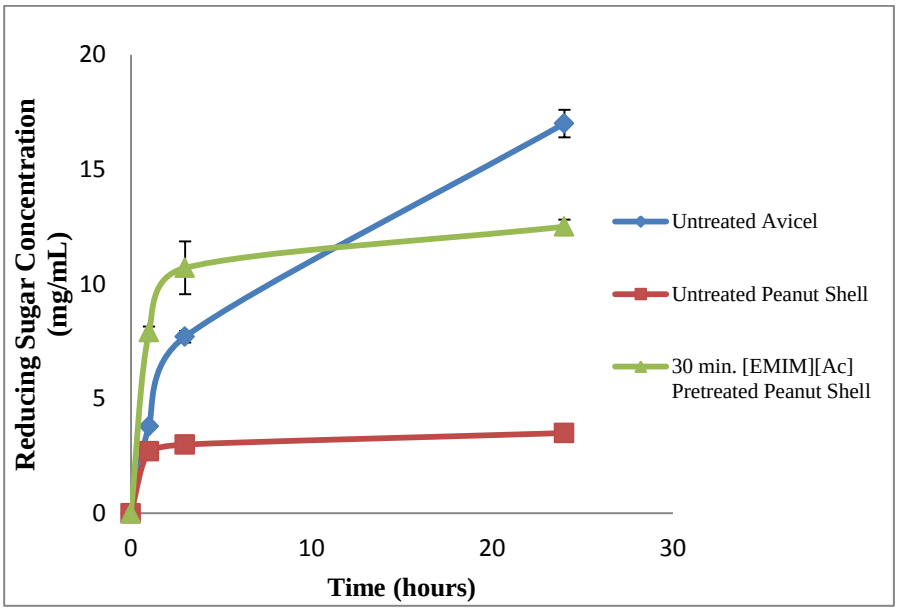


Figure 3.7 Reducing sugar concentrations of untreated avicel, untreated peanut shell and 30 min. [EMIM][Ac] pretreated peanut shell samples during enzymatic hydrolysis.

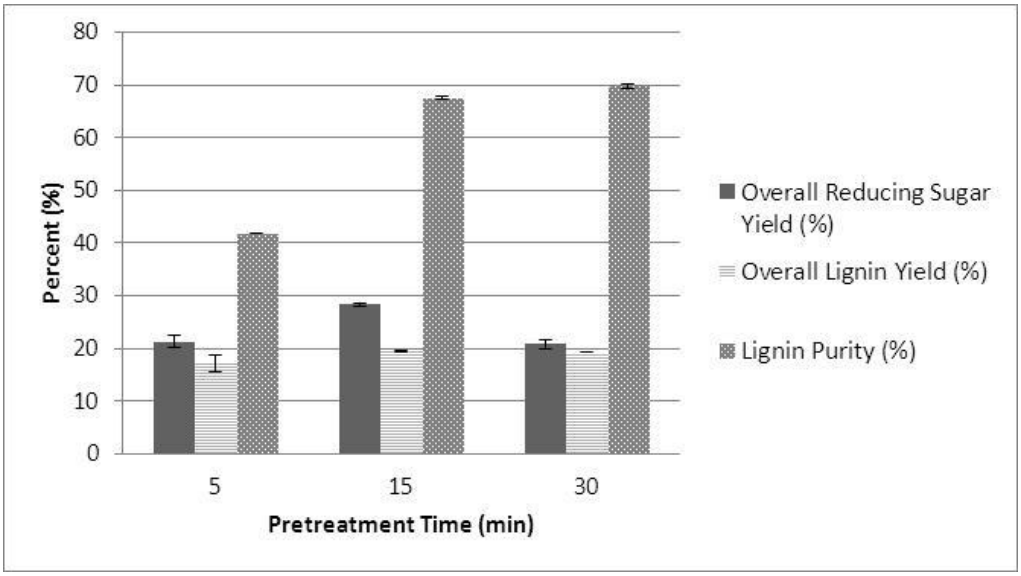


Figure 3.8 Effect of pretreatment time period on overall reducing sugar yield, lignin purtiy and overall lignin yield for [EMIM][Ac] pretreatment of peanut shells.

In Figure 3.8, [EMIM][Ac] pretreatments performed for 5, 15 and 30 minutes have different effects on the peanut shell at the same temperature. Increase in the pretreatment time did not necessarily increased the overall reducing sugar yield. This was probably related to the cellulose degradation at high temperatures. The longer the biomass exposed to high temperature ionic liquid, the more the cellulose polymer degraded. The decrease in the pretreatment solid recovery can be also an indication of this phenomenon. Also, the experiments were not performed under inert atmosphere. Instead, the experiments were performed at open atmosphere, because in a probable industrial application, the pretreatments would have not been performed in inert atmospheres like nitrogen or argon, which would increase the cost. As being performed in open atmosphere, the high temperature of the pretreatment medium could have effect the biomass degradation by O_2 exposure.

Due to the decrease in the solid recovery after pretreatment and the decrease in enzymatic hydrolysis, the overall reducing sugar yield decreased at 30 minutes, which is the longest pretreatment time period for [EMIM][Ac] experiment set. So, there is an optimum pretreatment time period for the maximum overall reducing sugar yield for [EMIM][Ac] pretreatment of peanut shells at 150 ^{o}C.

Overall lignin yield increased as pretreatment time increased; however, there was not an obvious trend as "increase in pretreatment time results in overall lignin yield". The trend could be seen in the lignin purity. Although there was not a very significant difference in the last two values, lignin purity had a tendency to increase as pretreatment time increased. Considering the little difference in the lignin purity values for the 15 minutes and 30 minutes pretreatments and the highest overall reducing sugar and overall lignin yield values were obtained in the 15 minutes pretreatment, 15 minutes was determined as the optimum pretreatment time period value.

3.3 EFFECT of TIME PERIOD ON IONIC LIQUID PRETREATMENT of PEANUT SHELLS USING [EMIM][Cl]

Like the Figure 3.4 in section 3.2, in Fiure 3.9, 15 minutes, 30 minutes and 60 minutes [EMIM][Cl] pretreated peanut shell samples showed similar appearances. However, [EMIM][Ac] and the [EMIM][Cl] pretreated peanut shells show different appearances. The ionic liquid type had different effects on the physical appearances of the pretreated peanut shells. [EMIM][Ac]pretreated peanut shell samples shows a more brownish, darker appearance, while [EMIM][Cl] pretreated peannut shell sample has a lighter - coloured apperance.

This simple image comparison may give idea about the different effects of the different ionic liquids had on the biomass during pretreatment. Nonetheless, appearances of both pretreated samples have changed and modified after the pretreatment process. The ground, particulate untreated material showed clustered, film – like structure when dried after pretreatment especially with [EMIM][Ac]. This film – like material should be grinded and made particulate again before the enzymatic hydrolys.In Table 3.8, effect of time period of [EMIM][Cl] pretreatments of peanut shells on solid recovery is shown.


a

b


c

Figure 3.9 Appearances of 15 min. (a), 30 min. (b) and 60 min. (c) [EMIM][Cl] pretreated peanut shells.

Table 3.8 Effect of time period of [EMIM][Cl] pretreatments on solid recovery.

Property	**15 minutes of [EMIM][Cl] pretreatment**	**30 minutes of [EMIM][Cl] pretreatment**	**60 minutes of [EMIM][Cl] pretreatment**
Pretreatment solid recovery (%)	76.7 ± 0.2 %	72.2 ± 1.2 %	66.2 ± 1.7 %

The pretreatment process was performed at 150 ^{o}C, 600 rpm of stirring rate and 5% (w/w) biomass loading.

Solid recovery decreased as the time period of the [EMIM][Cl] pretreatement increased as in the [EMIM][Ac] pretreatments, which is again probably due to the degradation of cellulose and hemicellulose parts of the biomass at high temperatures. However, the recovered solid quantity is higher in [EMIM][Cl] than in the [EMIM][Ac] pretreatments. In the [EMIM][Ac] pretreatments,

recovered solid quantity was 55 – 64 %, where it is 66 – 76 % in the [EMIM][Cl] pretreatments. This difference is probably due to the different solubilization effects of the ionic liquids. Hence, the ionic liquid type had effect on the pretreatment solid recovery.

Again, the recovered solid parts were exposed to enzymatic hydrolysis. In Table 3.9, effect of time period of [EMIM][Cl] pretreatments of peanut shells on enzymatic hydrolysis of recovered solid after pretreatment and lignin – rich solid is shown.

Table 3.9 Effect of time period of [EMIM][Cl] pretreatments on the enzymatic hydrolysis of recovered solid after pretreatment and lignin – rich solid.

Property	**15 minutes of [EMIM][Cl] pretreatment**	**30 minutes of [EMIM][Cl] pretreatment**	**60 minutes of [EMIM][Cl] pretreatment**
Enzymatic hydrolysis of pretreated solid (%)	16.4 ± 1.4 %	17.2 ± 0.6 %	25.0 ± 0.8 %
Overall reducing sugar yield (%)	12.5 ± 1.0 %	12.4 ± 0.2 %	16.5 ± 0.9 %
Solid recovery after enzymatic hydrolysis (%)	57.8 ± 0.0 %	53.7 ± 2.8 %	45.4 ± 1.6 %
Purity of lignin in the recovered hydrolysis residue (%)	40.7 ± 1.0 %	39.2 ± 0.5 %	48.5 ± 0.5 %
Overall lignin yield (%)	23.5 ± 0.6 %	21.1 ± 1.4 %	22.1 ± 0.6 %

The pretreatment process was performed at 150 ^{o}C, 600 rpm of stirring rate and 5% (w/w) biomass loading.

The reducing sugar concentrations obtained upon enzymatic hydrolysis of Avicel (control), untreated peanut shells (control) and pretreated peanut shells are given in figures 3.10, 3.11 and 3.12. The numeric data of the charts are given in the Appendix.

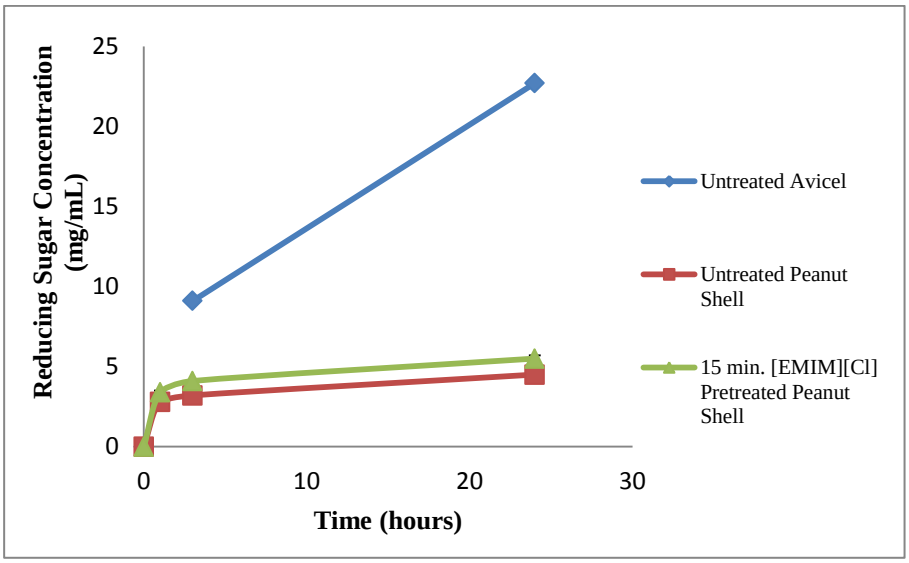


Figure 3.10 Reduing sugar concentrations of untreated avicel, untreated peanut shell and 15 min. [EMIM][Cl] pretreated peanut shell samples during enzymatic hydrolysis.

