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THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES**

MASTER’S THESIS

**EVALUATION OF THE POTENTIAL OF LOW-COST IONIC LIQUIDS FOR
THE PRETREATMENT OF HARDWOOD, HORNBEAM**

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DEPARTMENT OF ENERGY ENGINEERING

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ÖZET

Yüksek Lisans Tezi

SERT AĞAÇLARDAN GÜRGEN AĞACI ATIKLARININ ÖN İŞLEMİ İÇİN DÜŞÜK MALİYETLİ İYONİK SIVILARIN ARAŞTIRILMASI

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Bu çalışma, parçacık boyutunun (<0.15 mm, $0.15-1.18$ mm, $1.18-2.00$ mm ve >2.00 mm), biyokütle konsantrasyonunun (%10, %20, %30, %40 ve %50), iyonik sıvı geri dönüşümünün ve protik iyonik sıvı tipinin (TEAHSO_4 , HBIMHSO_4 ve HMMorPHSO_4); 150°C ve 3 saatte gerçekleştirilen gürgen ön işlemi ve biyokütlenin enzimatik sindirilebilirliğinin artırılması üzerindeki etkilerini araştırmayı amaçlamaktadır. Protik iyonik sıvılar arasında TEAHSO_4 ve HBIMHSO_4 , biyokütle yapısında ve enzimatik hidrolizdeki değişiklikler açısından gürgen üzerinde en çarpıcı etkiyi göstermiştir. İyonik sıvı ön işlem verimliliğinin lignoselülozik biyokütle parçacık boyutuna bağlı olduğu bulunmuştur. En yüksek selüloz içeriği $0.15-1.18$ mm arasındaki parçacık boyutunda %82.7 olarak elde edilmiştir. TEAHSO_4 ve HBIMHSO_4 ile ön işlem görmüş gürgenden glikoz verimleri sırasıyla %53.9 ve %87.5 olarak bulunmuştur. HBIMHSO_4 , biyokütleden ligninin %91'ini uzaklaştırırken, TEAHSO_4 %84'ünü uzaklaştırmıştır. %20 biyokütle konsantrasyonunda TEAHSO_4 ve HBIMHSO_4 ön işlemleri gerçekleştirilmiş, glikoz verimleri sırasıyla %59.5 ve %98.5 olarak bulunmuştur. TEAHSO_4 ve HBIMHSO_4 geri kazanılıp, tekrar kullanıldığında etkinliklerini korumuştur; protik iyonik sıvılar altıncı kez geri kazanıldığında dahi biyokütle yapısında önemli değişiklikler meydana getirmiştir. Selüloz içeriği yüksek katı malzeme SEM, XRD ve FTIR ile incelenmiş, bulgular protik iyonik sıvı ön işlemine tabi tutulan biyokütle örneklerinin bileşim ve enzimatik erişilebilirliğindeki farklılıkları doğrulamıştır.

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Anahtar kelimeler: Gürgen, protik iyonik sıvı, ön işlem, enzimatik hidroliz, glikoz.

ABSTRACT

Master's Thesis

EVALUATION OF THE POTENTIAL OF LOW-COST IONIC LIQUIDS FOR THE PRETREATMENT OF HARDWOOD, HORNBEAM

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This study aims to investigate the effects of particle size (<0.15 mm, 0.15-1.18 mm, 1.18-2.00 mm and >2.00 mm), biomass loading (10%, 20%, 30%, 40% and 50%), ionic liquid recycling and type of protic ionic liquid (TEAHSO₄, HBIMHSO₄ and HMMorpHSO₄) on hornbeam pretreatment conducted at 150°C for 3 h and the improvements in biomass enzymatic digestibility. Among, TEAHSO₄ and HBIMHSO₄ demonstrated the most striking effects on the hornbeam regarding the changes in biomass structure and enzymatic hydrolysis. Ionic liquid pretreatment was shown dependent on biomass particle size. The highest cellulose content was found as 82.7% for the biomass having particle size, 0.15-1.18 mm. 53.9% and 87.5% glucose yields were obtained from TEAHSO₄ and HBIMHSO₄ pretreated hornbeam, respectively. While HBIMHSO₄ removed 91% lignin from biomass, it was found 84% for TEAHSO₄. Following TEAHSO₄ and HBIMHSO₄ pretreatments at 20% biomass loading, 59.5% and 98.5% glucose yields were obtained respectively. Recycled and reused ionic liquids, TEAHSO₄ and HBIMHSO₄ preserved their performances; significant changes were observed in biomass structure even with ionic liquids recycled for the sixth time. Cellulose enriched solid material was analyzed through SEM, XRD and FTIR characterizations. The findings well verified the changes in biomass composition and enzymatic accessibility.

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Key Words: Hornbeam, protic ionic liquid, pretreatment, enzymatic hydrolysis, glucose.

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SYMBOLS AND ABBREVIATION

A_G	area of the peaks obtained for glucose
Au/Pd	gold/palladium
A_X	area of the peaks obtained for xylose
C_B	initial concentration of the biomass in the hydrolysis buffer that is subjected to enzymatic hydrolysis (g/L)
Cellulose _U	cellulose content of the pretreated biomass (%)
C_G	glucose concentrations (g/L)
CrI	degree of crystallinity (%)
C_X	xylose concentrations (g/L)
DF	dilution factor
L_{PRT}	lignin content of the biomass subjected to pretreatment (%)
L_{UT}	lignin content of untreated biomass (%)
LE	lignin extracted yield (%)
LPY	lignin precipitated yield (%)
ODW_{sample}	initial weight of the dry HB (g)
SR	solid recovery for biomass (%)
UV_{abs}	the absorbance at 205 nm
Volume _{filtrate}	initial volume of 72% (w/w) of sulfuric acid solution (mL)
W_{PRT}	weight of pretreated cotton stalks recovered after pretreatment (g)
W_P	weight of the precipitate obtained after PIL pretreatment (g)
W_{UL}	weight of lignin in the untreated biomass subjected to pretreatment (g)
W_{UT}	weight of untreated cotton stalks subjected to pretreatment (g)
$W_{(AIL+crucible)}$	weight of the with AIL (g)
W_{ash}	weight of ash (g)
$W_{(crucible\ tare)}$	empty crucible weight(g)
ϵ	absorptivity which is equal to 110 L/g.cm
2θ	Bragg angle
π	Dipolarity/polarizability ratio

α	Hydrogen bond acidity
β	Hydrogen bond basicity

Abbreviations

AFEX	Ammonia fiber explosion
AIL	Aprotic Ionic Liquid
AMIMCl	1-allyl-3-methylimidazolium chloride
ARP	Ammonia Recycled Percolation
ASL	Acid soluble lignin
ATR-FTIR	Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy
BHEAA	Bis(2-hydroxyethyl)ammonium acetate
BHEAB	Bis(2-hydroxyethyl)ammonium butyrate
BHEAP	Bis(2-hydroxyethyl)ammonium pentanoate
BHEAPr	Bis(2-hydroxyethyl)ammonium propionate
BHEMmesy	2-hydroxy-N-(2-hydroxyethyl)-N methylethanaminium methanesulfonate
BMIMCl	1-butyl-3-methyl imidazolium chloride
BMPYCl	1-butyl-3methylpyridinium- chloride
BMIMMESO ₄	1-butyl-3-methylimidazolium methylsulfate
C ₂ mimOAc	1-ethyl-3-methyl imidazolium acetate
C ₂ mim(MeO) ₂ PO ₂	N-ethyl-N'-methylimidazolium methylphosphonate
DMEAA	N, N-dimethyl ethanol ammonium acetate
DMEAF	N, N-dimethyl ethanol ammonium formate
DMEAG	N, N-dimethyl ethanol ammonium glycolate
DMEAS	N, N-dimethyl ethanolammonium succinate
DP	degree of polymerization
ECOENG	1,3 dimethyl imidazolium-dimethyl phosphate
EimCl	1-ethylimidazolium chloride
EimHCOO	1-ethylimidazolium formate
EimOAc	1-ethylimidazolium acetate
EMIMCl	1-ethyl-3-methylimidazolium chloride
EMIMAc	1-ethyl-3-methylimidazolium acetate

EMIMXS	1-ethyl-3-methyl-imidazolium xylene sulfonate
EtNH ₃ NO ₃	Ethylammonium nitrate
FAMEs	Methyl or ethyl esters of fatty acids
HB	hornbeam
HBIMHSO ₄	1-butylimidazolium hydrogen sulfate
HMIMHSO ₄	1-methylimidazolium hydrogen sulfate
HMMorpHSO ₄	4-methylmorpholinium hydrogen sulfate
HPLC	High Performance Liquid Chromatography
HPyCl	1-hexylpyridinium chloride
IL	Ionic Liquid
LA	Levulinic acid
NMR	Nuclear Magnetic Resonance Spectroscopy
NREL	National Renewable Energy Laboratories
P	Pine
PHB	Pretreated hornbeam
PIL	Protic Ionic Liquid
PP	Pretreated pine
SEM	Scanning Electron Microscopy
TEAHSO ₄	Triethylammonium hydrogen sulfate
TEAMeSO ₃	Triethylammonium methanesulfonate
UHB	Untreated hornbeam
UP	Untreated pine
UV-vis	Ultraviolet-visible spectroscopy
2-FDCA	Furan dicarboxylic acid
2HEAA	2-hydroxyethylammonium acetate
2HEAB	2-hydroxyethylammonium butyrate
2-HEAF	2-hydroxy ethylammonium formate
2HEAP	2-hydroxyethylammonium pentanoate
2HEAPr	2-hydroxyethylammonium propionate

m2HEAA	N-methyl-2-hydroxyethylammonium acetate
m-2HEAB	N-methyl-2-hydroxyethylammonium butyrate
m-2HEAP	N-methyl-2-hydroxyethylammonium pentanoate
m-2HEAPr	N-methyl-2-hydroxyethylammonium propionate
5-HMF	Hydroxymethylfurfural



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1. INTRODUCTION

Ionic liquids (ILs) can be utilized for a variety of applications including as media in batteries and supercapacitors, electrolytes in solar conversion technologies and biorefining of lignocellulosic biomass (Wishart, 2009). ILs have the capability to separate lignocellulosic biomass into its major fractions, cellulose, hemicellulose, and lignin and convert the building blocks of each constituent into valuable fuels, chemicals and materials, which is in agreement with the main characteristics of biorefineries.

Cellulose dissolved in ILs can be converted into regenerated cellulose and then into value-added products such as ethanol. On the other hand, lignin dissolved in ILs in the absence or presence of catalysts using heat can be converted into aromatic products. ILs have been broadly studied in literature and the studies demonstrated that different ILs are better suited for interaction with certain components of the lignocellulosic biomass.

Much work has been carried out on the potential of dialkyl imidazolium acetate ILs, however, there are still some critical issues to resolve, such as thermal stability of these ILs and their cost. “The Ionosolv process” has been proposed to solve these issues (Hallett et al, 2013) in which protic ionic liquids (PILs), as cost-effective alternatives to aprotic ionic liquids (AILs), are explored to process lignocellulosic feedstocks.

To date, PILs were found promising specifically towards lignin extraction during biomass pretreatment (Achinivu, 2018). PILs have the capability to rupture β -O-4 linkages effectively during the depolymerization of lignin (Cox and Ekerdt, 2012).

In recent years, there has been growing interest in the evaluation of PILs’ performances with respect to the structural variations in various lignocellulosic feedstocks. Accordingly, PILs have been studied to interact with potential sources of lignocellulosic biomass such as sugarcane bagasse (agricultural wastes) (Rocha et al., 2017), corn stover (agricultural wastes) (Achinivu et al., 2014), cotton stalks (agricultural wastes) (Semerci and Güler, 2018), switchgrass (energy crops) (Singh et al., 2017), willow (forest residues) (Weigand et al., 2017), pine (forest residues) (Semerci et al., 2019) and HB (forest residues) (Semerci et al., 2019).

While PILs have pretreated lignocellulosic biomass at different particle sizes and loadings, and even PILs' recycling has been previously shown as a sole parameter, PIL pretreatment of a lignocellulosic feedstock has not been fully evaluated with respect to these parameters. For this reason, HB, which is a typical hardwood in Turkey and has been rarely valorized in the biorefinery context, was particularly chosen for this thesis. As the possibility of PILs' use at higher biomass loadings and different particle sizes and their reuse have been demonstrated regarding the changes in the physical and chemical structure of HB and as well as, the improvements in the enzymatic conversion of biomass, a significant contribution was made to the literature.



2. LITERATURE SURVEY

2.1 The Significance of Lignocellulosic Biomass

Lignocellulosic biomass is an abundant, renewable, inexpensive and environmental friendly material. Biorefinery, which is an important component of renewable energy generation for the production of value-added fuels and chemicals from biomass, has gone through improvements in genetics, biotechnology, process chemistry, and engineering (Ragauskas et al., 2006).

Lignocellulosic biomass is highly functionalized and is the main fraction (~ 90%) of plant biomass (Makhubela et al., 2017). Lignocellulosic feedstocks mainly contain: (a) agricultural wastes, such as rice straw, rice husk, wheat straw, corn stover, bagasse, etc.; (b) forest residues which involve hardwoods like HB, oak, etc., and softwood like P, redwood, etc. and other by-products such as wood chips (Badgujar et.al., 2018) and sawdust and (c) energy crops, such as switchgrass, *Miscanthus giganteus*, grass etc. (Pandey et. al., 2019).

Lignocellulosic biomass predominantly consists a combination of carbohydrate polymers (cellulose and hemicellulose), lignin, extractives and ash. The amounts of carbohydrate polymers and lignin depend on the type of biomass. Many of the researchers have presented the compositions of lignocellulose from different hardwoods, softwoods, and agricultural residues in the publications. The percentages of these major components were found to change from one type to another as shown in Table 2.1.