Note: 1st hour result of reducing sugar concentration of avicel hydrolysis could not be retrieved due to experimental error.

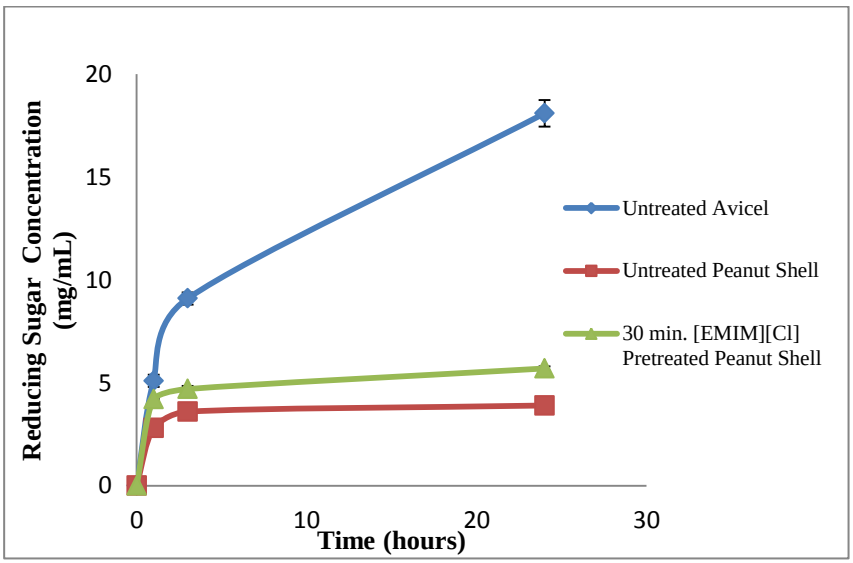


Figure 3.11 Reducing sugar concentrations of untreated avicel, untreated peanut shell and 30 min. [EMIM][Cl] pretreated peanut shell samples during enzymatic hydrolysis.

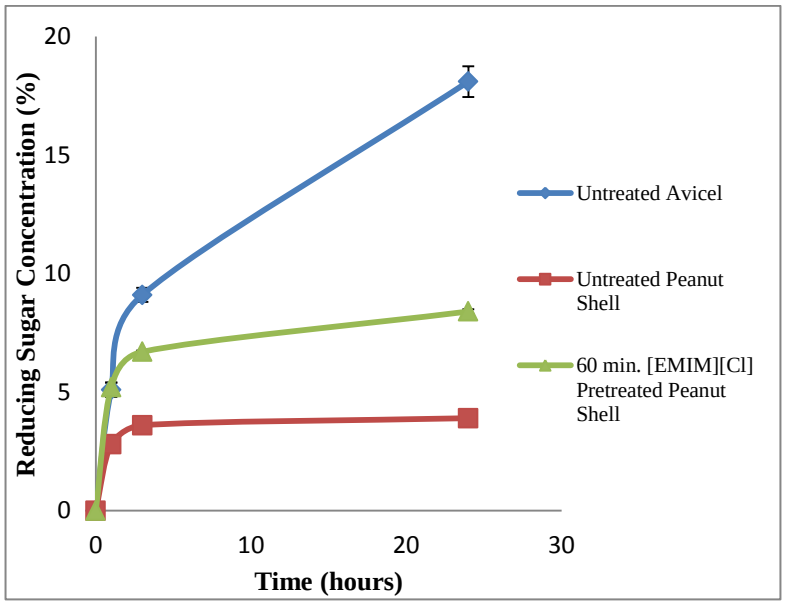


Figure 3.12 Reducing sugar concentrations of untreated avicel, untreated peanut shell and 60 min. [EMIM][Cl] pretreated peanut shell samples during enzymatic hydrolysis.

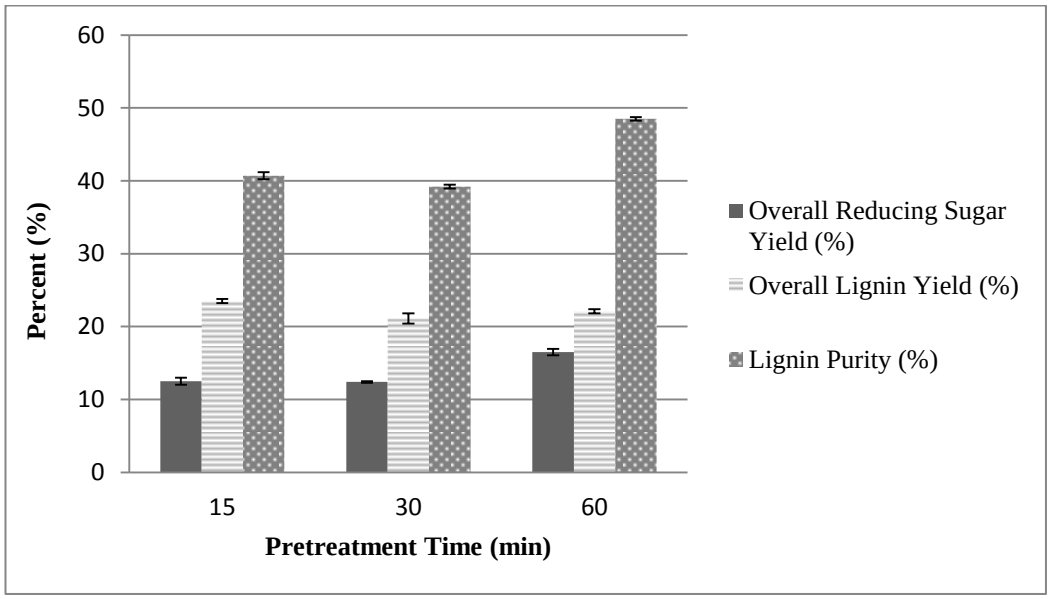


Figure 3.13 Effect of time period of [EMIM][Cl] pretreatments of peanut shells on overall reducing sugar yield, lignin purtiy and overall lignin yield.

In Figure 3.13, unlike the [EMIM][Ac] pretreatements, [EMIM][Cl] pretreatments had a general trend as "increasing values of as pretreatment time increses". In the [EMIM][Ac] pretreatements, a decrease in the overall reducing sugar yield was observed after 15 minutes, which was interpreted as the degradation of the cellulosic parts due to the high temperature effect. However, in [EMIM][Cl] pretreatments, even 60 minutes pretreatment showed increase in the overall reducing sugar yield. This was probably a result of differences in the dissolution effects of [EMIM][Ac] and [EMIM][Cl], as the literature implies [EMIM][Ac] has better solubility effects on biomass (Bahcegul et al., 2012). As the cellulosic polymer is dissolved more in [EMIM][Ac], degredation effects may be seen more rapidly. Cellulosic parts probably remain more intact in [EMIM][Cl], which delays the degredation.

With a multi product perspective, pretreatment of peanut shell with [EMIM][Cl] at 150 ^{o}C, longer pretreatment time provides better results for reducing sugar yield, lignin yield and lignin purity. Higher pretreatment times such as 90, 120 or 150 minutes; may have even better results. Longer pretreatment periods of these durations were not applied to the [EMIM][Cl] pretreatment of peanut shells since in this study; a short time period framework was aimed. There is a chance that a decrease in reducing sugar yield like in [EMIM][Ac] pretreatments could be observed after some time.

Overview:

A process flow diagram is conducted on ionic liquid pretreatment in Figure 3.14.

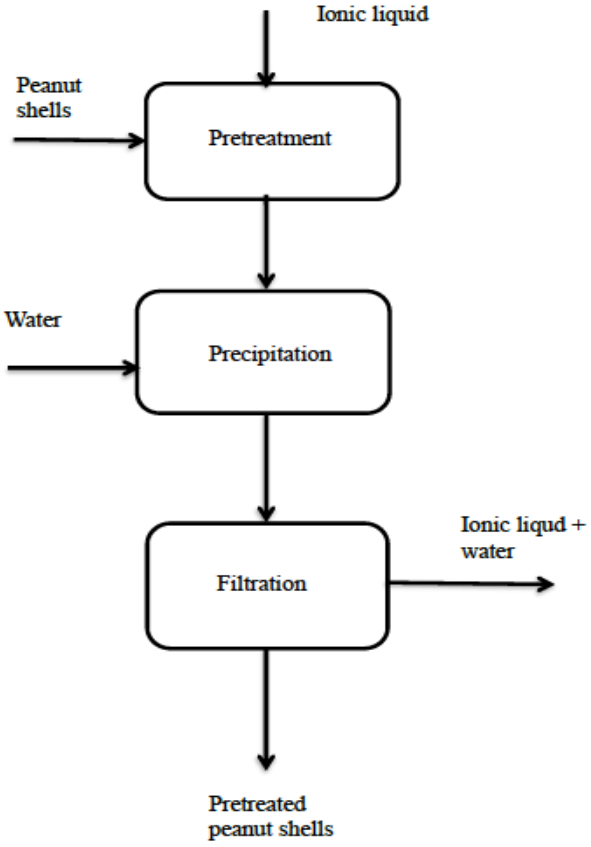


Figure 3.14 Flow diagram of ionic liquid pretreatment.

In this study, the output mass of the precipitated peanut shells were about 70% of the initial input mass of the peanut shells that are subjected to the pretreatment process. There are possible reasons for the mass loss. Firstly, the hemicellulose parts of the peanut shells can be degraded or hydrolyzed at 150 ^{o}C during pretreatment process, as hemicellulose can be hydrolyzed at high temperatures easily. Furthermore, the high temperature can cause the hemicellulosic part degrade. The hydrolyzed and degraded parts are then washed during the filtration. Moreover, hemicellulose is not the only part that can be degraded or hydrolyzed during the pretreatment. Cellulose can also be degraded and hydrolyzed even at small quantities during the pretreatment, since the temperature was as high as 150 ^{o}C. Also, during the washing and filtration steps, particles can be lost easily. The mass loss during pretreatment can be prevented by performing the pretreatment at lower temperatures (design choice) and minimizing the mass loss during the filtration and washing steps.

Comparisons of the [EMIM][Ac] and [EMIM][Cl] Pretreatments:

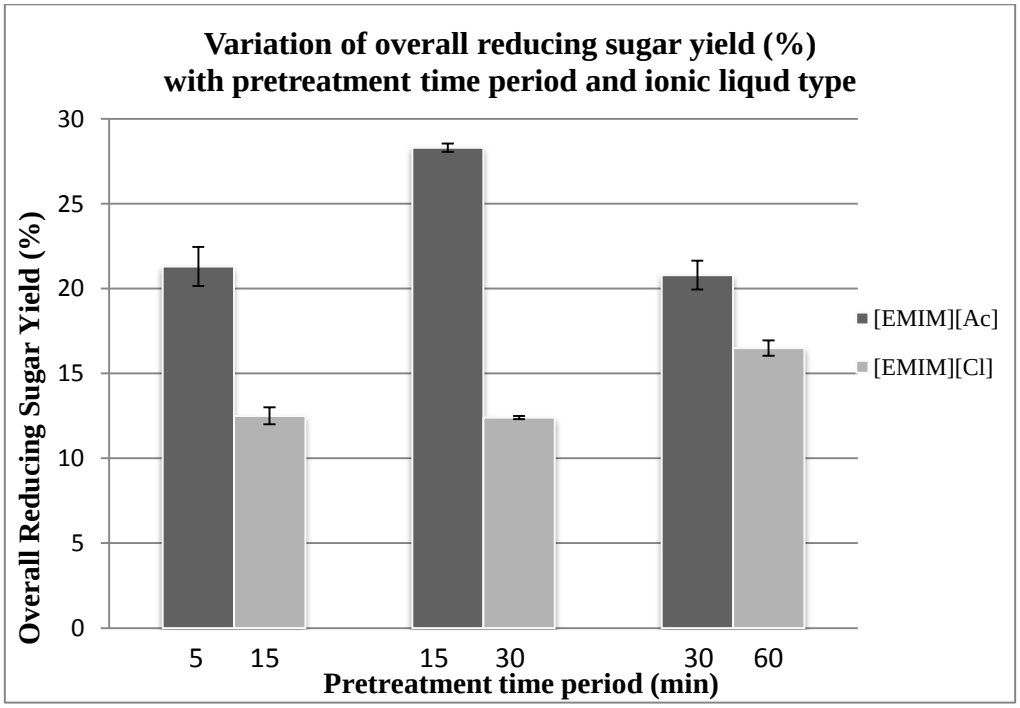


Figure 3.15 Graphical representation of the overall reducing sugar yield results of the [EMIM][Ac] and [EMIM][Cl] pretreatments, at 150 ^{o}C. Pretreatment time period is 5, 15 and 30 minutes for [EMIM][Ac] pretreatment, 15, 30 and 60 minutes for [EMIM][Cl] pretreatment.

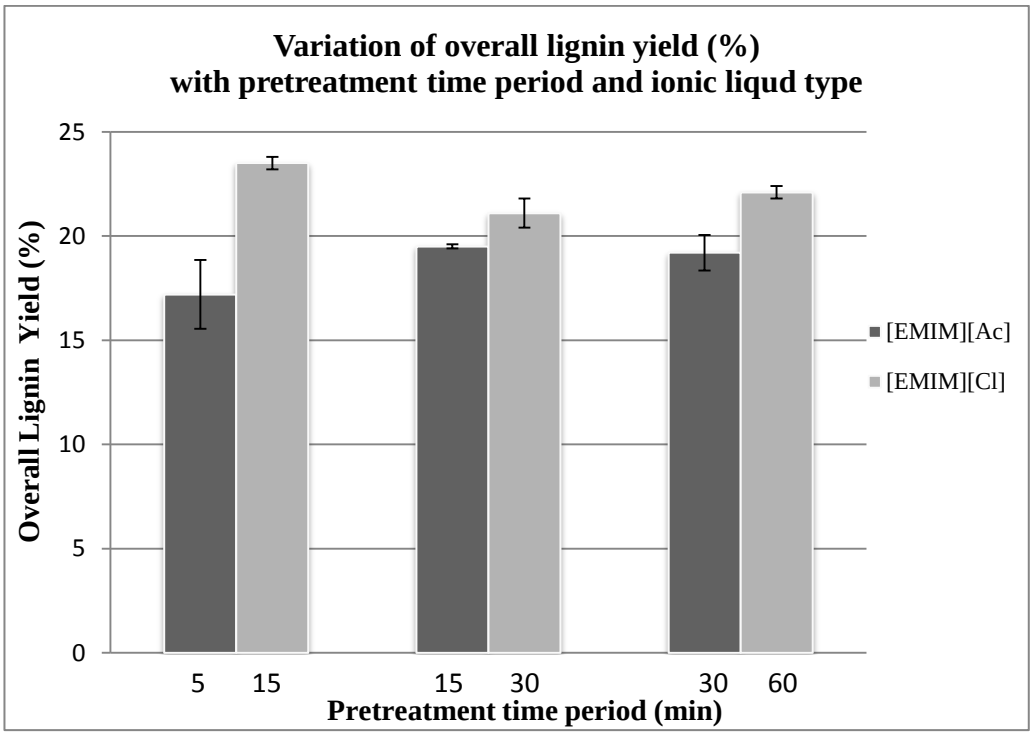


Figure 3.16 Graphical representation of the overall lignin yield results of the [EMIM][Ac] and [EMIM][Cl] pretreatments, at 150 ^{o}C. Pretreatment time period is 5, 15 and 30 minutes for [EMIM][Ac] pretreatment, 15, 30 and 60 minutes for [EMIM][Cl] pretreatment.

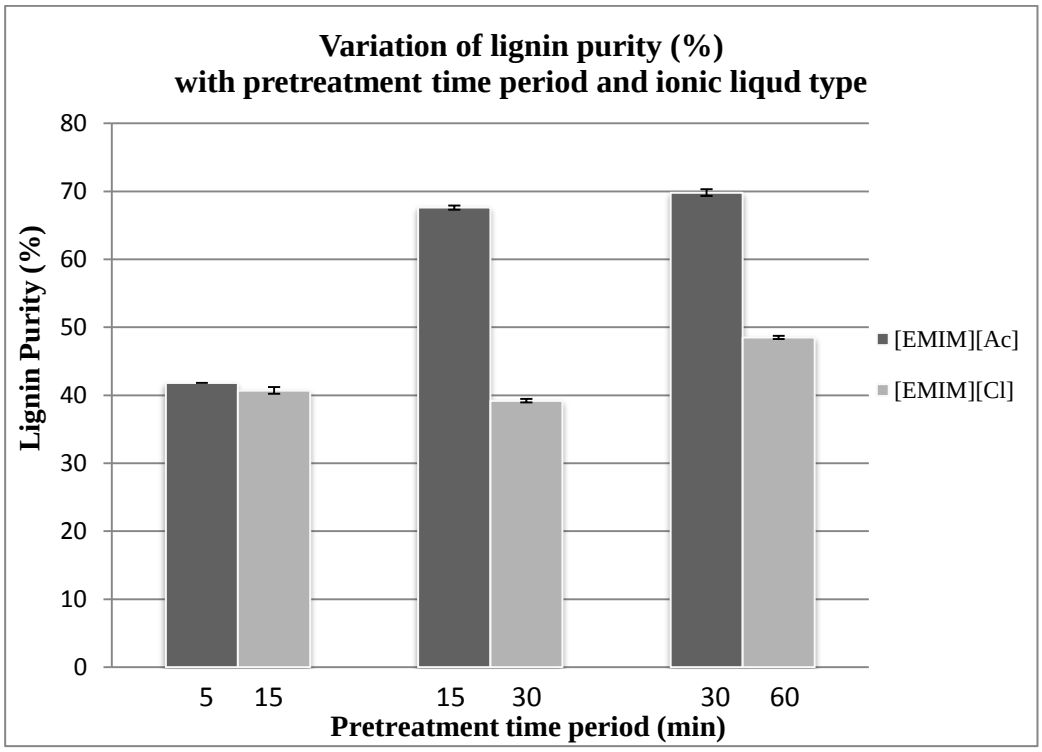


Figure 3.17 Graphical representation of the lignin purity results of the [EMIM][Ac] and [EMIM][Cl] pretreatments, at 150 ^{o}C. Pretreatment time period is 5, 15 and 30 minutes for [EMIM][Ac] pretreatment, 15, 30 and 60 minutes for [EMIM][Cl] pretreatment.

Firstly, it is clearly seen that, as pretreatment time increased, overall reducing sugar yield tended to increase in [EMIM][Cl] pretreatments. However, this was not the trend in [EMIM][Ac] pretreatments for three different time periods. This was obviously related to the degradation of the cellulosic component in the biomass, which was indicated by the solid recovery values as well.

Targeting a multi – product process, overall lignin yield was also another important parameter. With [EMIM][Cl] pretreatment, higher overall lignin yields were obtained in all pretreatment time periods. However, although the overall lignin yield with [EMIM][Ac] is lower than [EMIM][Cl], the difference is only about 5% at longer times. On the other hand, lignin purity of [EMIM][Ac] pretreatmtent products were higher than those of the [EMIM][Cl] pretreatment products.

It is also seen that, from the point of view of overall reducing sugar yield, [EMIM][Ac] performs better than [EMIM][Cl], which is a consistent result with the literature (Bahcegul et al., 2012). [EMIM][Ac] pretreatmtents resulted in higher than 20% overall reducing sugar yields, whereas the [EMIM][Cl] pretreatments resulted in maximum 15%. However, from the point of view of overall lignin yield, [EMIM][Cl] performed better than [EMIM][Ac]. [EMIM][Cl] pretreatments resulted in higher than 20% overall lignin yields, whereas [EMIM][Ac] pretreatments resulted in below 20%. This is probably due to the higher recovered solid material amounts, PSR% and the HSR% in the [EMIM][Cl] pretreatments. However, it should be noted that lignin purity of the [EMIM][Ac] products (~ 67%) are higher than that of the [EMIM][Cl] products (~ 40%), which actually indicates that hydrolysis residues of [EMIM][Cl] products have undigested cellulose and hemicellulose parts.

Finally, it can be concluded that:

- [EMIM][Ac] outperforms [EMIM][Cl] when pretreating peanut shells at 150 ^{o}C from the point of view of overall reducing sugar yield and lignin purity. Even 5 minutes of [EMIM][Ac] pretreatment had better results when compared to [EMIM][Cl] pretreatment for 60 minutes. This also shows the advantage of [EMIM][Ac] for a high throughput process, since higher number of batches of pretreatments can be conducted within the same time period.

- Only parameter that [EMIM][Cl] outperforms [EMIM][Ac] was overall lignin yield. However, considering there was only about 5% difference in the values and lignin purity was only around 45%. This parameter can be important only if the lignin is to be used as a precursor for other materials, such as in sulfonation or oxidation processes.

- It can be said that [EMIM][Ac] is a better pretreatment agent compared to [EMIM][Cl] regarding the pretreatments are conducted at 150 ^{o}C, from the point of multiple products.

- For [EMIM][Ac] pretreatments, it is not a generic rule that longer pretreatment time at constant temperature (at 150 ^{o}C) means better results from the point of multiple products. There is an optimum pretreatment time with respect to reducing sugar yields.

Hence, for a multi – product pretreatment process, in which peanut shells are pretreated at 150 ^{o}C with [EMIM][Ac], 15 minutes is the optimum time period redarding the reducing sugar and lignin yields. However, if purer (~5%) lignin product is desired, 30 minutes of pretreatment can be performed with a trade off as a slight decrease in reducing sugar yield. These parameters can be tuned more finely with further studies.

Thus, for the concrete admixture experiments, a lignin – rich product with a higher purity was desired. Peanut shells were pretreated at 150 ^{o}C for 30 minutes, and pretreated peanut shells were enzymatically hydrolyzed. The lignin – rich residue was used as the concrete admixture in the concrete admixture experiments. However the characterization studies of the untreated, pretreated and hydrolysis byproduct, the lignin – rich residue (LRR), were performed on the materials obtained from the 15 minutes of pretreatment.

3.4 XRD ANALYSIS

In Figure 3.18, diffractograms of untreated, 15 min. [EMIM][Ac] pretreated and lignin – rich residual samples are given.

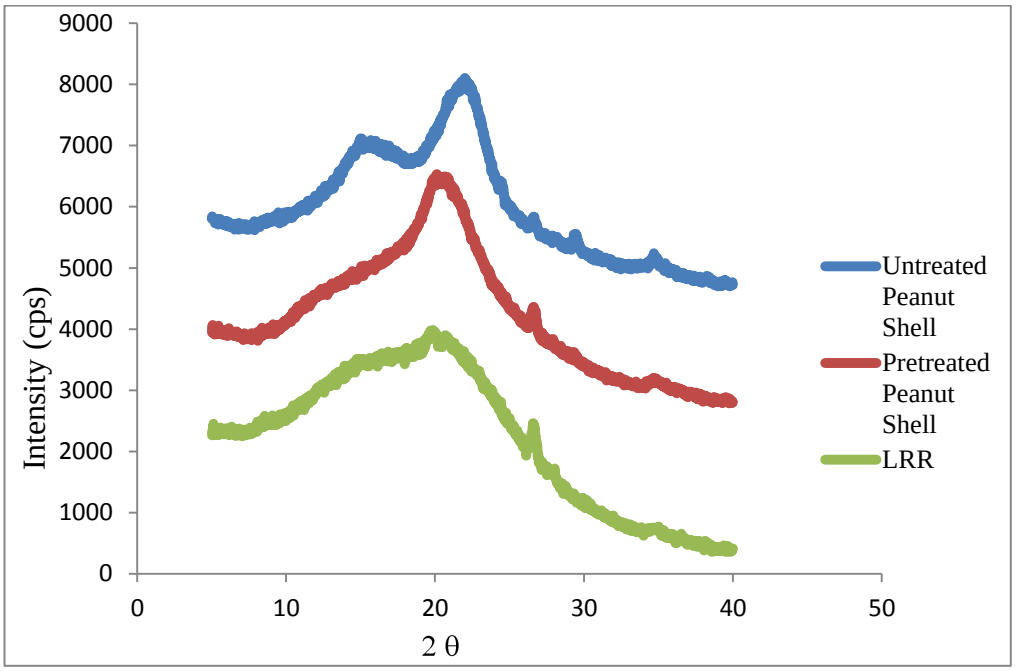


Figure 3.18 XRD diffractogram of untreated peanut shells, 30 min. [EMIM][Ac] pretreated peanut shells and LRR samples.