Table 2.1 Composition of various sources of lignocellulosic biomass

Lignocellulosic biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
<i>Agricultural wastes</i>				
Wheat straw	43.4	26.9	22.2	Shah and Ulah, 2019
Rice straw	38	32	12	Huang et al.,2016 Hassan et al.,2018
<i>Forest residues</i>				
Hardwood stems	40-55	24-40	18-25	Sun and Cheng, 2002
Softwood stems	45-50	25-35	25-35	Sun and Cheng, 2002
<i>Energy crops</i>				
Grasses	25-40	35-50	10-30	Sun and Cheng, 2002

2.2 Lignocellulosic Biorefinery Concept

The biorefinery concept is similar to petroleum refineries but instead of oil, biorefineries use biomass as the feedstock. Biorefineries include the processes and technologies required for the conversion of renewable resources into fuels, polymers, and other chemicals (Figure 2.1). Currently in a biorefinery, a product mixture including biochemicals, biomaterials and biofuels as well as heat and electricity generation can be obtained. Biorefineries focuses on the major challenges of different industrial sectors, including agriculture, food and chemicals, related value chains and products (Vertes et al., 2014).

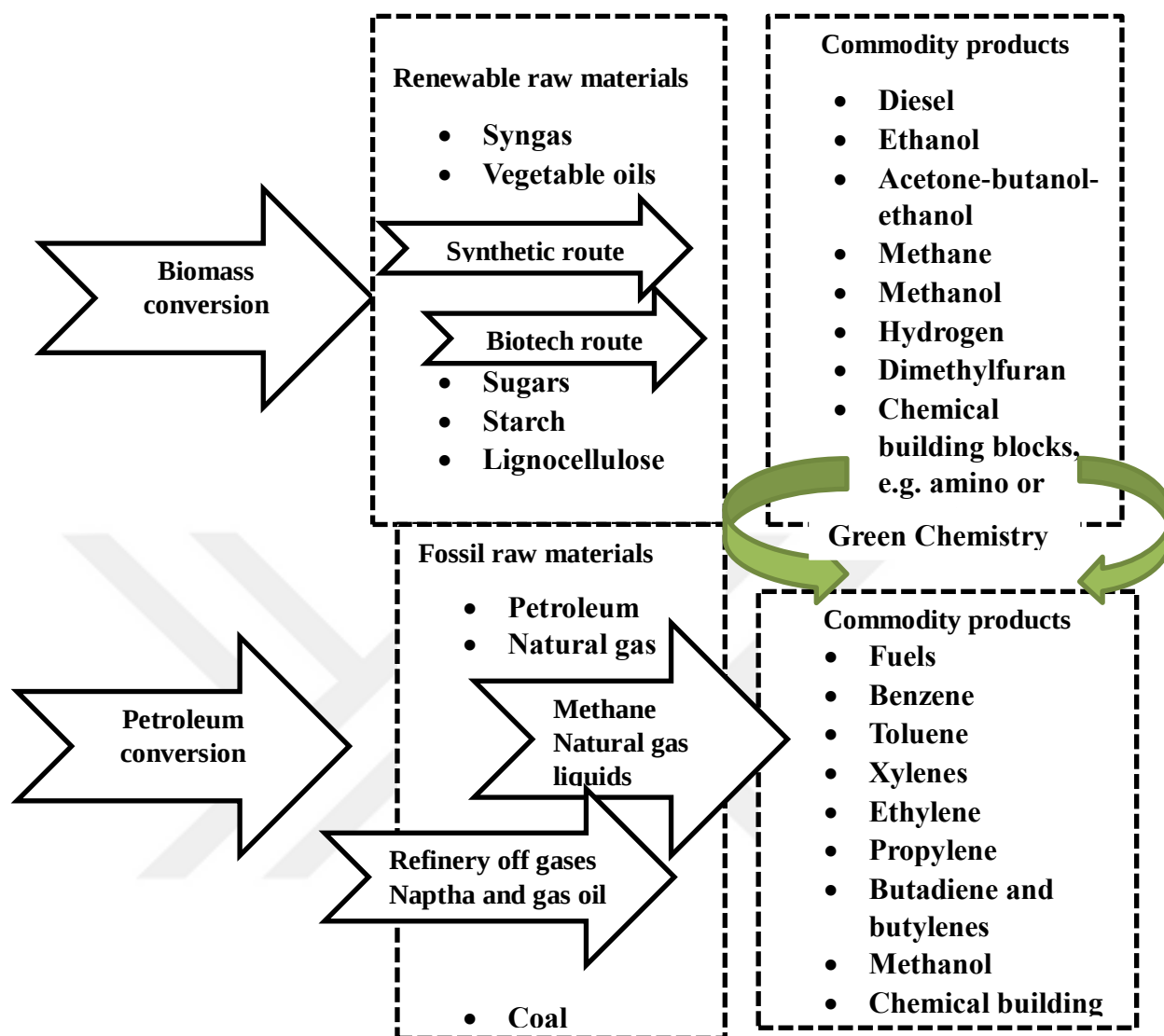


Figure 2.1 Comparison of the the petroleum refinery and the biorefinery

Biorefineries are expected to become energy and cost-competitive with petroleum refineries, when lignocellulosic biomass, which does not compete with food resources, can be utilized in a manner that makes the most value of its three different constituents: cellulose, hemicellulose, and lignin (Gurbuz and Semerci, 2016). In order to convert lignocellulosic biomass into valuable products, two biorefinery approaches have been exploited. The first approach include processes like gasification and pyrolysis offering simplicity of operation and lower operating costs. Syngas, a mixture of H_2 , CO , CO_2 and CH_4 in major, is produced by the process of gasification taking place at elevated temperatures and reduced oxygen concentrations. On the other hand, pyrolysis

conducted at a lower temperature range in the absence of oxygen leads to the conversion of biomass into pyrolysis oil, charcoal and a gaseous mixture with a composition similar to syngas (Cherubini, 2010). Other approach offers flexibility and allows each strategy to be tailored to the different chemical and physical properties of the fraction, making it particularly valuable for the production of different chemicals. The flexibility provided also requires a combination of biochemical and chemical approaches. Fermentation and anaerobic digestion are typical biochemical processes. While fermentation uses microorganisms and/or enzymes to convert a fermentable substrate into ethanol and organic acids, the latter provides the bacterial degradation of organic material into biogas (mixture of CH_4 and CO_2 in major) under oxygen deficient conditions around between 30 to 65 °C. Hydrolysis and transesterification are the most common chemical processes. Hydrolysis basically employ chemical agents such as mineral acids or alkalis or enzymes for the transformation of carbohydrates into glucose and subsequently into hexose dehydration products, furans such as hydroxymethylfurfural (5-HMF) and furan dicarboxylic acid (2-FDCA) and levulinic acid (LA). Transesterification, a chemical process by which vegetable oils can be converted to methyl or ethyl esters of fatty acids (FAMEs), leads to the production of biodiesel (Cherubini, 2010).

Catalytic routes used in the biorefineries have a vital role in the development of processes for efficient biofuel production. Figure 2.2 illustrates the major catalytic routes and corresponding products from lignocellulosic biomass (Gurbuz and Semerci, 2016).

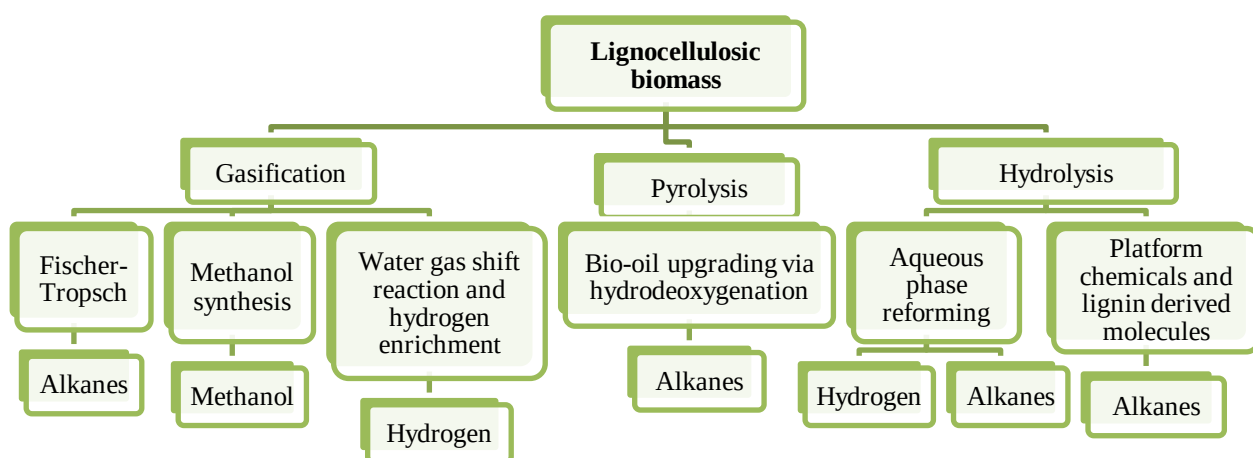


Figure 2.2 Major catalytic routes for the conversion of lignocellulosic biomass to biofuels (Gurbuz and Semerci, 2016)

2.3 The Lignocellulosic Feedstock Used

HB includes roughly 35% cellulose, 17% hemicellulose and 26% lignin (Semerci et. al., 2019).

HB (*Carpinus betulus*) is widespread in Europe and East Asia (Woodworkersinstitute, 2020). HB, which is composed of trunk, fruits and sprouts and leaves, is shown in Figure 2.3. Also spread in Turkey, it is generally found in the mixed forests of the Northern and Southern coastal regions. It possesses the longest fiber among the species used for producing tools, lathe-work as well as, fuel wood applications in Turkey. (OGM, 2020). HB has been previously used in the bioenergy field for the production of pyrolysis oil (Morali and Sensöz, 2015).

HB has been explored in terms of its pretreatment through a few traditional techniques such as steam explosion (Barbanera et al., 2018), AIL (Dotsenko et al., 2018), PIL (Semerci et al., 2019) pretreatments. Pretreatment of HB with PILs has limited number of studies in literature.

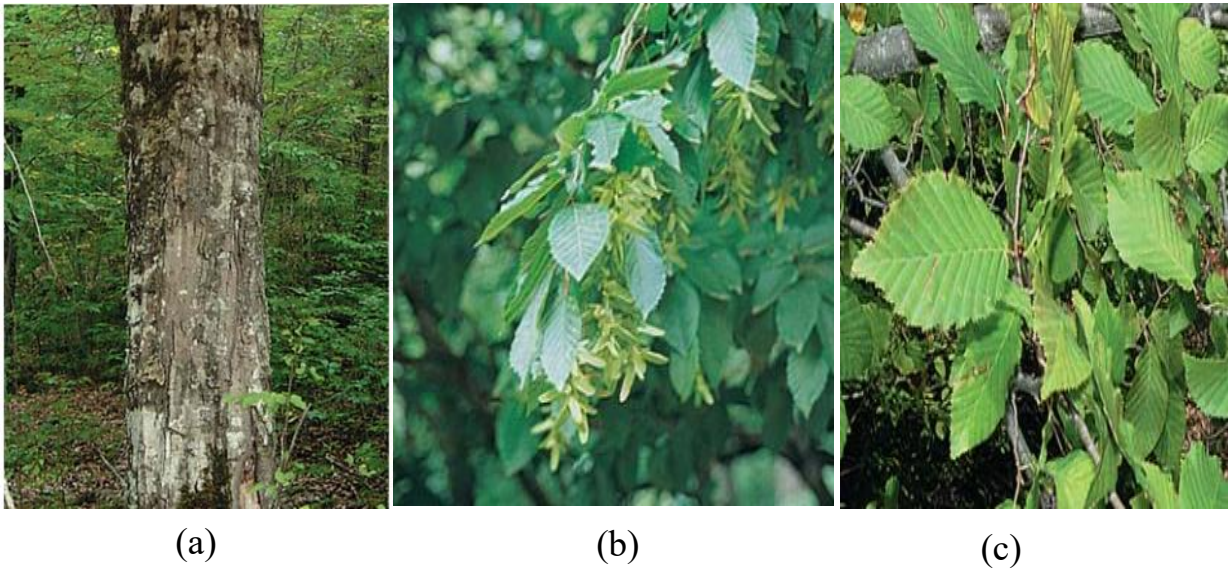


Figure 2.3 HB plant; (a) trunk, (b) fruits and sprouts, (c) leaves (OGM, 2020)

2.3.1 Cellulose

Cellulose is the most abundant organic polymer on earth. It is the major component of lignocellulosic biomass, which consists straight chains of D-glucose molecules linked through β -(1 $\rightarrow$ 4) glycosidic bonds with the formula $(C_6H_{10}O_5)_n$, where n is the degree of polymerization, ranging from thousands to tens of thousands (Figure 2.4) (Klemm et al., 2005) . Polymers of cellulose are interlinked through hydrogen, and Van der Waals bonds to form a microfibril, and presented in crystalline and amorphous form (Figure 2.5).

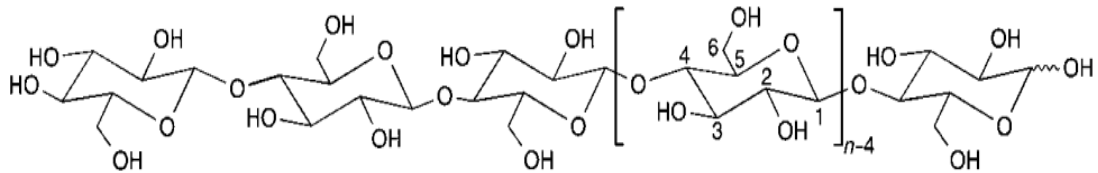


Figure 2.4 Molecular structure of cellulose (n = DP, degree of polymerization) (Klemm et al., 2005)

The major part of cellulose (around two-thirds of the total cellulose) is in the crystalline form. The intermolecular hydrogen-bonding and Van der Waals interactions between cellulose molecular chains, enable close and regular assembly of cellulose strings and give rise to the polymer's insolubility in water and in most solvents (Figure 2.6) (Hallett et al., 2013). Previously, the enzyme, cellulase was found to hydrolyze amorphous portions of cellulose to a greater extent compared to the crystalline fractions of the polymer. This indicated the need of decreasing the crystallinity of cellulose prior to enzymatic hydrolysis to attain higher sugar yields (Taherzadeh and Karimi, 2008). Because of its negative impact on the enzymatic reachability to cellulose, the cellulose crystallinity has been quantified by a variety of characterization methods, particularly via X-ray diffraction. (XRD) (Park et al., 2010).

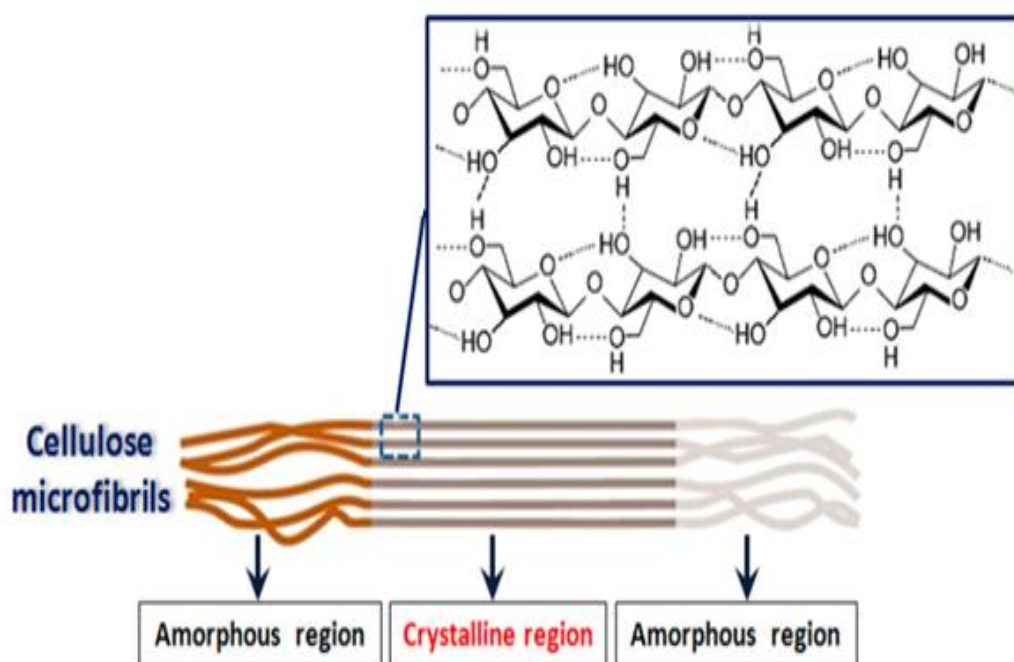


Figure 2.5 Interaction between cellulose molecular chains within the crystalline region of cellulose microfibrils (Zhou and Wu, 2012)

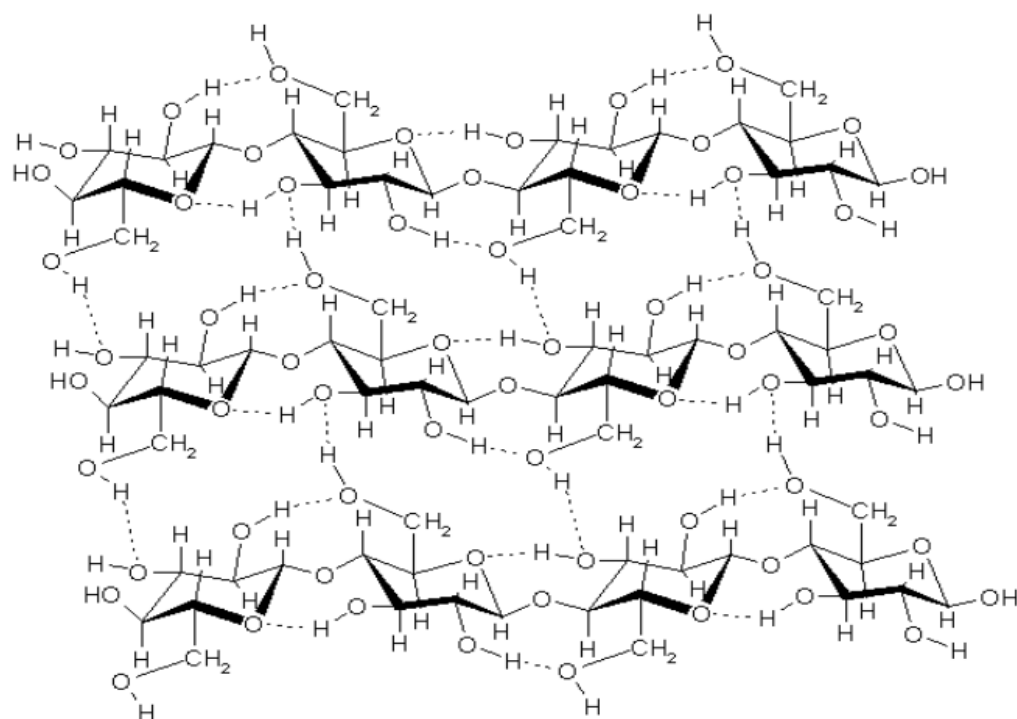


Figure 2.6 The hydrogen bonding in cellulose, depicted with the dashed lines (DoITPoMS, TLP Library, 2020)

2.3.2 Hemicellulose

Hemicellulose is similar to cellulose in regards to the presence of sugar-based polymer but it possesses a more complex and heterogeneous polysaccharide consisting units of five carbon monosaccharides (D-xylose and D-arabinose) and other six-carbon monosaccharides (D-mannose, D-galactose and D-glucose) (Figure 2.7).

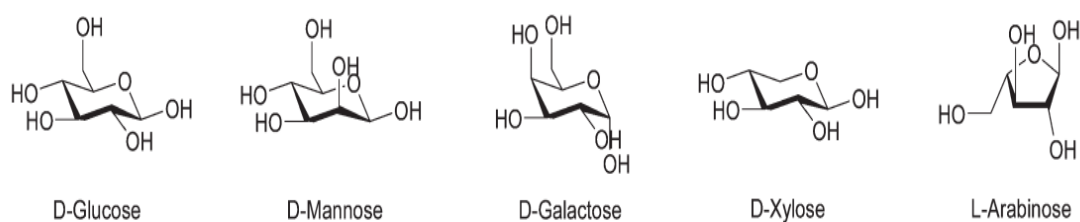


Figure 2.7 The hexoses and pentoses typically found in hemicellulose (Hallett et al., 2013)

Unlike cellulose, hemicellulose has a branched-chain structure which is amorphous and the degree of polymerization is only 50-200 (Guo et al., 2019). Therefore, hemicellulose is much easily decomposed compared to cellulose.

Hemicellulose is considered to join non-covalently to the surface of cellulose fibrils. It acts as an irregular form of material that keeps rigid cellulose fibrils in place. It has been proposed that the replacement with hydrophobic groups like acetyl and methyl groups improves the connection of hemicellulose to lignin, in this way, supports the cohesion between the three main lignocellulosic polymers (Hallett et al., 2013). The structure of hemicellulose relies on whether the species is a hardwood or softwood. As it is seen in Figure 2.8, the major hemicelluloses in softwoods are galactoglucomannans and arabinoglucuronoxylan, while in hardwood; the predominant hemicelluloses are glucuronoxylan (Pu et al., 2010).

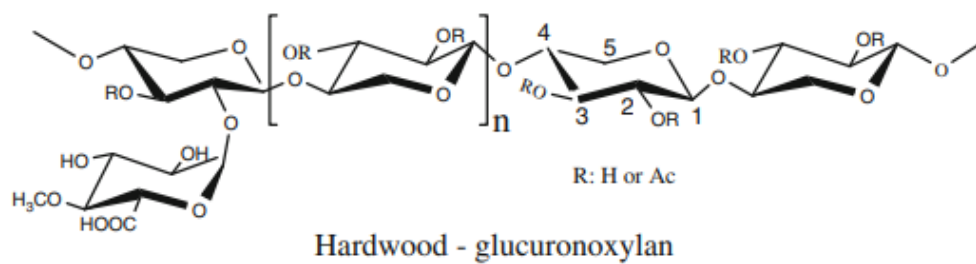
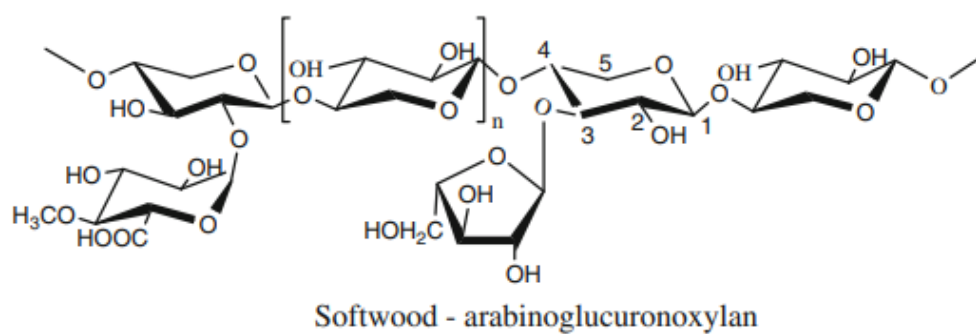
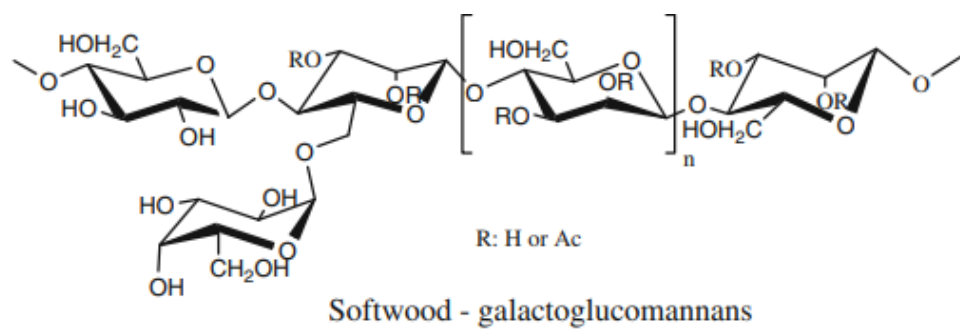


Figure 2.8 Hardwood and softwood hemicellulose structures (Pu et al., 2010)

2.3.3 Lignin

Of the three major components of lignocellulosic biomass, lignin is chemically different from the other macromolecular polymers. It contributes to hydrophobicity, resistivity to biological and physical attempts, and strengthening of structure. (Hallett et al., 2013). It is a compound comprising a three-dimensional combination of phenylpropanes and their derivatives. There are phenylpropane units referred to as monolignols: coniferyl, sinapyl, and *p*-coumaryl alcohol (Pu et al., 2010) (Figure 2.9). These subunits are identified by their aromatic ring structure.

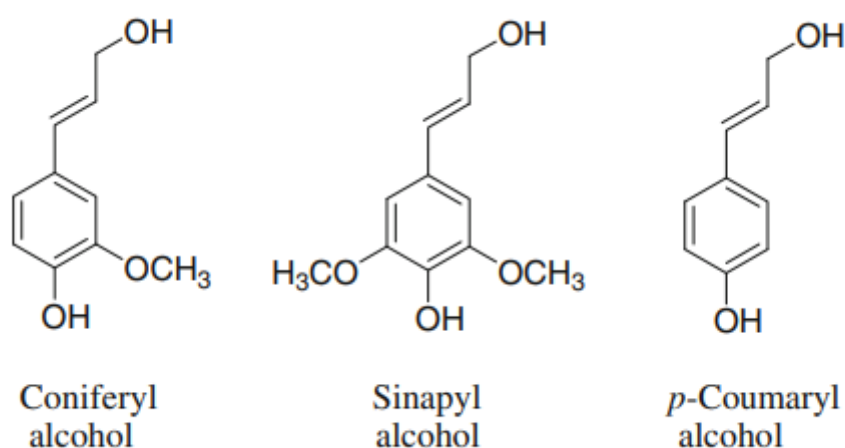


Figure 2.9 Three phenylpropane precursors of lignin

The lignin polymer includes a broad variety of linkages among β -O-4 ether bonds are the most common ones. This bond is present in almost 50% of all inter-subunit bonds. β -O-4 ether bonds give rise to the linear stretching of the polymer. Other linkages such as C-O and C-C linkages are lesser and branching happens when lignification progressed. The variety of linkages of the lignin polymer is illustrated in Figure 2.10 (Hallett et al., 2013). Table 2.2 illustrates the abundance of major linkages found in hardwood lignin. The amount and composition of lignin varies based on the type and origin the plant (conifer wood, deciduous wood or grass) (Szalay et al., 2020) (Figure 2.11).