In the diffractogram, the peak at $2\theta = 15^{o}$ observed for the untreated peanut shell, disappeared in [EMIM][Ac] pretreated and lignin – rich residual sample. However, the main peak at $2\theta = 21.8^{o}$ in the untreated sample remained same for the [EMIM][Ac] pretreated sample, altough there is a peak broadening and a shift to lower angles, to 20^{o}. On the other side, the peak at $2\theta = 21.8^{o}$ is broadened widely in the lignin – rich residue. The peak shift at $2\theta = 21.8^{o}$ and disappearance at $2\theta = 15^{o}$ were due to due to changes in cellulose structure. Cellulose I, which is the native form of cellulose, is converted to Cellulose II during pretreatment and, which is the form of the cellulose in regenerated cellulose fibers. (Maki-Arvela et al., 2010; Wang et al., 2011; Bahcegul et al., 2012). The widely broadening of the peak at $2\theta = 21.8^{o}$ in the lignin – rich residual sample was probably due to the digestion of the cellulosic structure in the biomass during enzymatic hydrolysis. Similar results have been reported in the study of Cheng et al., (2012)andBahcegul et al., (2012) for [EMIM][Ac] pretreatment of switchgrass, pine and eucalyptus in the former, for cotton stalk in the latter.

In this study, although there is a peak shift seen, intensity counts for both pretreated peanut shell and LRR samples are counted higher than that of the untreated peanut shell sample. This could have happened due to some possible reasons. Assuming that the peanut shell samples contain native Cellulose I structure and the Cellulose I was transformed into Cellulose II during the ionic liquid pretreatment. However, it is stated that drying of cellulose fibers may cause the fibers collapse, and with wetting, specific surface area increases and water is known to increase the crystallinity of the

cellulose by causing the amorphous cellulose to go into re-crystallization (Taherzadeh et al., 2008). Although the experimental procedures are applied strictly in order to avoid deviations in experimental results, if there has been an excessive drying after pretreatment and enzymatic hydrolysis, or out – of – control drying with any high - temperature effect, a re – crystallization of the amoprhous Cellulose II may have occured.

The XRD patterns of Avicel and mercerized cellulose are given in Figure D1 and XRD parameters for Cellulose I and Cellulose II are given in Table D1.

3.5 FTIR ANALYSIS

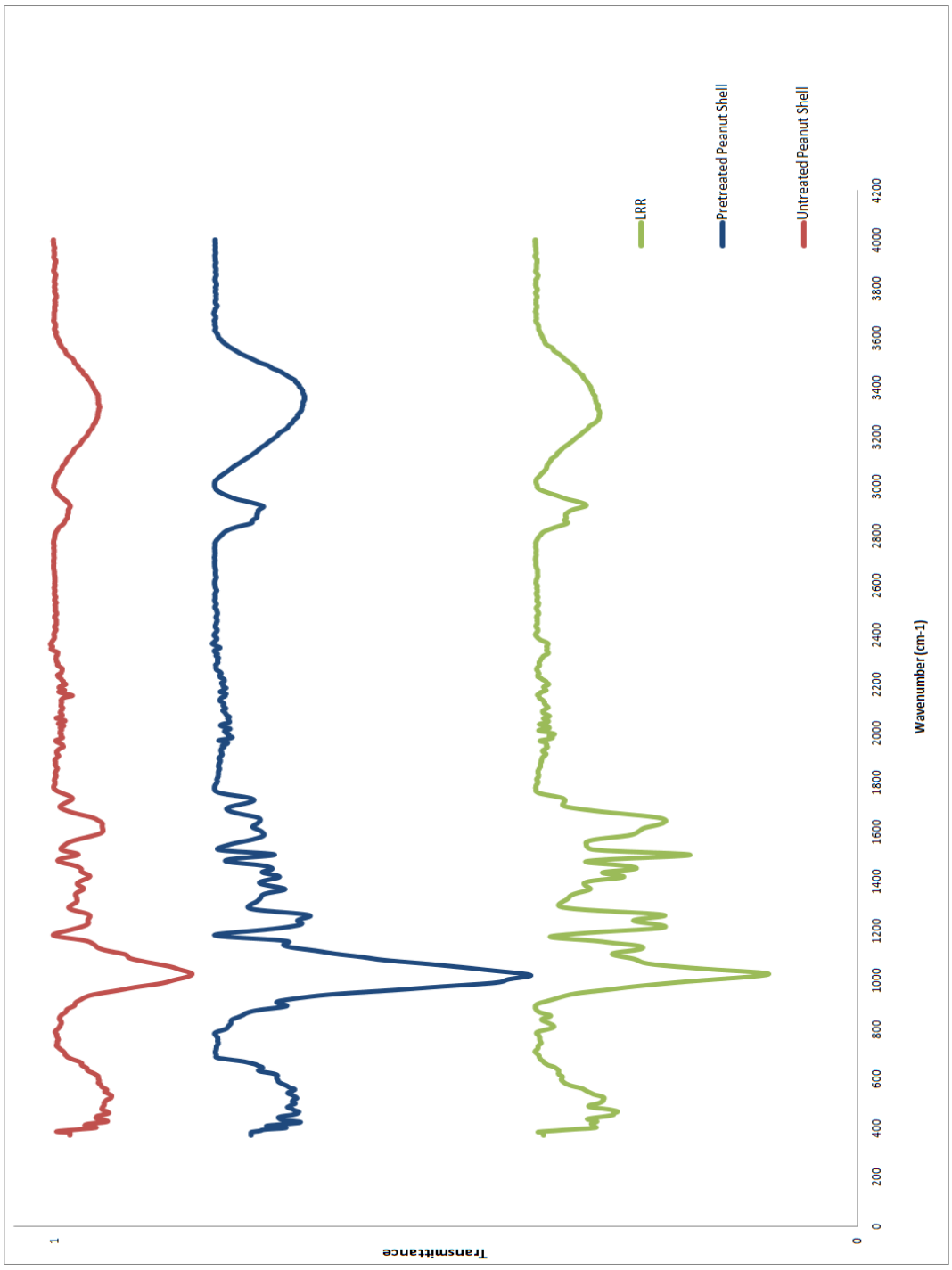


Figure 3.19 FTIR spectra of untreated peanut shell (red), 30 minutes [EMIM][Ac] pretreated peanut shell (blue) and lignin rich residue (green).

In order to have a better understanding, a closer look was given to the FTIR spectra. 400 – 900 cm^{-1}, 900 – 1800 cm^{-1} and2070 – 3070 cm^{-1} and bands were investigated closely. Firstly, the wide band in the 3000 – 3600 cm^{-1} is assigned to O – H stretching vibrations. There are no significant bands between 1800 – 2700 cm^{-1}. Stewart and Morrison (1992) stated that lignin spectrum does not have absorption bands in 1800 – 2700 cm^{-1} region.

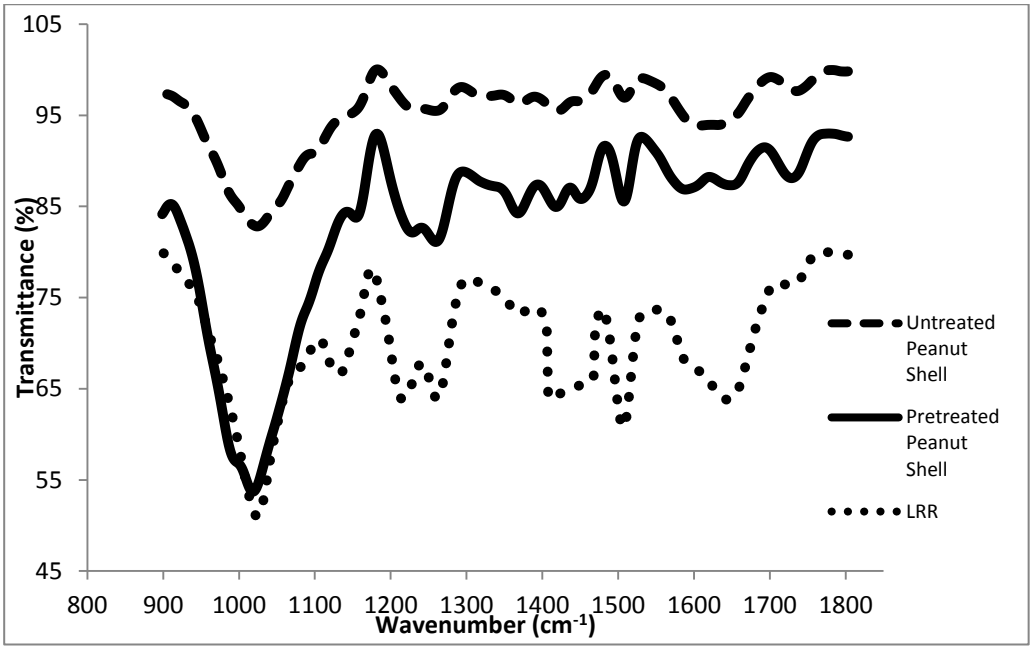


Figure 3.20 FTIR spectra of untreated peanut shell(blue), 30 minutes [EMIM][Ac] pretreated peanut shell (red) and lignin rich residue (green) samples in 900 – 1800 cm^{-1}

When the 900 – 1900 cm^{-1} region is investigated,the three spectra show similar patterns. However, LRR shows different peaks and bands in some regions. For example, the band at 1260 cm^{-1} exists in three spectra, however, the band at 1214 cm^{-1} existsonly in LRR spectra. The 1214 cm^{-1} band is assigned to assymmetric vibrations of C – O – C in ethers and esters (Bhat et al., 2009). The band at 1735 cm^{-1} is assigned to the unconjugated C = O stretching in the hardwood lignins, and this band only exists in the untreated spectrum. There is a 1728 cm^{-1} band in the pretreated spectrum, which may be due to a band shift because of the pretreatment since 1725 cm-1 is also assigned to the C = O stretching in the softwood lignins. However, there is no band existing in the 1725 – 1735 cm^{-1} region in the LRR spectra. The three spectra show bands in 1506 – 1509 cm^{-1} region. According to Casas et al., (2009) the vibrations at 1513 – 1504 cm^{-1} are characteristic aromatic skeletal vibrations for lignin. It can be seen that the intensity increases for pretreated and LRR relative to the untreated sample. Again, LRR intensity is higher than the pretreated sample. At 896 cm^{-1} only pretreated spectra shows a band which is assigned to the glucose ring vibration in cellulose. (Casas et al., 2009) This may be due to the pretreatment effect. FTIR band assignments for softwood and hardwood lignins are given in Appendix B (Casas et al., 2012).