Table 2.2 Abundance of major linkages in hardwood lignins (Ragauskas and Yoo, 2018)

Linkage type	Approximate percentage
β -O-4	60-62
β -5	3-11
5-5	3-9
4-O-5	7-9
β -1	1-7
β - β	3-12
Dibenzodioxocin	0-2

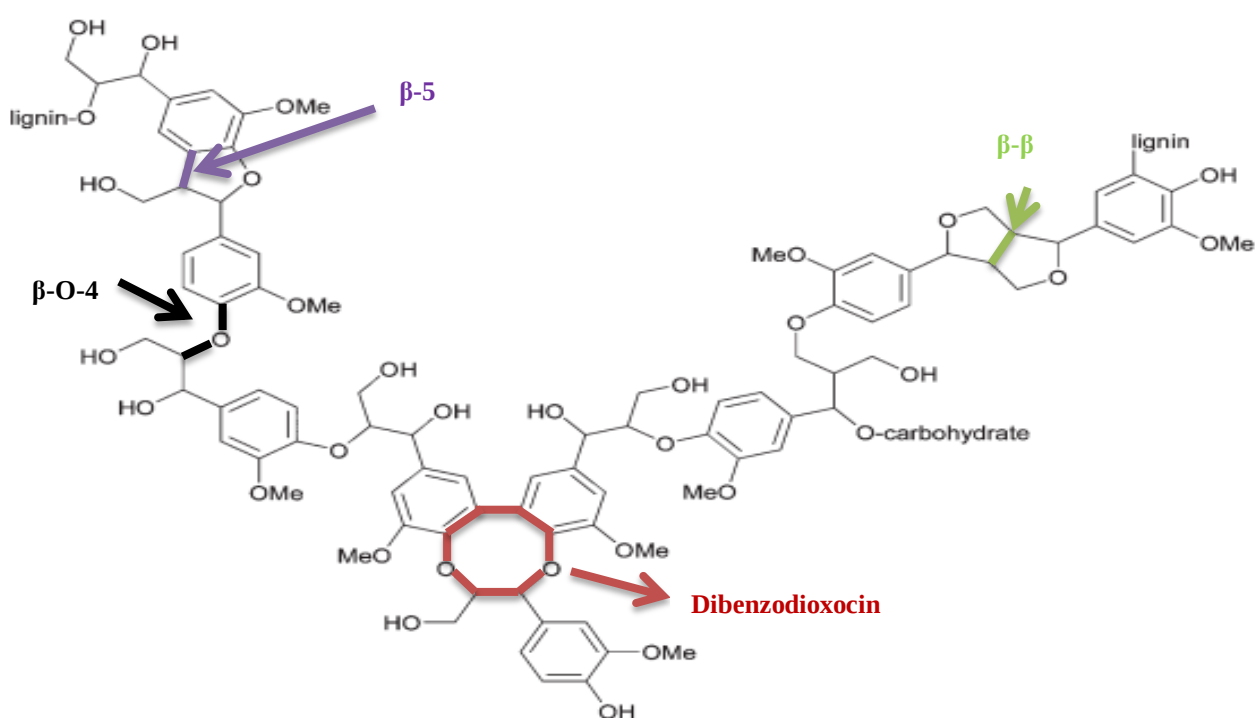


Figure 2.10 Depicted generally of native lignin with different linkages such as C-O and C-C (Hallett et al., 2013)

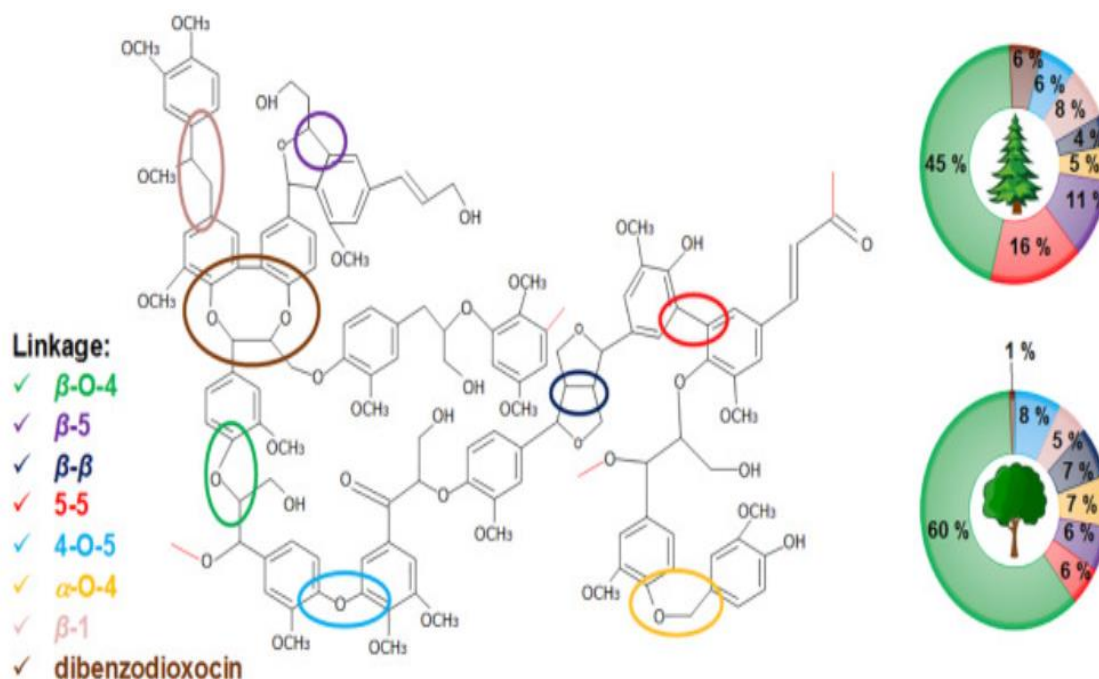


Figure 2.11 A fragment of Freudenberg's model of the structure of lignin, with schematic differentiation of the content of particular bond types in the biopolymer structure (Szalaty et al., 2020)

Lignin provides structural rigidity along with cellulose and hemicellulose and prevents swelling of lignocelluloses. Lignin is also responsible for the recalcitrant structure of lignocellulosic biomass as a barrier to enzymatic degradation due to the restriction of cellulase adsorption on cellulose, and thus the delignification has great potential to enhance the rate and extent of cellulose to glucose conversion. Nevertheless, hemicellulose also gets hydrolyzed and leaves the structure during delignification (Wyman, 1996). To separate lignin, researchers have put an enormous effort to develop an effective depolymerization process for lignin valorization. Lignin removal is feasible with diverse pretreatment conditions, especially with the inclusion of alkaline agents. In addition to alkaline reagents, chemical pretreatments which include ammonia recycle percolation (ARP), organosolv process, lime, and IL pretreatments, were referred to as leading technologies for lignin removal (Doherty et al., 2011). The Kraft or sulfate process is the basic conventional method that generates massive amounts of lignin as a by-product (Smook, 2002). This process has been employed for papermaking and other relevant products. Sodium sulfide and sodium hydroxide are employed under strong

alkaline conditions in order to break the ether bonds within lignin. The delignification process comprises three stages. The initial stage occurs roughly at 150 °C and is controlled by dispersion and then temperature is elevated to around 170 °C during the second stage. While the final stage happens at even elevated temperatures results in the deriving of black liquor that chiefly comprised decomposed lignin fragments, by decreasing the pH to between 5 and 7.5 with acidic agents (usually, sulfuric acid) or CO₂ (Koljonen et al., 2004). The Ammonia Recycled Percolation (ARP) is pretreatment method that raises the enzymatic digestibility and also achieves high degree of delignification. Ammonia is an efficient delignification reagent in this process. The primary factors influencing the reactions occurring in the ARP are reaction time, temperature, ammonia concentrations, and the amount of liquid throughput. ARP of corn cobs/stover mixture gave the best results when aqueous ammonia (10% concentration) at 1 mL/min was used at 170 °C for 15–60 min. 74–80% lignin removal, and higher glucan conversions (even with respect to the hydrolysis of α -cellulose) were achieved (Iyer et al., 1996). Since then, researchers have tried various conditions for ARP process such as prolonged reactions, high liquid throughput rates, two steps pretreatment, etc. (Kim et al., 2006).

As shown in Table 2.2, β -O-4 ether bonds being the most dominant linkage in lignin structure can also be broken with the PIL-biomass interactions. Solubilization of lignin and other components of the lignocellulosic structure other than cellulose as the core mechanism of PILs is dependent on the complex of bonds formed within the structure (Rashid et al., 2016; Achinivu, 2018). The action of PILs on lignin depolymerization stimulating the breakage of β -O-4 linkages under mild conditions (110-150 °C) has been previously shown (Cox and Ekerdt, 2012).

Also, lignin is acceptable as a bioresource which can be used for the production of a wide range of products relying on its alterations and depolymerizations while its removal from the biomass. (Sannigrahi et al., 2010). These products can be attained from lignin which are adhesives, adsorbers, dispersants, carbon fibers additionally solvents like BTX chemicals benzene, toluene, and xylene (Zhang, 2008). Vanillin (3-methoxy-4-hydroxybenzaldehyde) (Figure 2.12) which is used in the food, beverage, and cosmetic industry can also be produced from natural lignin present in the wood

(Borregaard, vanillin, 2020). Currently, Borregaard, Norway is the only company, is the world's largest producer of bio-based vanillin and the only producer of vanillin from wood harvested from sustainable forests. Borregaard has been carrying on this process for over 50 years (Borregaard, vanillin, 2020). EuroVanillin Supreme is the closest to natural product with minimum CO₂ footprint in vanillin (Smart Vanillin, EuroVanillin).

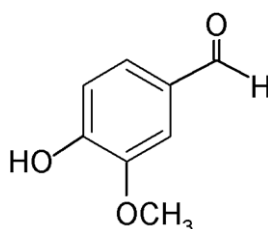


Figure 2.12 Chemical structure of vanillin

2.4 Pretreatment of Lignocellulosic Biomass

2.4.1 Why is there a need for pretreatment?

Pretreatment, which is the name given to a series of processes that include chemicals, microorganisms, heat, and pressure and lignocellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars. Pretreatment operations aim to alter or deconstruct the lignocellulosic structure to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicellulose. On the other hand, pretreatment processes are to rupture the lignin seal and disrupt the crystalline structure of cellulose (Mosier et al., 2005).

After pretreatment, the lignin seal is ruptured and enzymes can reach cellulose and hemicellulose part of biomass as shown in Figure 2.13.

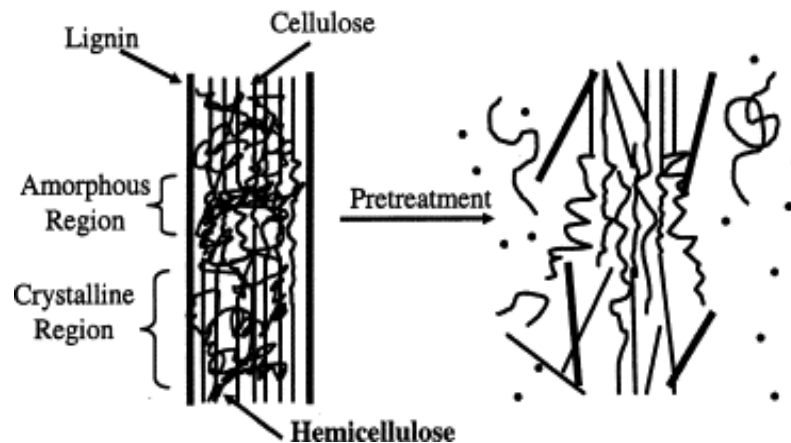


Figure 2.13 Schematic representation of biomass pretreatment (Mosier et al., 2005)

A traditional and widely studied way is to change lignocellulosic biomass into value-added products, especially ethanol, and this studied way includes the pretreatment of lignocellulosic biomass and subsequently enzymatic hydrolysis of pretreated material to attain sugars, especially glucose. A certain pretreatment process can be perused with the changes important in enzymatic hydrolysis happening in the lignocellulosic structure. It is desirable to increase the surface areas within reach of enzymes, decreasing cellulose crystallinity, extracting hemicellulose and lignin, and modification of the natural lignin structure at the end of a certain pretreatment type; because these increase the performance of enzymes using to rupture cellulose in lignocellulosic biomass (Mosier et al., 2005). In addition to the glucose production via enzymatic hydrolysis, another significant aspect for pretreatment is its impact on the fermentation step applied through microorganisms for the products such as ethanol or other fermentative products (Hendriks and Zeeman, 2009).

Pretreatment methods affect the cost of the functional steps that are enzyme loading, enzymatic hydrolysis rates, toxicity, and lignin extraction. Due to the effect on the fermentative production process, pretreatment chemistry is important. Efficient pretreatment methods that have been identified as substantial parameters such as the low energy demand, sugar improvement yield, and the reduction of particle size (Yang and Wyman, 2008).

Below are the detailed pretreatment key points that provide cost reduction and progress in pretreatment technologies (Alvira et al., 2010);

1. High yield for multiple products, site ages, harvest times; have shown that various pretreatment methods are more suitable for certain feedstocks. For example, alkaline pretreatment has been effective towards herbaceous plants and agricultural residues reducing the lignin content of the lignocellulosic biomass. (Silverstein et al., 2007). Acid base pretreatment, dilute acid hydrolysis, has less impact on lignin but more favorable for fermentable sugar formation from switchgrass (Sánchez and Cordona, 2008).
2. Highly digestible pretreated solid; for instance, in a previously reported study, enzymatic hydrolysis of AFEX-treated switchgrass showed almost 93% glucan conversion whereas untreated samples showed 16%, also shown that effective enzymatic hydrolysis of AFEX-treated biomass at enzyme loadings as low as 15 FPU/ g glucan (Alizadeh et al., 2005).
3. Fermentable sugars should not be degraded.
4. The liquid hydrolysate achieved by hydrolysis must be fermentable, and highly yielded. Toxic compounds depend on feedstock and harshness of pretreatment. Furan derivatives, carboxylic acids, and phenolic compounds are the three major toxic compounds that might occur as a result of a specific type of pretreatment (Hendriks and Zeeman, 2009). The base furan components are furfural and 5-hydroxymethylfurfural (HMF), weak acids including largely acetic, formic and levulinic acids and phenolic compounds containing aldehydes, ketones, acids, and alcohols. The compound furfural occurs as a result of the dehydration of xylose and is therefore associated with the hemicellulose component; 5-HMF is caused by the dehydration of glucose, which is formed from the cellulose fraction of lignocellulosic biomass. Phenolic compounds, on the other hand, are not of carbohydrate origin and are caused by depolymerization of the lignin component of lignocellulosic biomass. Partial hemicellulose and lignin degradation that lead to toxic components formation give rise to undesirable conditions during pretreatment. To diminish the impact of toxic compounds, the inhibitors must be removed through detoxification methods such as anion exchange, overliming, solvent extraction or fermenting yeasts highly tolerant to inhibitors.

5. The reduction of the particle size of the biomass by milling or grinding is not only to enhance surface area but also to reduce the crystallinity of the biomass. However, milling process was reported to be a cost intensive technology.
6. Pretreatment reactors should be made at a reasonable size and a low-cost by reducing their volume. Besides, appropriate materials for extremely caustic chemical environment and against severe pressures and temperatures should be used.
7. Formation of solid waste residues during hydrolysis and fermentation should be effectively treated.
8. To diminish energy consumption during pretreatment, biomass must avoid high moisture. The moisture of the biomass should be reduced, air-dried, before pretreatment.
9. After pretreatment, enzymatic hydrolysis should attain high sugar yield which is above 90%.
10. Sugar dispersion regaining between pretreatment and ensuing enzymatic hydrolysis should be suitable for the fermentation of pentoses (arabinose and xylose) in hemicellulose.
11. Lignin and other components should be recovered in order to be converted into precious co-products.
12. The requirements of heat and power have to be reduced so that certain overall energy efficiency can be maintained.

Over the years, different pretreatment technologies have been suggested. They are biological, physical (grinding, irradiation, extrusion, among others), chemical (dilute acid, alkalis, ILs, oxidizing agents, organosolv) and physicochemical (steam explosion, hydrothermal, fibre expansion with ammonia) pretreatments (Kumar et al., 2009; Brodeur et al., 2011). Due to the diversity in the hemicellulose, cellulose, and lignin composition, lignocellulosic biomass chosen also affects the selection of the pretreatment process. (Dahadha et al., 2017). Different pretreatment methods are compared in Table 2.3.

Table 2.3 The comparison of the main lignocellulosic biomass pretreatment methods

Pretreatment Method	Feedstock	Operating Condition	Mechanism of Action	Pros	Cons	References
Biological (Microbial)	Agricultural residuals: wheat straw, rice straw Softwood	Various environmental conditions	Hemicellulose and lignin decomposition	Cost effective Low energy requirement Modest reaction conditions; No catalyst; No toxic compounds generated	Low hydrolysis rate; Needs large sterile area	Tu and Hallett, 2019, da Silva et al., 2019
Steam explosion	Agricultural waste: corn stover, wheat straw, sugarcane bagasse Hardwood	160–260 °C with 0.69–4.83 MPa for several seconds to a few minutes	Hemicellulose breakdown at 150°C Lignin rupture at 180°C and above	Cost-efficient Lower hazards Hemicellulose solubilisation and lignin transformation	Partial degradation of hemicellulose Inhibitor compounds formed Incomplete breakdown of lignin carbohydrate matrix	Tu and Hallett, 2019, Sun and Cheng, 2002, da Silva et al., 2019
Physical	Wood, forestry biomass, straw	180–240 °C	Smaller particle size and higher surface area	Reduce crystallinity, lower DP	High energy consumption, lengthy process	Tu and Hallett, 2019, Alvira et al., 2010, Hendriks and Zeeman, 2009
Dilute acid	Wheat plant, switchgrass	High temperature (e.g. 180 °C) during a short period of time, low temperature (e.g. 120 °C) for longer retention time (30-90 min)	Blocking enzyme accessibility to the substrate for sugar production	Hemicellulose solubilisation, disrupt lignin	Generation of inhibitory sugar degradation products	Alvira et al., 2010, Singh et al., 2010

2.5 Ionic Liquid Pretreatment

IL pretreatment is a new technique compared to other chemical pretreatments such as dilute acid and alkaline, and considered as promising pretreatment. The first important study was done by Swatloski et al. (2002) who found that dissolution of cellulose with

ILs under heating conditions. They have shown IL, 1-butyl-3-methylimidazolium chloride (BMIMCl) dissolved cellulose. This was verified with Scanning Electron Microscopy (SEM) images which show disappearance of regular structure of cellulose fibers (Figure 2.14). They have concluded that ILs are capable of breaking up cellulose's neat hydrogen bonded network (Swatloski et al., 2002).

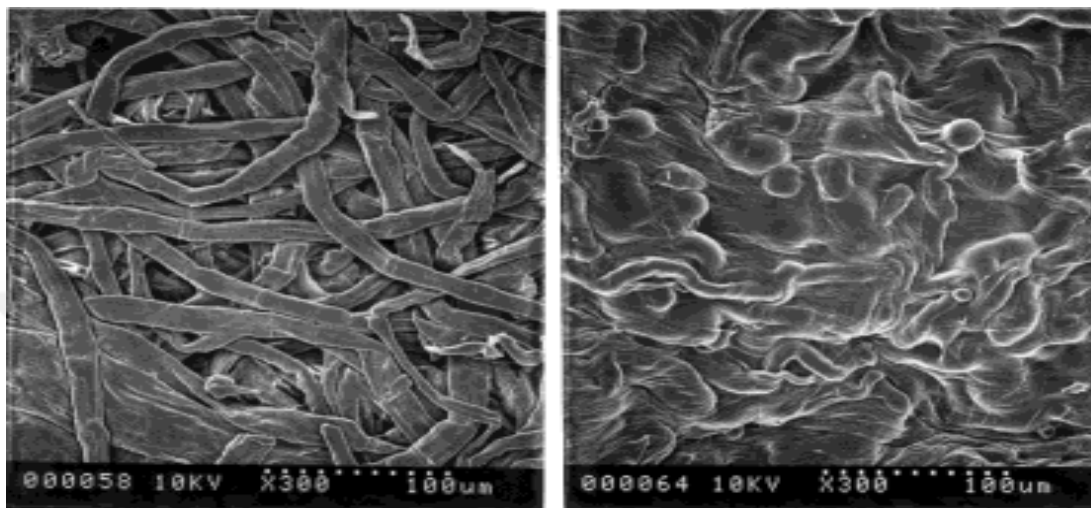


Figure 2.14 (a) native cellulose and (b) regenerated cellulose, by SEM images (Swatloski et al., 2002)

2.5.1 Ionic liquids

ILs are considered to be green alternatives to volatile organic solvents, as no hazardous chemicals are formed by their application and ~100% recovery of the IL used is possible (Brandt-Talbot et al., 2017). They usually possess low melting points, generally under 100 °C (Mora-Pale et al., 2011) and also, have a wide liquidus range that Huddleston et al. (2001) reported BMIMCl with a melting point of 41 °C and decomposition temperature of 254 °C. Other useful properties of ILs are their high thermal stability, high ionic conductivity, large electrochemical window, miscibility, water stability, density, viscosity, odorlessness, polarity and refractive index (Huddleston et al., 2001). They are a class of organic salts, comprised entirely of cations (usually organic) and anions (usually inorganic) (Vancov et al., 2012). Since 1914, when the first IL was described as EtNH_3NO_3 (Wilkins and Sugden, 1929), ILs have

been receiving the attention of many engineers and scientists, as shown by the increasing number of papers published in recent years.

They are referred to as “designer solvents” owing to their suitability of using different anion and cation combinations, adjusting their chemical and physical properties (viscosity, melting point, polarity and hydrogen bond basicity). Figure 2.15 shows the schematic design of ILs; different colors and shapes represents different functional groups and IL (Mai and Koo, 2016). The miscibility of ILs with water is controlled by the selection of anion and cation. Water mainly interacts with anion through the formation of hydrogen bonds. The cation contributions are secondary, acting as a weak hydrogen bond donor (Murugesan and Linhardt, 2005). Haykir (2013) demonstrated the distinctive outcome for the regenerated (precipitated) cellulose after adding water in IL (Figure 2.16).

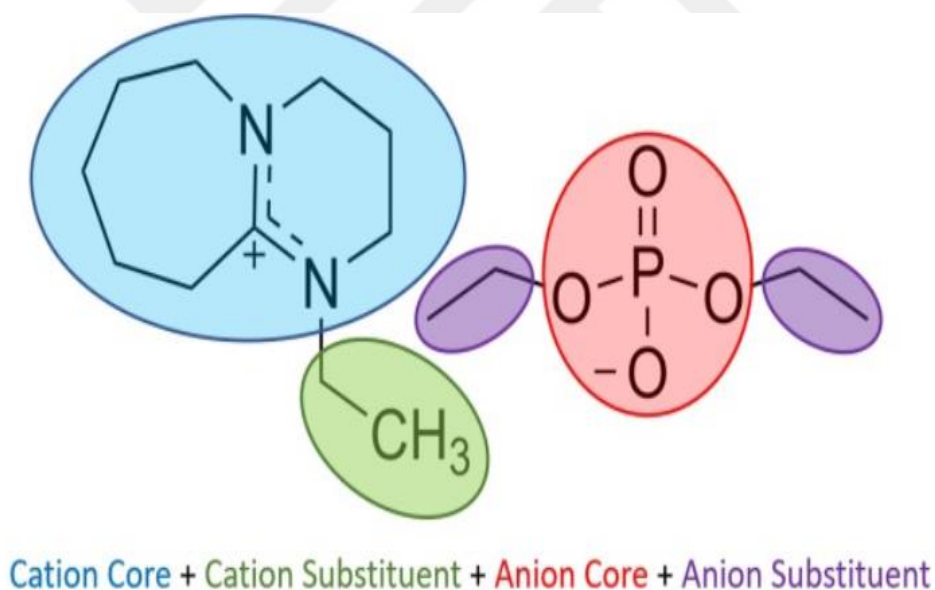


Figure 2.15 ILs with dissimilar design and colors stand for different functional groups by schematic sampling (Mai and Koo, 2016)

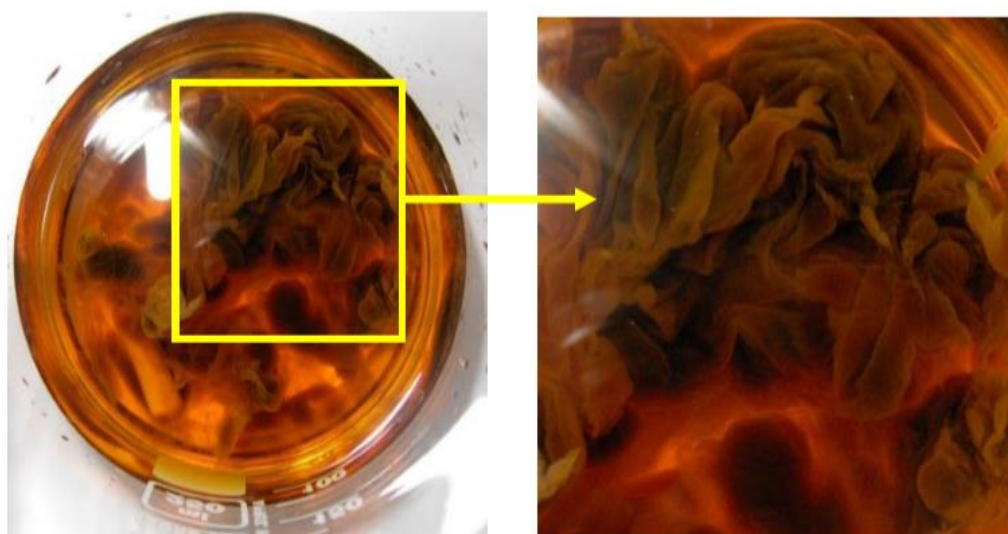


Figure 2.16 Regenerated cellulose after precipitation by adding water to the solution of IL-cellulose (Haykir, 2013)

High viscosity of ILs, which can represent a barrier to mass transfer, has been regarded as a drawback for processes including ILs (Mora-Pale et al., 2011). This will clearly result in ineffective biomass pretreatment. Huddleston et al. (2001) reported that the viscosity of ILs varying from 10 cP to 500 cP.

The Kamlet-Taft (1976) parameters (α , β , π^*) have an effect on the processing of biomass with IL. Important indicators of solvent to receive or donate hydrogen bonds are hydrogen bond acidity (α) and basicity (β). The magnitude of these parameters directly plays a role in cellulose dissolution (Mora-Pale et al., 2011). β is the deciding factor for the dissolution capability of ILs. ILs with β values higher than 0.8 are required for the dissolution of cellulose with 10% or higher loading in IL (Doherty et al., 2010; Brandt et al., 2013). All ILs have mostly high dipolarity/polarizability (π) values (Brandt et al., 2010).

ILs can be divided into two broad categories: AILs and PILs. Recently, Brandt et al., (2013); Pinkert et al. (2011); Sun et al. (2009) have studied the efficiency of delignification of AILs. AILs' dissolution properties are responsible for the proper selection of cations and/ or anions, as well as environmentally friendly features have a

remarkable role in this process (Mikkola et al., 2014). The short alkyl chain AILs are preferred and the most typical one is imidazolium acetate due to the higher solubility of lignin (Zakrzewska, Bogel-Lukasik, & Bogel-Lukasik, 2010). However, there are many obstacles, such as high process temperature ($>100^{\circ}\text{C}$), low efficiencies ($<50\%$), solid waste accumulation, recovery challenges due to high viscosity and high cost of AILs (Rashid et al., 2016). Alternatively, the literature has included the use PILs. PILs are less expensive and more straightforward to synthesize than their aprotic counterparts and synthesized by a single step reaction between a Brønsted acid and base as a result of a simple proton exchange mechanism. This mechanism leads to lignin extraction from the lignocellulosic biomass and substantial changes in the aromatic polymer's structure. The relationship between PILs and lignocellulosic biomass will be explained in detail in the next section.

2.5.2 Processing of cellulose with ionic liquids

ILs interact with cellulose, indicating cellulose dissolution. The mechanism of dissolving cellulose via ILs primarily includes the degradation of cellulose's hydrogen bond network. Zhang et al. (2005) proposed that 1-allyl-3-methylimidazolium chloride (AMIMCl), has been used for the dissolution and regeneration of cellulose. Figure 2.17 shows how with the typical IL AMIMCl processing of cellulose. AMIMCl dissociated to individual $[\text{AMIM}]^+$ and $[\text{Cl}]^-$ ions. It is discovered that cation $[\text{AMIM}]^+$ interacted with the oxygen and $[\text{Cl}]^-$ attached to the hydrogen of OH^- during IL- cellulose interaction. This mechanism leads to the dissolution of cellulose. This effect resulted in a decrease in cellulose crystallinity and thereby, improvement in its enzymatic conversion.