3.6 SEM ANALYSIS

For a detailed understanding of morphological changes in the biomass structure due to pretreatment, a scanning electron micrograph study was performed. Some of the detailed micrographs mentioned in this part are in Appendix.

In Figure 3.21 a and b, and Figure C1, SEM micrographs of the untreated peanut shell samples are shown. The untreated peanut shell samples have a rigid structure with a fiber – like patterns on the surface.

In Figure 3.21 c and d, Figure C2 and Figure C3, SEM micrographs of the [EMIM][Ac] pretreated peanut shell samples are shown. The pretreatment had effects on the surface of the peanut shells. The surface has undergone an appearance change from a smooth shape to a more amorphous rough appearance. The [EMIM][Ac] pretreated peanut shell samples do not have a flat and smooth surface and the surface and does not show the fiber – like appearances anymore. On the contrary, there are cavities and empty spaces on the surface. Especially in Figure C3, an exceptional image, the particle has almost a mesh – like shape.

Afterwards, the hydrolysis residue, LRR was analyzed with SEM. In Figure 3.21 e and f, and in Figure C4 and C5, SEM micrographs of LRR are shown. The micrographs show that the particles look like the [EMIM][Ac] pretreated peanut shell samples, with cavities and empty spaces on the surfaces and they do not show the untreated peanut shell samples' rigid and smooth surface appearance. Moreover, the amorphous appearance dominates. In Figure C4 and C5, cavities on the surfaces are clearly seen. Since it is the LRR, the hydrolysis residue, the cavities may indicate the hydrolyzed parts of the lignocellulosic complex.

Lastly, the SEM micrographs of the untreated, [EMIM][Ac] pretreated peanut shell samples and LRR samples are shown together below together with 800 and 4000 times magnification in order to have a better visual appearance comparison. Figures on the left column are the 800 times magnifications and on the right column are the 4000 times magnifications, being the top images are the untreated peanut shell samples, the middle images are the [EMIM][Ac] pretreated peanut shells and the botto images are the LRR samples.

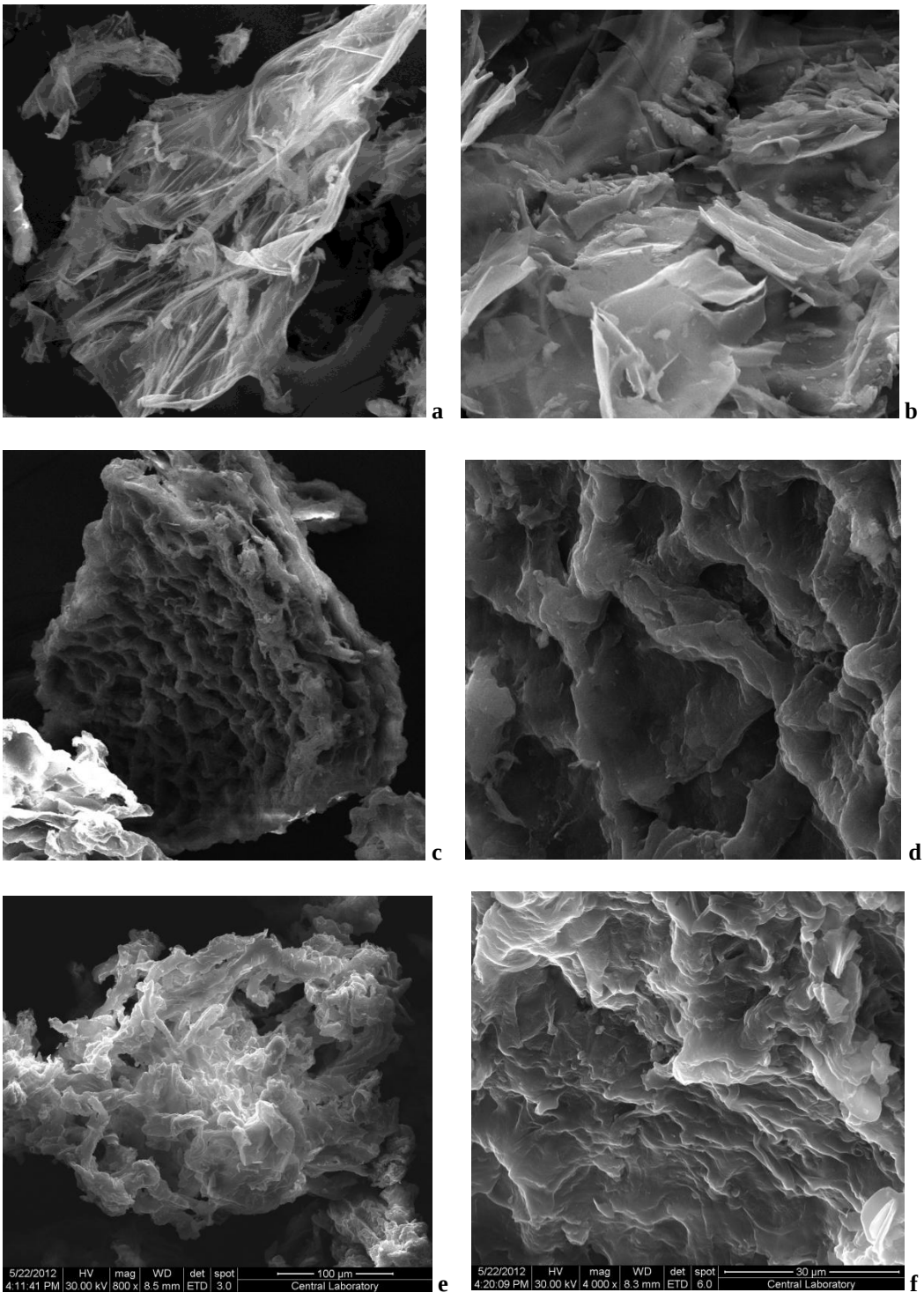


Figure 3.21 SEM micrographs of untreated peanut shell (a), [EMIM][Ac] pretreated peanut shell (c) and LRR (e) with 800 times magnification and untreated peanut shell (b), [EMIM][Ac] pretreated peanut shell (d) and LRR (f) with 4000 times magnification.

To conclude, the micrographs show that pretreatment had changing effects on the surface of the biomass. The interaction between the biomass particles and the ionic liquid resulted in increase in the biomass surface area, which enhances the enzymatic hydrolysis via improving the enzyme attack on the cellulosic parts, as more surface is now susceptable to enzymes. From the micrographs, it can be said that the pretreated and the LRR has cavities, hollows and lumps on the surface, making an amorphous – like structure, implying the increased surface area when compared to the relatively rigid, solid like surface of the untreated native sample. Moreover, the pretreated and the LRR samples look alike, with more cavities, hollows and empty spaces on the surfaces, may be the result of the enzymatic hydrolysis, removing the cellulosic parts. In order to understand the real surface area comparison with numbers, more study should be performed on the samples such as BET analysis.

3.7 THERMOGRAVIMETRIC ANALYSIS

The three samples, untreated peanut shell, [EMIM][Ac] pretreated peanut shell and LRR were subjected to thermogravimetric analysis. According to Carrier (Carrier et al., 2011), hemicellulose is the first component of the woody biomass to decompose at inert atmospheres. Hemicellulose starts to decompose at around 250 – 300 ^{o}C. After the hemicellulose, celluose starts to decompose at around 300 – 350 ^{o}C. Lastly, the decomposition of the lignin occurs. Lignin starts to decompose at around 300 – 500 ^{o}C.

In Figure 3.22, it is seen that, untreated peanut shell and pretreated peanut shell samples showed similar weight loss behaviours. Around 100 ^{o}C, a weight loss about 5% is observed, which is attributed to the evaporation of water content. After that, untreated peanut shell started to lose weight slightly earlier than the pretreated peanut shell. This can be related to the higher hemicellulose content in the untreated peanut shell. Since the pretreatment occured at 150 ^{o}C, it can be assumed that during the pretreatment, hemicellulose content of the peanut shell can be either decomposed or dissolved in the ionic liquid medium and washed over with water. Carrier also states that a peak in the 200 – 300 ^{o}C band confirms the hemicellulose presence. This can be concluded as since the pretreated peanut shell sample did not show an apparent weight loss in the 200 – 300 ^{o}C band as the untreated peanut shell sample, there is less hemicellulose in the pretreated peanut shell, which is consistent with the literature. Moreover, the untreated and the pretreated peanut shell samples showed maximum weight loss at 300 – 400 ^{o}C band.

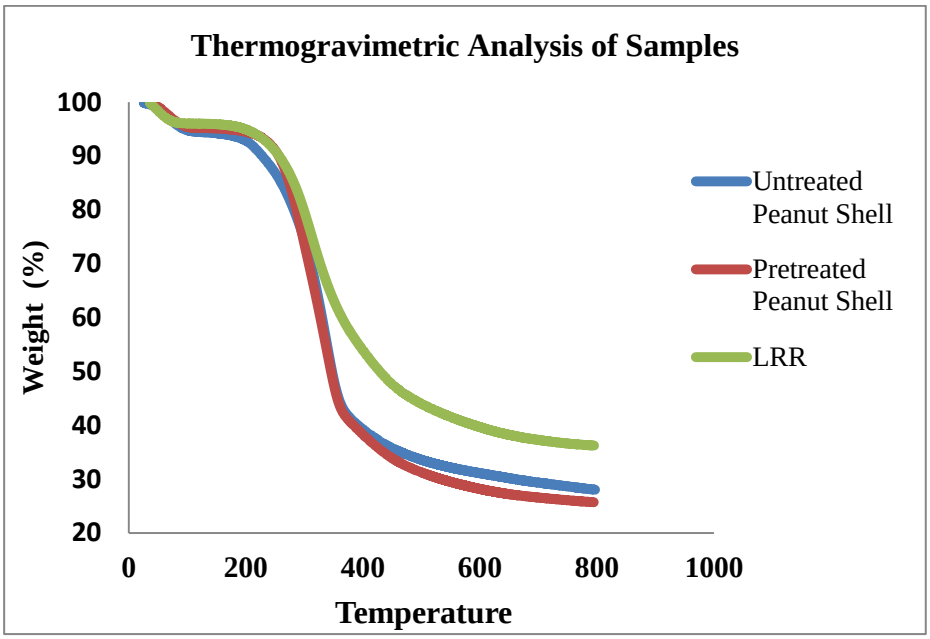


Figure 3.22 Thermogravimetric analysis of untreted peanut shell (blue), pretreated peanut shell (red) and LRR (green).

On the other hand, the LRR sample shows the weight loss in a broader temperature range. The weight loss of the LRR during pyrolysis starts at the same temperatures with the pretreated peanut shell sample. There is also a significant weight loss at around 350 ^{o}C, which indicates that the LRR sample has cellulose content. Another difference is the sharp curve at the 380 ^{o}C in the untreated peanut shell and pretreated peanut shell samples. LRR does not have this sharp curve, rather is has a smoother curve. This can be an indication of purer content of the LRR from point of view of hemicellulose and cellulose, since the constant weight loss at around 350 ^{o}C could be related to the cellulose and hemicelluloses more than the lignin.

The untreated peanut shell had a total weight loss of 72.0%, the pretreated peanut shell had a total weight loss of %74.3, the LRR had a total weight loss of 63.8%. It can be claimed that the LRR had less content of hemicellulose and cellulose content compared to the untreated peanut shell and pretreated peanut shell. Although lignin continutes to decompose after 400 ^{o}C, the hemicellulose and cellulose contents would have been decomposed after this tempreature. Hence, the LRR had less weight loss since it had less cellulose and hemicellulose, and more lignin content.

3.8 FLOW TESTS

After characterization of the peanut shell lignin obtained via ionic liquid pretreatment and enzymatic hydrolysis, it was tested as a mortar admixture along with cotton stalk lignin and alkali lignin.