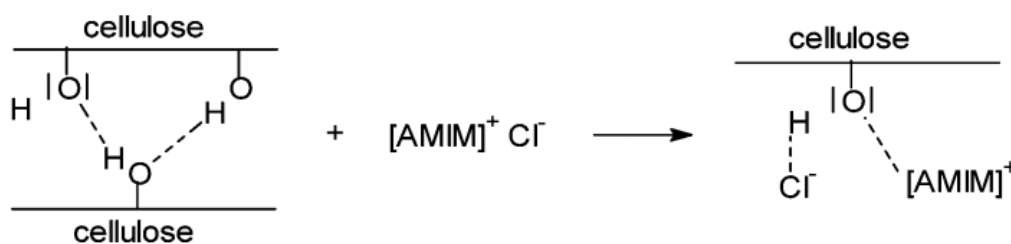


Figure 2.17 The mechanism of dissolving cellulose via AMIMCl (Zhang et al., 2005)

Maki-Arvela et al. (2010) compiled many studies on the dissolution of cellulose in a variety of ILs. One of the comprehensive studies was performed by Zavrel et al. (2009) 21 different ILs were explored by a high-throughput system for their ability to dissolve cellulose. This study also enabled the comparison of ILs with respect to their performance of dissolution capability and rate. Six of these ILs, which were AMIMCl, EMIMCl, EMIMAc, BMIMCl, 1,3 dimethyl imidazolium-dimethyl phosphate (ECOENG) and 1-butyl-3methylpyridinium-chloride (BMPYCl), completely dissolved 5% (w/w) of cellulose in 12 hours at 90°C. The most efficient solvent for cellulose was obtained as EMIMAc.

In another study, Fukaya et al. (2008) investigated the effect of the anion structure of ILs on the solubilization of cellulose under mild conditions. They prepared ILs with a series of alkylimidazolium salts including dimethyl phosphate, methyl methylphosphonate, or methyl phosphonate as anions with a fixed cation, N-ethyl-N'-methylimidazolium (C₂mim), characterized and tested as potential solvents for cellulose dissolution. The study revealed that N-ethyl-N'-methylimidazolium methylphosphonate (C₂mim(MeO)₂PO₂) showed maximum cellulose solubility of 10 wt% at 45°C after 30 min of submersion time.

Dadi et al. (2006) demonstrated that microcrystalline cellulose subjected to 1-*n*-butyl-3-methylimidazolium chloride (BMIMCl) pretreatment at temperatures between 130 °C and 150 °C from 10 to 180 min gave 90% glucose conversion of enzymatic hydrolysis at t=48 h. However, 60% was achieved for native microcrystalline cellulose. The same group also found (Dadi et al., 2007) that regenerated cellulose obtained upon cellulose dissolution in either 1-allyl-3-methylimidazolium chloride (AMIMCl) or BMIMCl, resulted in reduced crystallinity compared to untreated cellulose. Complete dissolution in both AMIMCl and BMIMCl was obtained at 5% cellulose lodings whereas cellulose was entirely dissolved in BMIMCl and partially in AMIMCl at 10% of loading.

Simmons group (2009) utilized a laser scanning confocal microscope to track the dynamic solubilization mechanisms during ionic liquid pretreatment of switchgrass. They observed that IL quickly swelled the secondary cell walls (Figure 2.18). This swelling may be a result of breaking the inter- and intra-molecular hydrogen bonding

responsible for the rigid and highly compact crystalline cellulose polymer structure within the biomass.

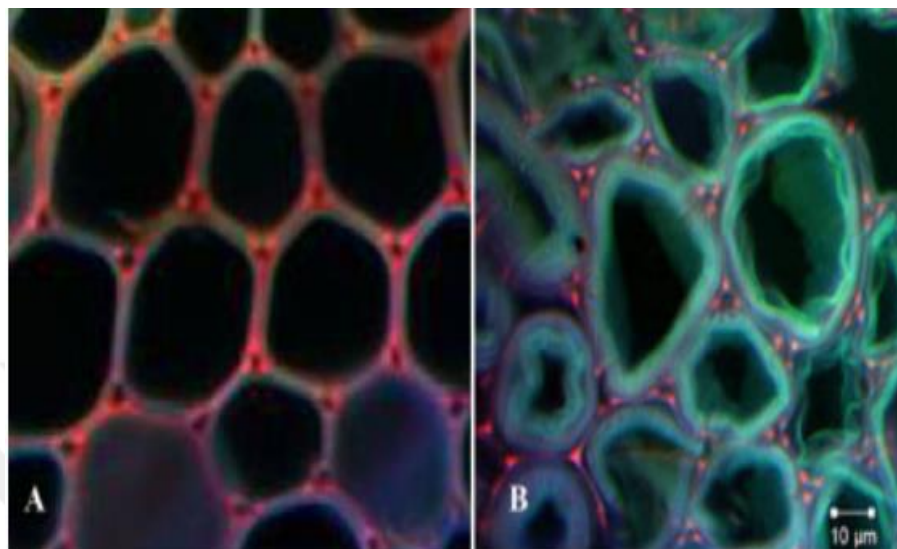


Figure 2.18 Confocal fluorescence images of parenchyma cell wall, (A) before pretreatment and (B) swollen cell wall after 10 min pretreatment with EMIMAc at 120 °C (Simmons et al., 2009)

After all, either the type of anion or cation in ILs has an impact on cellulose dissolution which determines the generation of bonds between ILs and OH⁻ groups of the cellulose and thereby, disrupts crystalline cellulose.

2.5.3 Processing of lignin with ionic liquids

Besides cellulose dissolution, ILs dissolve or extract lignin during the pretreatment of lignocellulosic biomass. Lignin is solvated in ILs with the contribution of both ions. The solubility of lignin in ILs not only increases the enzymatic accessibility of cellulose in biomass, but also benefits the conversion of lignin into high value-added products.

Pu et al. (2007) carried out the dissolution of a residual softwood kraft lignin in several different ILs. They discovered that ILs solubilized high lignin at room temperature. Also, a big difference in cation and anion sizes results in the solubilization of lignin

well in ILs. They especially investigated that the $[\text{BMIM}]^+$ cation is good dissolution of the biopolymer. To this respect, it can be deduced that the four-carbon chain replaced the imidazole cation can support the 'relaxation' of the lignin structure, thus increasing the efficiency of its dissolution. Moreover, it was shown that the resolution of lignin increases with respect to the following anion series: $[\text{MeSO}_4]^- > \text{Cl}^- \approx \text{Br}^- \gg \gg [\text{PF}_6]^-$. Interestingly, 1-butyl-3-methylimidazolium methylsulfate (BMIMMESO_4) dissolved 344 g/L of lignin at 50°C, which is quite high (Pu et al., 2007).

Fort and his co-workers (2007) studied the dissolution of lignin from woods such as pine, poplar, eucalyptus, and oak in 1-n-butyl-3-methylimidazolium chloride (BMIMCl). They also achieved 40-50 % of the cellulose extraction through BMIMCl dissolution at 100 °C for 24 h. They demonstrated that extraction levels for hardwoods are lower than for softwoods depending on differences in morphological properties and complexity of their lignocellulosic matrices.

Tan and coworkers (2009) studied extraction of lignin using EMIMXS (1-ethyl-3-methyl-imidazolium xylene sulfonate) at atmospheric pressure and elevated temperatures (170–190 °C). The authors achieved the extraction of lignin yield more than 93% from sugarcane bagasse dissolution.

In another study, pretreatment with (EMIMAc) and five other ILs such as BMIMCl , EMIMAc, DMEAF, DMEAS, DMEAG were carried out for the extraction of lignin from triticale and wheat straw and flax shives. BMIMCl was less efficient than EMIMAc considering delignification of straw. EMIMAc yielded higher lignin removal in which roughly 52% alkali insoluble lignin was removed upon triticale dissolution in EMIMAc at 150 °C for 90 min. In addition, cellulose digestibility of the residue yielded >95% at 150 °C after 90 min (Fu et al., 2010). Lee et al. (2009) studied lignin extraction from wood flour in EMIMAc. 40% lignin removal and more than 90% of cellulose digestibility was reported for the maple wood flour. This study also demonstrated that EMIMAc could be reused at least up to four times retaining the digestibility of cellulose.

In 2009, Sun et al. used EMIMAc for the pretreatment of softwood (southern yellow pine) and hardwood (red oak). This study also demonstrated that EMIMAc was better

solvent than BMIMCl for wood dissolution and 31% of the original lignin was recovered from southern yellow pine.

EMIMAc is still the most attractive option for disrupting biomass crystallinity and removing a certain amount of lignin. An alternative to this aprotic IL has not been explored yet.

2.5.4 Interaction of protic ionic liquids with lignocellulosic biomass

In the literature studies, various lignocellulosic raw materials have been pretreated with both PILs and AILs in order to increase biomass conversion to fermentable sugars. In this part, the literature studies, interaction of the aforementioned PILs with lignocellulosic biomass, will be discussed. The ability of PILs to dissolve cellulose and lignin depends on how PILs act on the complexity lignocellulosic structure (Achinivu et al., 2014; Rashid et al., 2016).

Brandt-Talbot and her co-workers (2017) showed the dissolution of *Miscanthus* in PIL. This is the first demonstration of an efficient and repeated lignocellulose fractionation with a truly low-cost IL, and it opened a path to an economically viable PIL-based pretreatment process. The PIL, triethylammonium hydrogen sulfate TEAHSO₄, containing 20 wt% of water showed a delignification yield over 85 wt% at 393.15 K and was reused four times for pretreatment of *Miscanthus*.

Hossain, Rawal, and Aldous (2019) investigated three imidazolium-based PILs, 1-ethylimidazolium acetate (EimOAc), 1-ethylimidazolium formate (EimHCOO), and 1-ethylimidazolium chloride (EimCl) for pine pretreatment. They also compared the performances of aprotic (EmimOAc and EmimCl) and PILs. EimCl has been demonstrated to be capable of whole biomass dissolution and could be easily recovered by distillation; thereby, was repeatedly used (ref-Hossain et al., 2019).

Miranda et al. (2019) studied that pineapple fibres pretreatment with the twelve PILs (2-hydroxyethylammonium acetate (2HEAA), 2-hydroxyethylammonium propionate (2HEAPr), 2-hydroxyethylammonium butyrate (2HEAB), 2-hydroxyethylammonium pentanoate (2HEAP), bis(2-hydroxyethyl)ammonium acetate (BHEAA), bis(2-

hydroxyethyl)ammonium propionate (BHEAPr), bis(2-hydroxyethyl)ammonium butyrate (BHEAB), bis(2-hydroxyethyl)ammonium pentanoate (BHEAP), N-methyl-2-hydroxyethylammonium acetate (m2HEAA), N-methyl-2-hydroxyethylammonium propionate (m-2HEAPr), N-methyl-2-hydroxyethylammonium butyrate (m-2HEAB), and N-methyl-2-hydroxyethylammonium pentanoate (m-2HEAP)) and found enhanced cellulose and hemicellulose quantities, lower lignin amounts after pretreatment. Among the twelve PILs, BEAHPr demonstrated the highest efficiency with 92% lignin and 48% hemicellulose removal and gave the highest crystallinity index (CrI) (70%).

In another study, cellulose I material from corn straw was separated by low-cost (about \$1000/ t) polyhydric PILs. Cellulose I materials with 2-hydroxy-N-(2-hydroxyethyl)-N-methylethanaminium methanesulfonate (BHEMmesy) pretreatment was obtained with high purity (≥ 90 wt%) at 140 °C for 6 h. Also, the study revealed that IL, BHEMmesy was recycled for utilization for five times (Yang et al., 2018).

Pin et al., (2019) investigated eleven PILs for pretreatment of sugarcane bagasse. In the study, the performances of PILs were evaluated with respect to cation effect using hydrogen sulfate anion and acetate anions. PILs with acetate anion were more efficient, leading to enzymatic hydrolysis yields of up to $72.1 \pm 1.2\%$ of glucose and $45.9 \pm 6.0\%$ of xylose yields with (Me(NH₂)(CH₂)₂OH) OAc) pretreatment at 160 °C for 3 h. Lu et al. (2014) and Rocha et al. (2017) approved that the cation structure affected cellulose solubility for ILs having acetate anion and that cations possessing larger alkyl groups affected fewer each other cellulose indeed, possibly due to existing of these groups steric hindrance.

Sun et al. (2017) demonstrated one-pot integrated biofuel production using a low-cost (~\$1 per kg) and biocompatible PIL, ethanolamine acetate (EOAOAc), not requiring solid/ liquid separations or pH adjustments for the first time. The study also compared EOAOAc to other PILs which displayed the best performance, and the sugar yields (glucose, 57.9%; xylose 18.7%).

Hallett et al., (2019) studied the low-cost PIL, triethylammonium hydrogen sulfate (TEAHSO₄) for the pretreatment of rice straw, rice husk, wheat straw and sugarcane bagasse. An optimal pretreatment was obtained at 170 °C with the aim of maximizing

enzymatic saccharification yields and minimizing residence time. While the glucose yield for the rice husk after enzymatic saccharification, were obtained roughly 90%, it was 73% for rice straw, wheat straw, and sugarcane bagasse.

Semerici and Güler (2018) investigated several PILs' performances relating to impacts on the pretreatment of cotton stalks at a comparatively high biomass loading (15 wt %). We revealed that HBIMHSO₄ evaluated cotton stalks which are an important commercial product waste; this means it hasn't been studied before in literature.

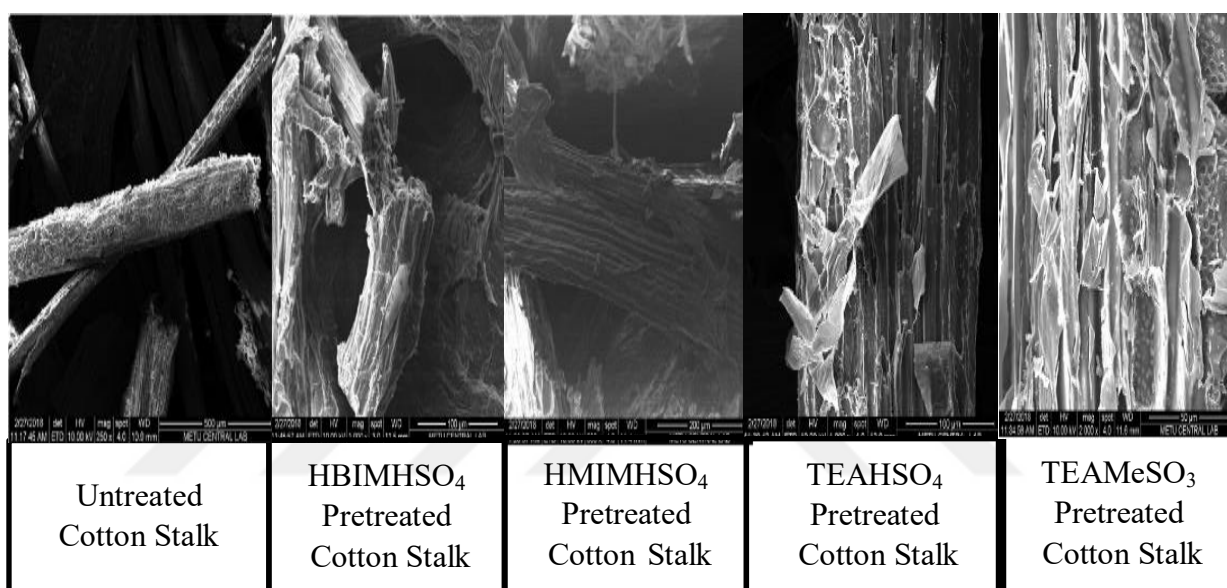


Figure 2.19 SEM images of untreated, HBIMHSO₄ pretreated, HMIMHSO₄ pretreated, TEAHSO₄ pretreated and, TEAMeSO₃ pretreated cotton stalks (Semerci and Güler, 2018)

As shown in Figure 2.19, untreated cotton stalks showed an intact and rigid macrofibril structure. The most typical changes were attained when biomass pretreated with HBIMHSO₄. Compact biomass structure was totally devastated and uneven structure was obtained. The linkages within the structure ruptured. Next image is pretreated HMIMHSO₄ cotton stalks. These changes weren't as significant as those obtained for HBIMHSO₄. Another one presented that TEAHSO₄ had also drastically affect the

structure of the cotton stalk. According to the last image minimal modifications observed for TEAMeSO₃ pretreated samples.

The study performed by Dias et al. (2019) evaluated the potential use of 2-hydroxy ethyl ammonium-based PILs (2-hydroxy ethylammonium formate (2-HEAF), 2-hydroxy ethylammonium acetate (2-HEAA) and 2-hydroxy ethylammonium propionate (2-HEAPr)) for dissolving cellulose, xylose, and lignin. The study showed that xylose and lignin could be dissolved by the PILs while cellulose could not be dissolved. Figure 2.20 showed that there was no change between three images, which indicates that 2-HEAF didn't dissolve cellulose, and the same results were achieved in the other PILs. Among the PILs, 2-HEAF which demonstrated better ionicity was the most appropriate one. They also showed that using an anion with a short alkyl carbon chain, lignin dissolution could be faster.

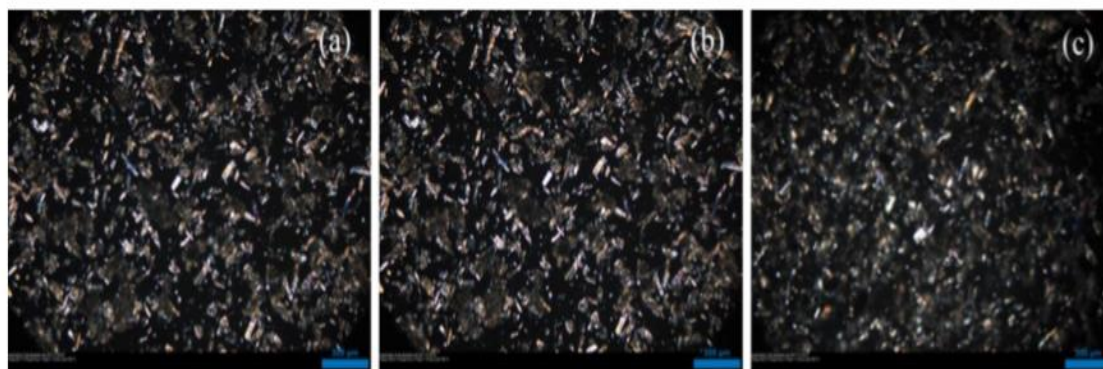


Figure 2.20 The cellulose (1 wt %) images in 2-HEAF using heating rate of 1 °C min⁻¹ by optical microscopy. (a) T = 30.0 °C; (b) T = 69.7 °C; (c) T = 100.0 °C (Dias et al., 2019)

Most recently, we published (2019) a paper that studied the effects of temperature and time during TEAHSO₄ pretreatment on biomass varieties which were HB and P. We obtained 150 °C, 3h as the best conditions; PHB converted into glucose with 97 % yield. For this reason, these parameters were chosen as optimum for HB pretreatment for this master thesis.

2.6 Enzymatic Hydrolysis

Enzymatic hydrolysis has been a vital step for the production of fermentable sugars and other value-added products from biomass subjected to lignin and crystallinity reductions through pretreatment. Enzymatic hydrolysis is usually carried out at milder operation conditions (e.g. pH 4.5-5.0 and temperature 40–50°C). That is the reason why low corrosion problems and low toxicity of the hydrolyzates are expected in this step (Sun and Cheng, 2002).

In the enzymatic conversion of the pretreated lignocellulosic biomass, enzymes are used to breakdown the cellulose polymer, especially down its to monomer, glucose. Two important stages control cellulose hydrolysis with enzyme catalysis. These are the adsorption of enzymes on cellulosic particles, and the formation of enzyme-substrate complexes (Mansfield et al., 1999; Chandra et al., 2007). The inhibition by products (cellobiose and D-glucose), thermal stability, synergism, and adsorption are enzyme related factors while the existence of hemicellulose and lignin, cellulose crystallinity, and DP, accessible external and internal surfaces of cellulose are substrate-related factors.

Cellulase and hemicellulase enzymes (glycosylhydrolases) are specific enzymes that are used in enzymatic hydrolysis of cellulose and hemicellulose, respectively. This enzyme group contains 15 protein families and some subfamilies of cellulase which are also called cellulolytic enzymes comprised of three main classes of enzymes: endo-glucanases (EC 3.2.1.4), exo-glucanases, such as 1,4- β -D-glucan glucanohydrolases (also known as cellodextrinases) (EC 3.2.1.74) and 1,4- β -D-glucan cellobiohydrolases (cellobiohydrolases) (EC 3.2.1.91), and β -glucosidases (EC 3.2.1.21) (Taherzadeh and Karimi, 2007). The endo-glucanases act on the amorphous region of the cellulose fiber and generate the free-chain ends while the exo-glucanases, which aim at the crystalline regions, act on the ends of cellulose chains and liberate either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as main products. β -glucosidases produce glucose, cleaving the cellobiose (Lynd et al., 2002). Illustrate in Figure 2.21 shows, the hydrolysis of cellulose to glucose by cellulolytic enzymes (Taherzadeh and Karimi, 2007). Compared to other glycoside hydrolases, cellulases are differentiated

due to their ability to hydrolyze β -1,4-glucosidic bonds between glucosyl residues. For example, Figure 2.22 shows that endo- β -1,4-glucanase cleaves internal β -1,4-glucosidic bonds to generate small oligomers (Linton,2020).

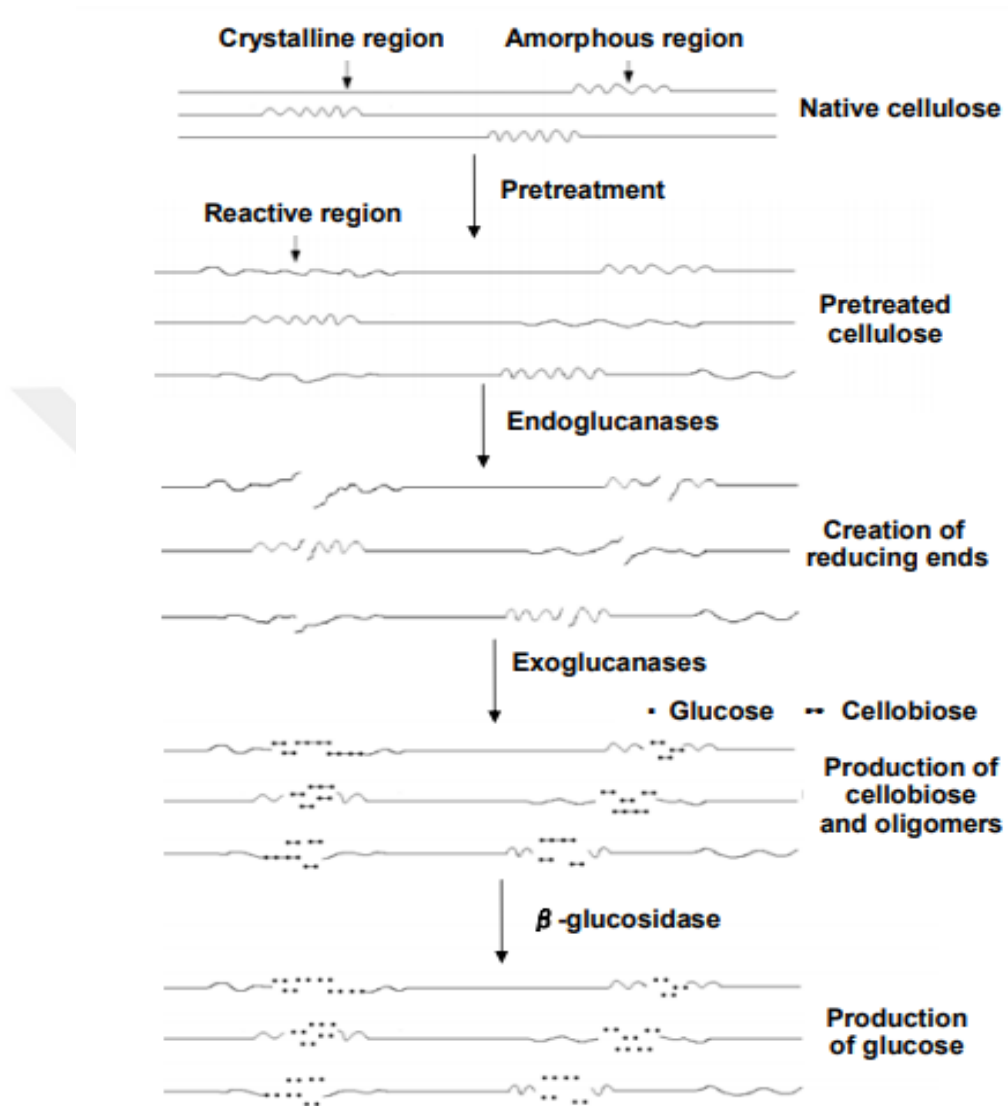


Figure 2.21 A schematic representation cellulose hydrolysis to glucose with cellulolytic enzymes (Taherzadeh and Karimi, 2007)

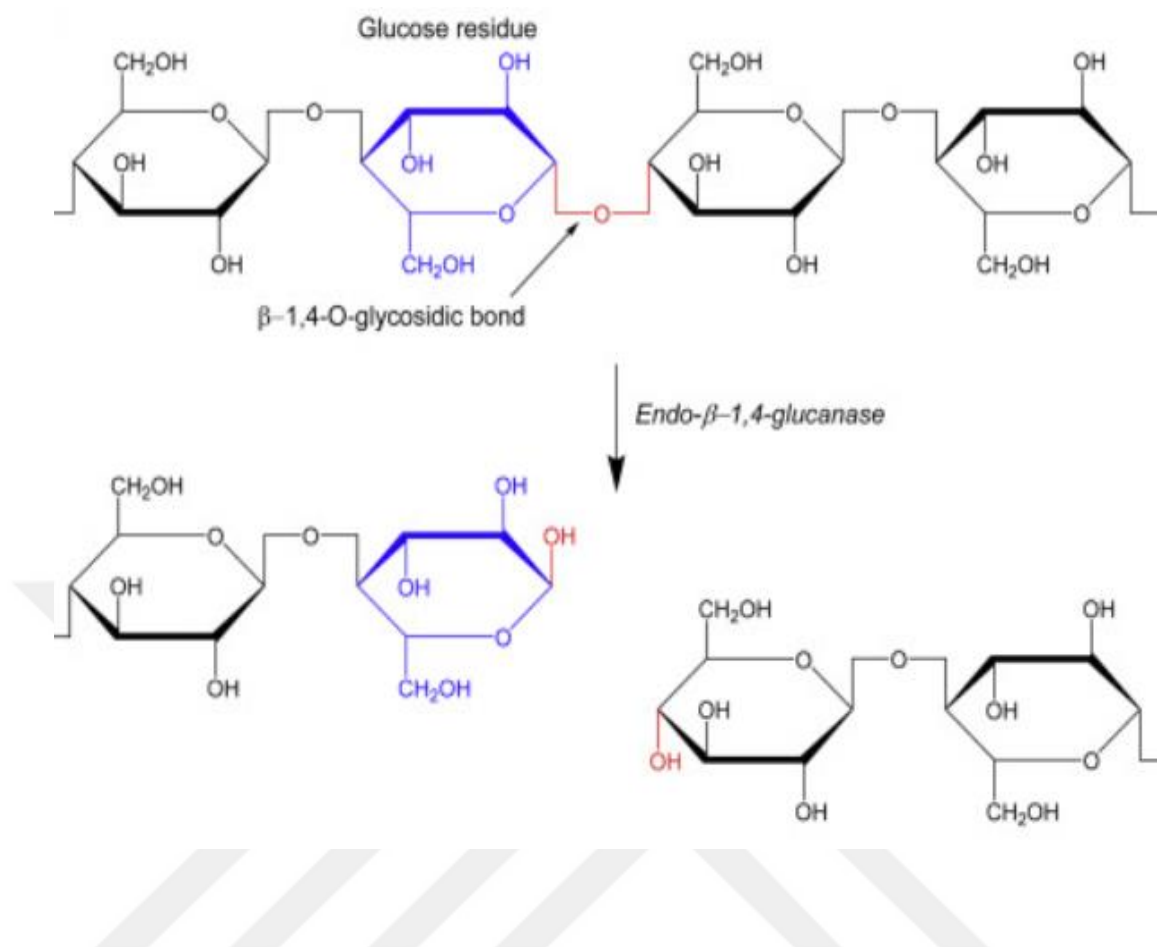


Figure 2.22 A schematic representation hydrolysis reactions by endo- β -1,4-glucanase. The red glycosidic bonds represent to be the hydrolyzed (Linton, 2020)