Table 3.10 Flow diameters and densities of the control mixture, concrete mixture with alkali lignin, concrete mixture with cotton stalk lignin and concrete mixture with LRR.

Specimen ID	**Flow Diameter (mm)**	**Density (g/cm3)**	**Admixture Percentage %(w/w)**
Control	**150**	**2.19**	**0**
Alkali Lignin	**155**	**2.19**	**0.22**
Cotton Stalk Lignin	**162**	**2.21**	**0.22**
LRR	**159**	**2.21**	**0.22**

It is seen that alkali lignin improved the flow of the concrete mixture by 3.3%, whereas the cotton stalk lignin improved the flow by 8%. LRR had an improvement of the flow by 6%. Although the alkali lignin's particle size is smaller than the cotton stalk lignin and the LRR, it is seen that the effect of the lignins obtained from cotton stalk and peanut shells with ionic liquid pretreatment had more effect on the flow of the concrete. This can be attributed to the differences in the lignin structures originated from the lignin source and process. Nadif stated that the source of the lignin (biomass) and the process upon which lignin is obtained has effects on the lignin's effect on flow of mortars (Nadif et al. 2002). It is clear that the cotton stalk and the LRR lignins were obtained via ionic liquid processes, as the alkali lignin is a laboratory grade product, which is obtained via alkali pulpilng processes.

The lignin admixtures were 0.22 (w/w) % in the concrete. The admixtures were 1 (w/w) % in lignin. Hence, the admixture concentration is really low in the total concrete mass. However, it is seen that the flows are increased by 6% and 8% in LRR and cotton stalk lignin. If the concentrations of the solutions are increased to higher levels, it is possible that the flow values would also incrase. This part of the study has a promising way for deeper research.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this thesis work, a conceptual biorefinery process development was studied. Containing high amount of lignin, an important agricultural and industrial byproduct, peanut shells were pretreated with two ionic liquids, [EMIM][Ac] and [EMIM][Cl] at constant temperature for different pretreatment time periods, hence the impact of pretreatment time period was seen on derivation of multiple products, reducing sugar and lignin-rich solid product. These two products were obtained in a single step ionic liquid pretreatment. In other studies, in order to produce lignin, a second anti solvent addition and precipitation steps were required. In this study, this step was not necessary for lignin production, which brought advantages of less chemicals, less energy, i.e., less cost.

With only one step of ionic liquid ([EMIM][Ac]) pretreatment and enzymatic hydrolysis, reducing sugars and a lignin-rich solid product were produced. Both the pretreatment and enzymatic hydrolysis steps were conducted at mild conditions in contrast to acid and alkali pretreatments. There was no need to corrosion – resistant materials, additive chemicals for neutralization or pressured vessels. Hence, [EMIM][Ac] was shown to be a promising pretreatment agent for a green, economical bioprocess with a multi-product approach.

It was seen that there was material loss in the pretreatment step. Approximately 60 – 70% of the biomass was recovered in the ionic liquid pretreatment step. The material loss was mostly due to cellulose and hemicellulose degradation. The mass loss due to the polymeric material degradation could be decreased by decreasing the pretreatment temperature. However, it should be noticed that decreasing the pretreatment time period could decrease the enzymatic digestibility of the biomass in hydrolysis step. Another cause of material loss was the direct loss during filtering process when the materials are washed. Better filtering systems can be employed in order to decrese the material loss in this step.

The lignin-rich solid product was determined as a porous, cellulose containing material with different characteristics of crystallinity than that of native peanut shell. Furthermore, it was shown to be effective as a concrete admixture by increasing the flow of the concrete by 6%.

The lignin-rich solid product could also be used as a precursor material for lignin-based bioprocesses. Valuable products such as vanillin, anti-oxidants, lignosulfonates can be producted by using the lignin-rich solid product as a raw material. This can also add more value to the pretreatement process. Moreover, Hemicellulases can be employed in order to obtain pentoses along with glucose and other reducing sugars. Pentose – fermenting microorganisms can be employed for further fermentation processes, or hemicellulose itself can be used in production of different products such as biodegradable films.

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APPENDIX A

Reducing Sugar Concentrations

Table A1. Reducing sugar concentrations of the enzymatic hydrolysis of the untreated avicel, untreated peanut shells and 5 min [EMIM][Ac] pretreated peanut shells.

Emzymatic hydrolysis time (hrs.)	**Untreated avicel**	**Untreated peanut shells**	**5 minutes [EMIM][Ac] pretreated peanut shells**
0	0.0 mg/mL	0.0 mg/mL	0.0 mg/mL
1	4.7 ± 0.0 mg/mL	3.1 ± 0.0 mg/mL	8.0 ± 1.4 mg/mL
3	9.3 ± 0 .0mg/mL	3.4 ± 0.0 mg/mL	10.4 ± 1.8 mg/mL
24	20.2 ± 0.0 mg/mL	4.1 ± 0.0 mg/mL	11.2 ± 1.7 mg/mL

Pretreatment was performed at 150 ^{o}C, 1.0 g of peanut shells were combined with 20 g of ionic liquid at 600 rpm for 5 minutes. Enzymatic hydrolysis was performed at 50 ^{o}C with 3 (w/v) % biomass loading; 50 FPU/g biomass Celluclast 1.5L and 60 cellobiose units/g biomass Novozyme 188.

Table A2. Reducing sugar concentrations of the enzymatic hydrolysis of the untreated avicel, untreated peanut shells and 15 min [EMIM][Ac] pretreated peanut shells.

Emzymatic hydrolysis time (hrs.)	**Untreted avicel**	**Untreated peanut shells**	**15 minutes [EMIM][Ac] pretreated peanut shells**
0	0 mg/mL	0 mg/mL	0 mg/mL
1	5.3 ± 0.7 mg/mL	3.1 ± 0.2 mg/mL	11.0 ± 0.1 mg/mL
3	9.2 ± 1.0 mg/mL	3.4 ± 0.4 mg/mL	14.2 ± 0.1 mg/mL
24	19.2 ± 0.7 mg/mL	4.1 ± 0.1 mg/mL	15.8 ± 0.3 mg/mL

Pretreatment was performed at 150 ^{o}C, 1.0 g of peanut shells were combined with 20 g of ionic liquid at 600 rpm for 15 minutes. Enzymatic hydrolysis was performed at 50 ^{o}C with 3 (w/v) % biomass loading; 50 FPU/g biomass Celluclast 1.5L and 60 cellobiose units/g biomass Novozyme 188.

Table A3. Reducing sugar concentrations of the enzymatic hydrolysis of the untreated avicel, untreated peanut shells and 30 min [EMIM][Ac] pretreated peanut shells.

Emzymatic hydrolysis time (hrs.)	**Untreated avicel**	**Untreated peanut shells**	**30 minutes [EMIM][Ac] pretreated peanut shells**
0	0 mg/mL	0 mg/mL	0 mg/mL
1	3.8 ± 0.3 mg/mL	2.7 ± 0.2 mg/mL	7.9 ± 0.5 mg/mL
3	7.7 ± 0.5 mg/mL	3.0 ± 0.1 mg/mL	10.7 ± 2.3 mg/mL
24	17.0 ± 1.2 mg/mL	3.5 ± 0.1 mg/mL	12.5 ± 0.6 mg/mL

Pretreatment was performed at 150 ^{o}C, 1.0 g of peanut shells were combined with 20 g of ionic liquid at 600 rpm for 30 minutes. Enzymatic hydrolysis was performed at 50 ^{o}C with 3 (w/v) % biomass loading; 50 FPU/g biomass Celluclast 1.5L and 60 cellobiose units/g biomass Novozyme 188.

Table A4. Reducing sugar concentrations of the enzymatic hydrolysis of the untreated avicel, untreated peanut shells and 15 min [EMIM][Cl] pretreated peanut shells.

Emzymatic hydrolysis time (hrs.)	**Untreated avicel**	**Untreated peanut shells**	**15 minutes [EMIM][Cl] pretreated peanut shells**
0	0 mg/mL	0 mg/mL	0 mg/mL
1	-	2.8 mg/mL	3.4± 0.2 mg/mL
3	9.1 mg/mL	3.2 mg/mL	4.1 ± 0.1 mg/mL
24	22.7 mg/mL	4.5 mg/mL	5.5 ± 0.4 mg/mL

Pretreatment was performed at 150 ^{o}C, 1.0 g of peanut shells were combined with 20 g of ionic liquid at 600 rpm for 15 minutes. Enzymatic hydrolysis was performed at 50 ^{o}C with 3 (w/v) % biomass loading; 50 FPU/g biomass Celluclast 1.5L and 60 cellobiose units/g biomass Novozyme 188.

Table A5. Reducing sugar concentrations of the enzymatic hydrolysis of the untreated avicel, untreated peanut shells and 30 min [EMIM][Cl] pretreated peanut shells.

Emzymatic hydrolysis time (hrs.)	**Untreated avicel**	**Untreated peanut shells**	**30 minutes [EMIM][Cl] pretreated peanut shells**
0	0 mg/mL	0 mg/mL	0 mg/mL
1	5.1 ± 0.6 mg/mL	2.8 ± 0.4 mg/mL	4.2 ± 0.1 mg/mL
3	9.1 ± 0.6mg/mL	3.6 ± 0.2 mg/mL	4.7 ± 0.3 mg/mL
24	18.1 ± 1.3 mg/mL	3.9 ± 0.1 mg/mL	5.7 ± 0.2 mg/mL

Pretreatment was performed at 150 oC, 1.0 g of peanut shells were combined with 20 g of ionic liquid at 600 rpm for 30 minutes. Enzymatic hydrolysis was performed at 50 oC with 3 (w/v) % biomass loading; 50 FPU/g biomass Celluclast 1.5L and 60 cellobiose units/g biomass Novozyme 188.

Table A6. Reducing sugar concentrations of the enzymatic hydrolysis of the untreated avicel, untreated peanut shells and 60 min [EMIM][Cl] pretreated peanut shells.

Emzymatic hydrolysis time (hrs.)	**Untreated avicel**	**Untreated peanut shells**	**60 minutes [EMIM][Cl] pretreated peanut shells**
0	0 mg/mL	0 mg/mL	0 mg/mL
1	5.1 ± 0.6 mg/mL	2.8 ± 0.4 mg/mL	5.2 ± 0.1 mg/mL
3	9.1 ± 0.6mg/mL	3.6 ± 0.2 mg/mL	6.7 ± 0.3 mg/mL
24	18.1 ± 1.3 mg/mL	3.9 ± 0.1 mg/mL	8.4 ± 0.2 mg/mL

Pretreatment was performed at 150 ^{o}C, 1.0 g of peanut shells were combined with 20 g of ionic liquid at 600 rpm for 60 minutes. Enzymatic hydrolysis was performed at 50 ^{o}C with 3 (w/v) % biomass loading; 50 FPU/g biomass Celluclast 1.5L and 60 cellobiose units/g biomass Novozyme 188.

APPENDIX B

FTIR Band Assignments Lignins:

Table B1. FTIR band assignments for softwood and hardwood lignins (Casas et al., 2012).

Band (cm^{-1})		**Assignment**
Softwood Lignin	**Hardwood Lignin**	
1725	1735	C=O stretching (unconjugated)
1660	1658	C=O stretching (conjugated)
1596	1603	Aromatic skeletal vibration breathing with C=O stretching
1510	1510	Aromatic skeletal vibration
1463	1462	C-H deformations asymmetric
1423	1425	Aromatic skeletal vibrations combined with C-H in plane deformation
1375	1375, 1328	Syringyl unit breathing with C=O stretching and condensed guaiacyl rings
1269	1269	Guaiacyl ring breathing with carbonyl stretching
1221	1220	C-C plus C-O plus C=O stretch
1140	1140	C-H in plane deformation of guaiacyl ring plus secondary alcohols plus C=O stretch ether-O-
-	1116	Aromatic C-H deformation in syringyl ring
1086	1086	C-O deformation in secondary alcohols and aliphatic esters
1032	1033	Aromatic C-H in plane deformation plus C-O deformation in primary alcohols okys C=O stretch (unconjugated)
858	-	C-H out of plane in positions 2, 5 and 6 of guaiacyl rings
-	835	C-H out of plane deformation in positions 2 and 6 of syringyl rings.

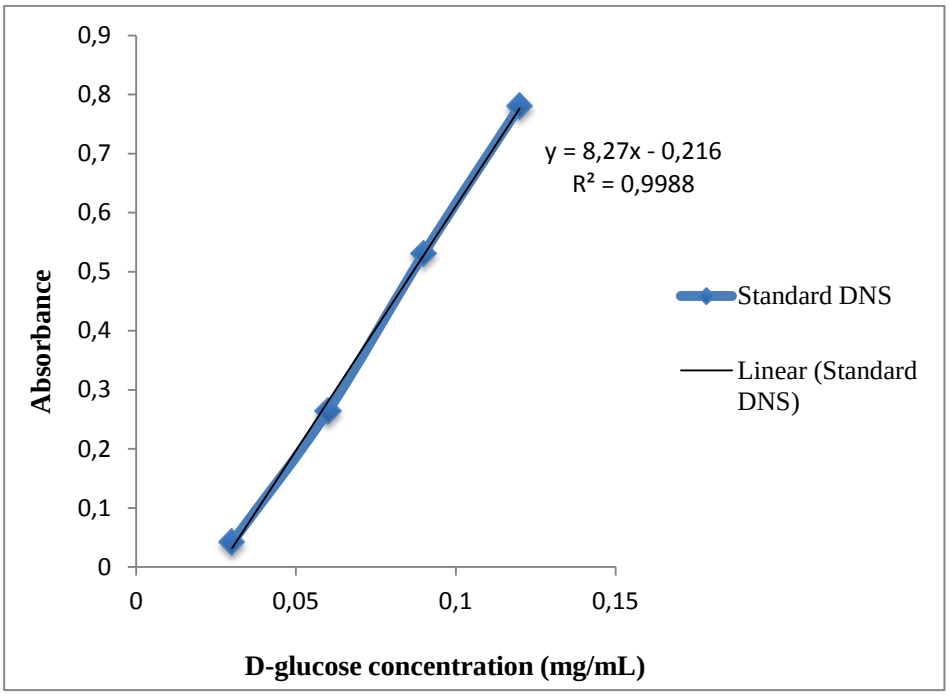


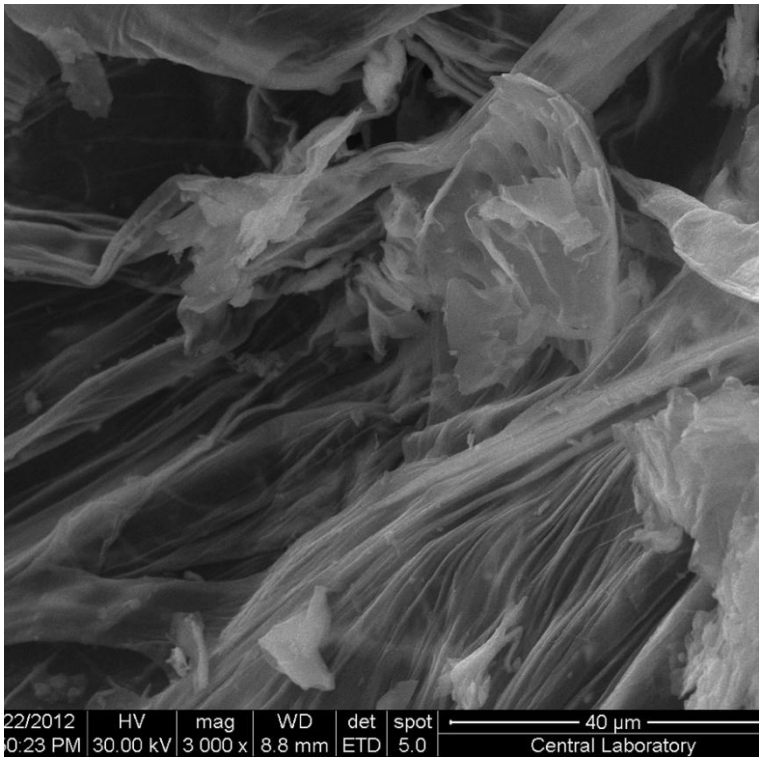
Figure B1. A sample Absorbance vs. D-glucose concentration graph.

When performing Miller's DNS method (1959), standard solutions of D-glucose were prepared and absorbance values of these standard solutions were recorded. Afterwards, Absorbance vs. Concentration graph is plotted and a linear equation is obtained. Finally, absorbance of an unkown – concentration sample from hydrolysis medium is read on the UV – visible spectrometer. The reducing sugar concentration of the sample is calculated by the same equation.

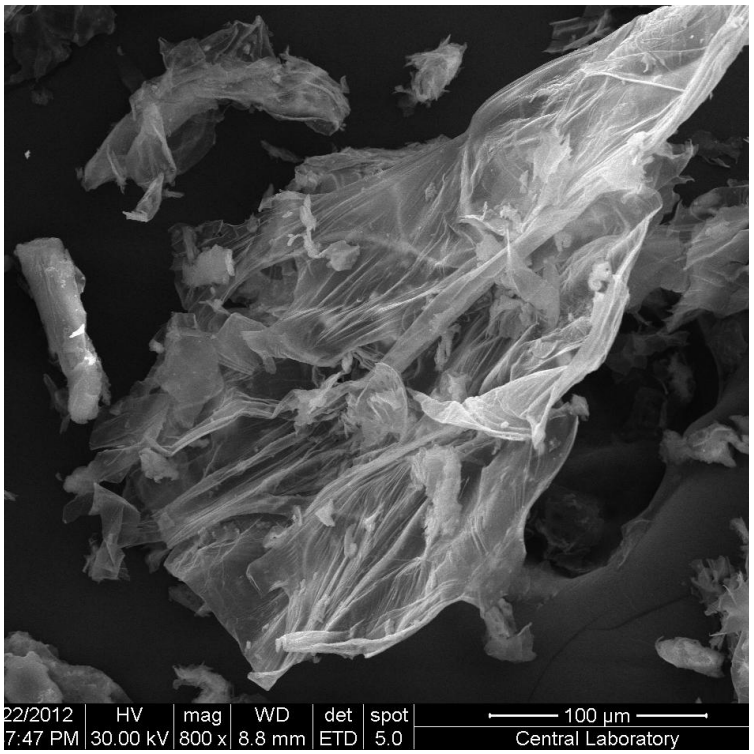
APPENDIX C

SEM MICROGRAPHS

In Figure C1, SEM micrograph of the untreated peanut shell samples is shown with 800 and 3000 times magnification.



a)



b)

Figure C1 SEM micrographs of the [EMIM][Ac] untreated peanut shell samples with 3000 times (a) and 800 times (b) magnification.

In Figure C2, SEM micrographs of [EMIM][Ac] pretreated peanut shell samples with 800 times magnification are shown.

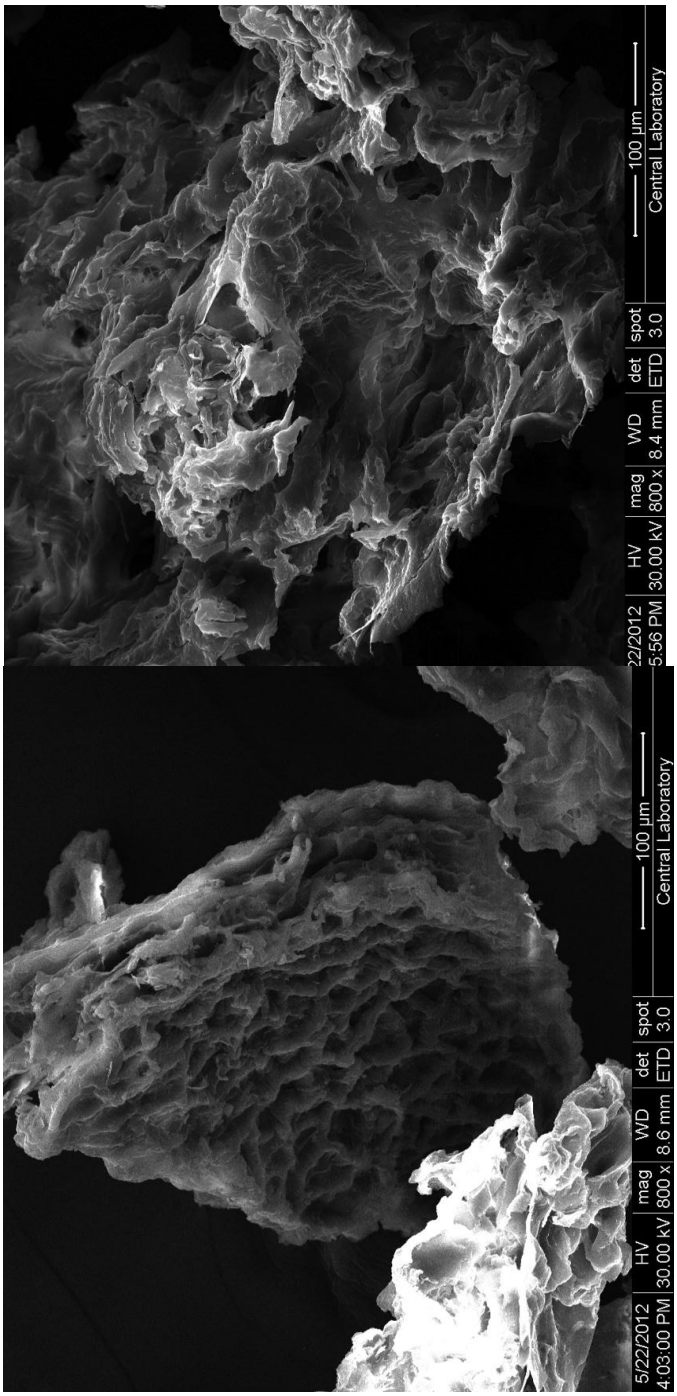


Figure C2 SEM micrographs of [EMIM][Ac] pretreated peanut shell samples with 800 times magnification.

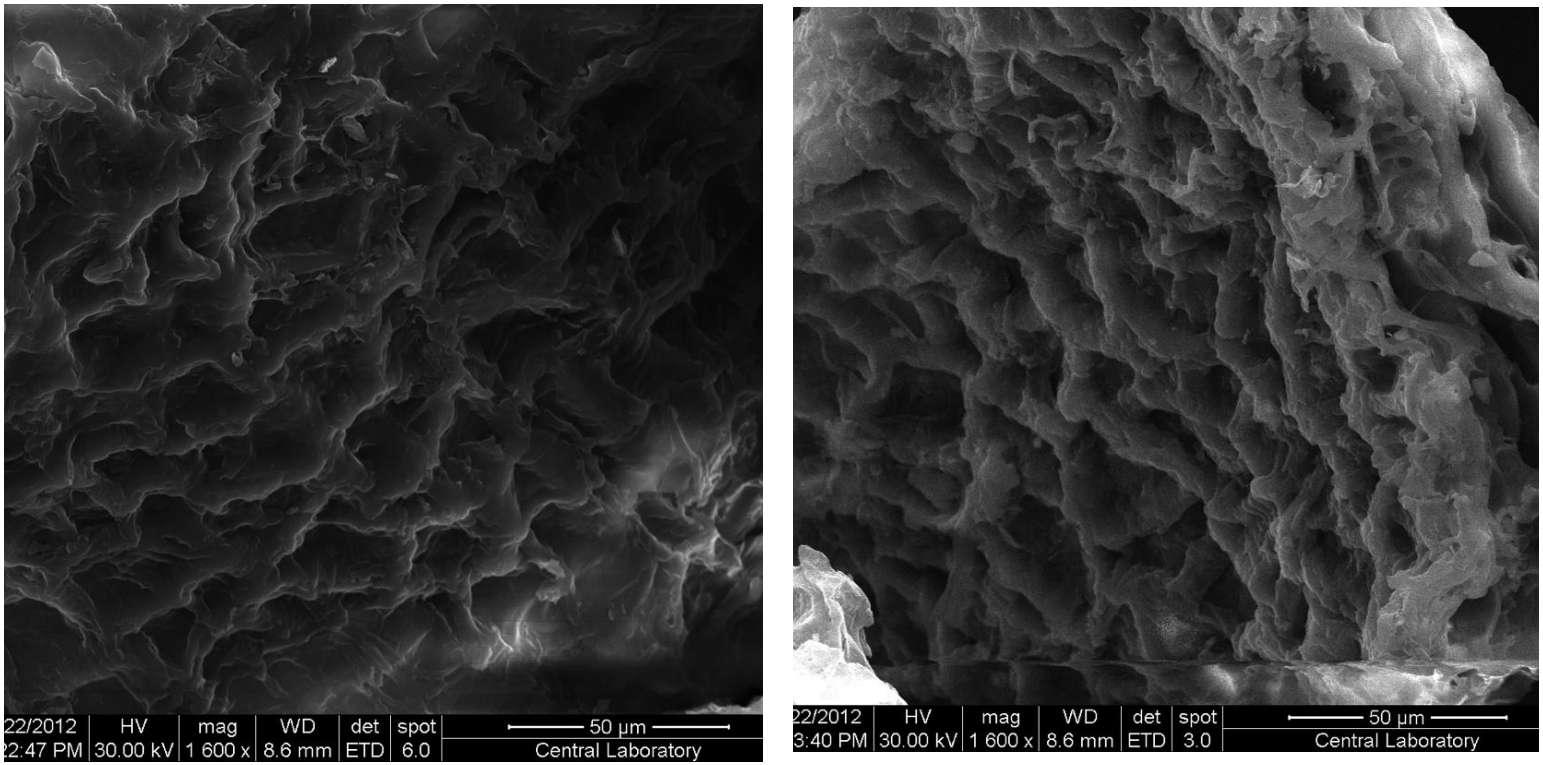


Figure C3 SEM micrographs of [EMIM][Ac] pretreated peanut shell samples with 1600 times magnification.

In Figure C4, SEM micrographs of LRR with 800 times magnification are shown and in Figure C4, SEM micrographs of LRR with 1600 times magnification are shown.

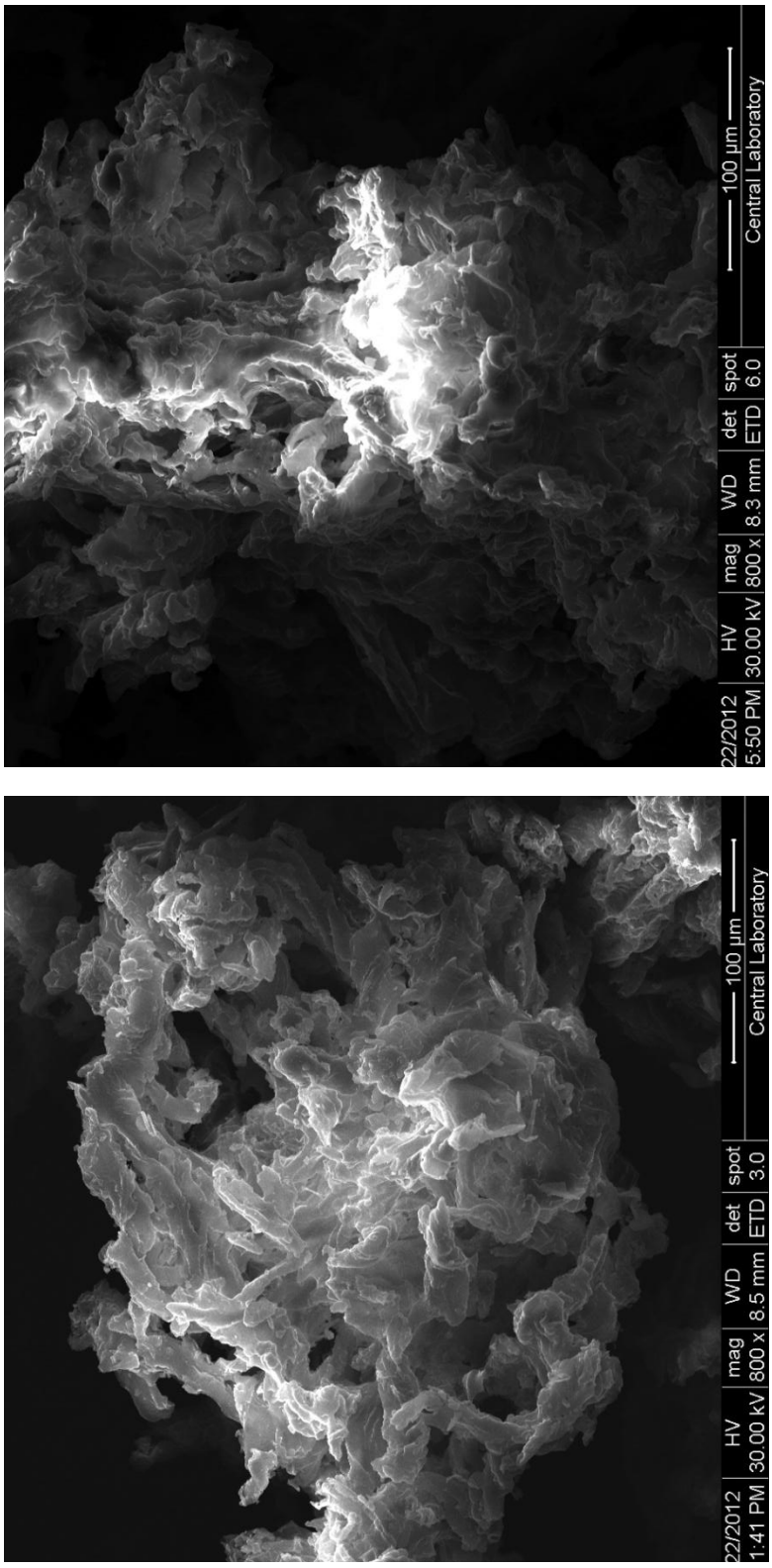


Figure C4 SEM micrographs of LRR sample with 800 times magnification.

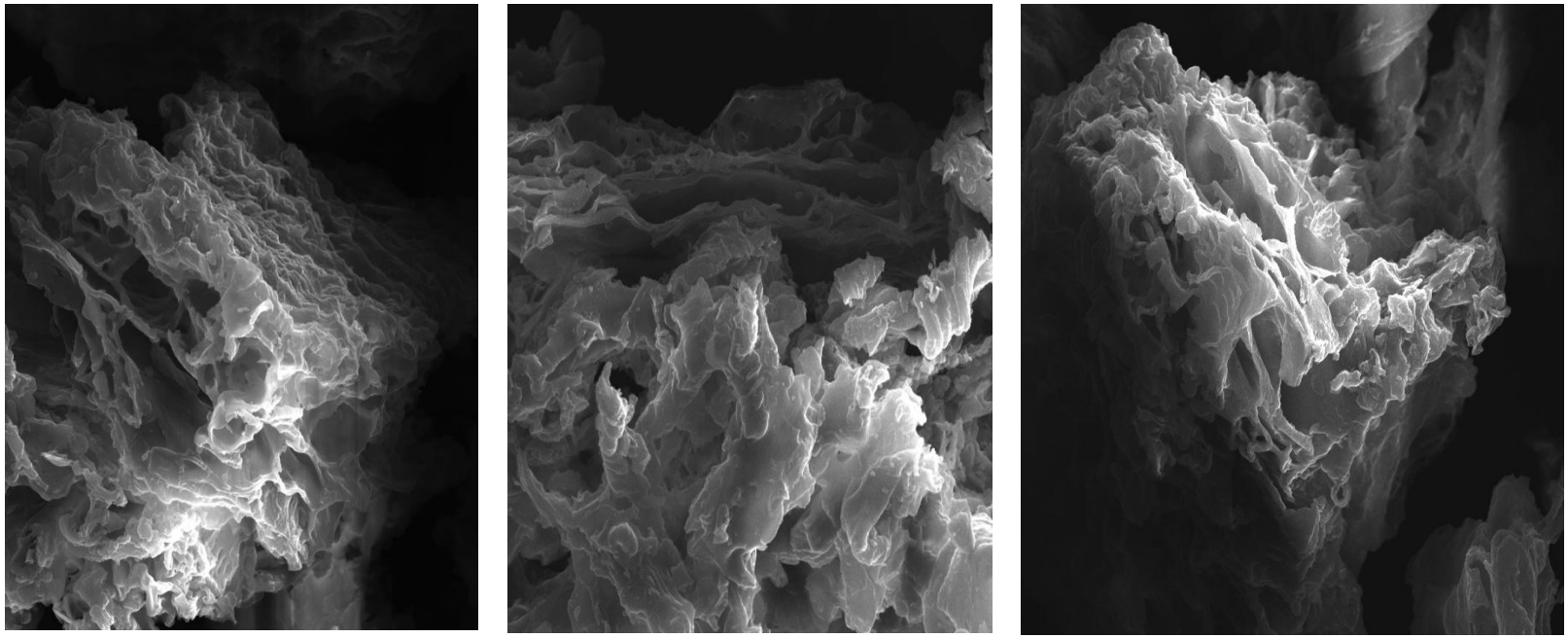


Figure C5 SEM micrographs of LRR samples with 1600 times magnification.

APPENDIX D

XRD Patterns for Cellulose I and Cellulose II:

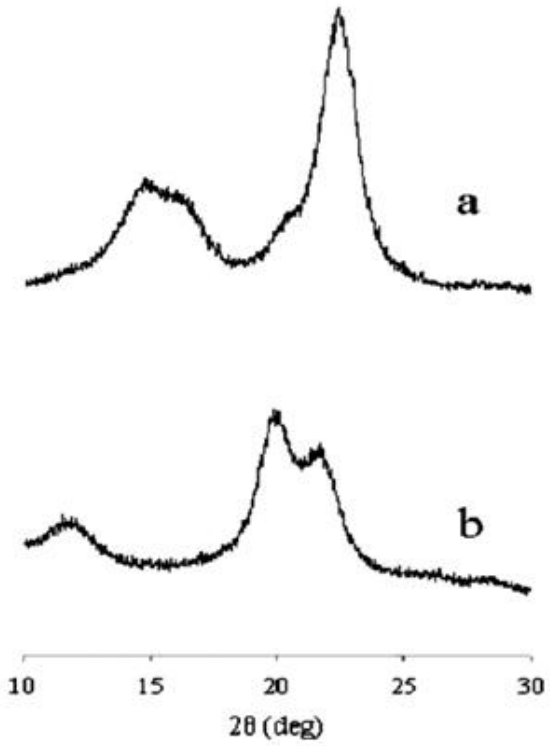


Figure D1 XRD patterns of avicel (a) and mercerized cellulose (b) (Hattori et al., 2012).

Table D1 XRD parameters of cellulose polymorphs (Ford et al., 2010).

	Lattice Plane				
	101	101	021	002	040
	2θ (°)	2θ (°)	2θ (°)	2θ (°)	2θ (°)
Cellulose I	14.7	16.6	20.6	22.5	34.7
Cellulose II	12.3	20.1	-	21.9	34.7