Also, hemicellulases are a class of enzymes. Hemicellulose comprises various sugar units and thus hemicellulases contain more complex enzyme classes than cellulases. Hemicellulases contain xylanase (EC 3.2.1.8) that hydrolyzes β -1,4-linked xylose backbone, whereas, β -xylosidase (EC 3.2.1.37) clips xylose monomers starting from the nonreducing end of xylooligosaccharides and xylobiose. The xylan backbone adorned with the arabinose, glucuronyl, galactouronyl, galactosyl and mannose substituents cleavage by the action of α -arabinofuranosidase (EC 3.2.1.55), α -glucuronidase (EC 3.2.1.139), α -galactosidases (EC 3.2.1.22), and β -mannosidases (EC 3.2.1.25), respectively. The acetyl xylan esterases (EC 3.1.1.72), ferulic acid esterases (EC 3.1.1.73), and *p*-coumaric acid esterases (EC 3.1.1.10) involved in de-esterification are also needed for complete hemicellulose

deconstruction (Chadha et al., 2019). Figure 2.23 involves enzymes used in the hydrolysis of xylan (Collins et al., 2005).

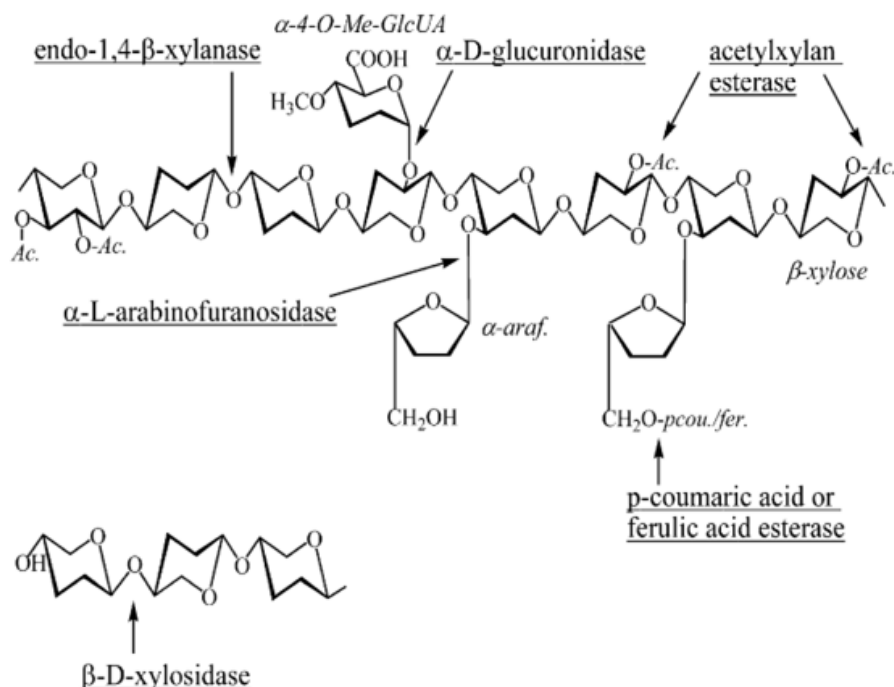


Figure 2.23 Xylan degrading enzymes (Collins et al., 2005)

Cellulases and hemicellulases can be produced from both bacteria and fungi for the hydrolysis of lignocellulosic materials. There are several species of bacteria such as *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Bacillus*, *Bacteriodes*, *Ruminococcus*, *Erwinia*, *Acetovibrio*, *Microbispora*, and *Streptomyces*, and fungi such as *Trichoderma*, *Penicillium*, *Fusarium*, *Phanerochaete*, *Humicola*, and *Schizophillum* spp., for the synthesis of cellulases and hemicellulases (Sun and Cheng, 2002). *Trichoderma reesei* or *Trichoderma viride* and *Aspergillus niger* are the potential producer microorganisms of cellulose degrading enzymes and have been extensively studied (Zhang and Lynd, 2004). The *T. reesei* cellulases have even been announced to be “the industry standard” for enzymatic lignocellulose hydrolysis (Merino and Cherry, 2007). The cellulase produced by *Trichoderma* has some advantages such as resistance to chemical inhibitors, being stable under the enzymatic hydrolysis conditions (Taherzadeh and Karimi, 2007).

The amount of enzyme adsorbed on the cellulose is related to the rate and the extent of the cellulose hydrolysis and thus adsorption kinetics acts on hydrolysis. The most typical model for cellulase adsorption kinetics is Langmuir isotherm; derived assuming that adsorption can be defined by a single adsorption equilibrium constant and a specified adsorption capacity (Zhang and Lynd, 2004).



3. MATERIALS AND METHODS

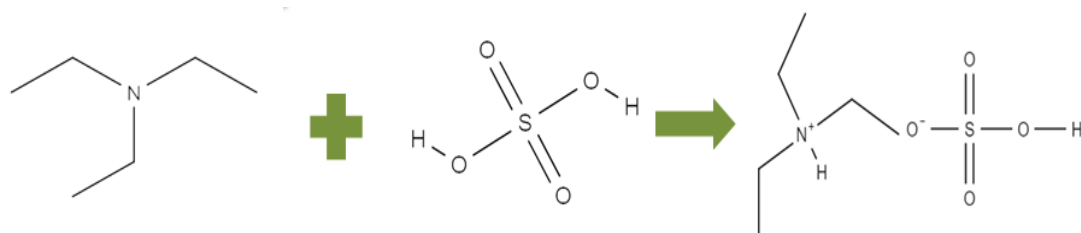
3.1 Materials

HB residues, directly obtained from a sawmill in Manisa, Turkey. These were air dried, before pretreatment. The particles were then sieved to sizes (<0.15 mm, 0.15 mm-1.18 mm, 1.18-2.00 mm and >2.00 mm) and stored at room temperature for pretreatment. Technical ethanol, sulfuric acid, calcium carbonate, sodium citrate were purchased from Merck (Darmstadt, Germany). Triethylamine, 1-butylimidazole, 4-methylmorpholinium were purchased from Sigma-Aldrich (St. Louis, MO, USA). The cellulase enzyme, Cellic Ctec2 was provided by Novozymes (Bagsværd, Denmark).

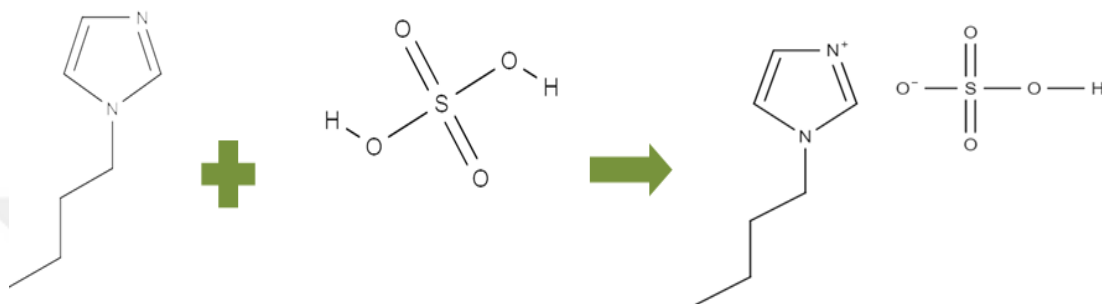
3.2 Methods of Experiments

3.2.1 PILs synthesis

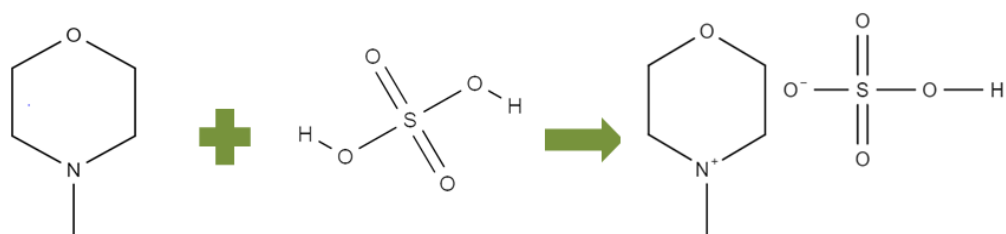
PILs synthesis was carried out via an acid-base reaction. 1:1 the acid and the base ratio were used in the synthesis of PILs. The base was prepared in a round bottom flask and mixed with an equal volume of water for one hour. This experimental setup was cooled via ice bath and was put on a magnetic stirrer. The acid solution was prepared under the same conditions but the solution of sulfuric acid with a four-fold higher volume of water was prepared and added drop wise to the base solution that was under constant stirring and cooled with an ice bath overnight. So as to eliminate water, the resultant solution was kept in a rotary evaporator at 70°C . The process was continued until 20 percent of the water left in the medium. PILs which are TEAHSO_4 , HBIMHSO_4 , HMMorpHSO_4 were prepared according to the above mentioned this procedure. Figure 3.1 shows how PILs synthesized structurally.



(a)



(b)



(c)

Figure 3.1 Synthesis of PILs used in this study: (a) TEAHSO₄ (b) HBIMHSO₄ (c) HMMorpHSO₄

3.2.2 Pretreatment of HB with PILs

HB with three different PILs which are TEAHSO₄, HBIMHSO₄, HMMorpHSO₄ was pretreated according to Ionosolv protocol. The Ionosolv protocol has been reported by Hallett (2016). All pretreatments were conducted in 50 mL Schott tubes with teflon-lined red screw caps. 10 g of PIL was combined with 1 g of oven-dried HB (10% biomass loading). Each tube was shaken using the IKA vortex mixer until the biomass and each of PILs were thoroughly mixed. The tubes containing the PIL-biomass mixture were placed into the JSR oven at 150°C for 3 hours. Once removed from the JSR oven, the samples were allowed to rest at room temperature for roughly 1 h and ethanol was added to precipitate the pretreated biomass, in other words, the solid fraction. Solid fraction, which was washed 30 ml ethanol by the IKA stirrer and the residual, was recovered by filtration with a roughing filter. Finally, the recovered biomass was dried at 70 °C under vacuum for 24 h before it was subjected to enzymatic reaction and characterizations. Solid weight was recorded to determine the percentage solid recovery (SR) as follows:

$$SR(\%) = \left(\frac{W_{PRT}}{W_{UT}} \right) \times 100 \quad (3.1)$$

W_{PRT} is the weight of biomass recovered after pretreatment (g) and W_{UT} is the weight of untreated biomass subjected to pretreatment (g). All pretreatments were performed in duplicate.

The liquid fractions which possess PIL and solubilized lignin were decanted into a 50 mL falcon tube and then 30 mL water added so as to precipitate the lignin. The day after, lignin suspension was visible in the solution. The falcon tubes centrifuged for 45 min at 6000 rpm. After, the liquid mixture having recycled PIL was decanted. This process of lignin washing was repeated for a total of three water washes. The lignin with some water was decanted into petri dishes, and then the petri dishes placed into (NUVE EV 018) drying oven (usually 24 h) before removing and recording the weight of the lignin. The decanted liquid mixture of PIL and water was kept at 80°C using BUCHI rotary evaporator to recover the ionic liquid.

Percentage lignin extracted was calculated in below equation (3.2) and lignin precipitated yield (%) was calculated in equation (3.3).

$$LE(\%) = \left[\left(L_{UT}(\%) - \left(SR(\%) \times \frac{L_{PRT}(\%)}{100} \right) / L_{UT}(\%) \right) \right] \times 100 \quad (3.2)$$

where L_{UT} (%) is the lignin content of untreated biomass, SR (%) is the solid recovery after pretreatments and L_{PRT} (%) is the lignin content of the biomass subjected to pretreatment (Haykir et al., 2013).

$$LPY(\%) = [W_P / W_{UL}] \times 100 \quad (3.3)$$

where W_P is the weight of the precipitate obtained after PIL pretreatment and W_{UL} is the weight of lignin in the untreated biomass subjected to pretreatment.

The pretreatment of HB was studied also various at biomass loading (20%, 30%, 40%, 50%) and studied at different four particle sizes (0.15 mm, 0.15-1.18 mm, 1.18-2.00 mm and >2.00 mm). In addition, the impact of IL recycling on biomass pretreatment was evaluated.

3.2.3 Compositional analysis

Compositions analysis was used to specify the sugar and lignin content of the untreated and pretreated samples from the experiments carried out in this thesis. This procedure followed the National Renewable Energy Laboratories (NREL) guidelines outlined by Sluiter et al. (2008). Figure 3.2 shows the general flow diagram of compositional analysis.

In preparation for analysis, filter crucibles were placed in the oven at least 4 h and heated to 575 °C. After heating, the crucibles were placed in a desiccator to cool for 20 min. The crucible's tares were recorded and they were placed back into the oven. This process was repeated until the crucible's recorded weights were constant. Similarly, HB was incubated in oven at 105 °C at least 4 h to attain constant weight for the biomass. All compositional analysis was conducted in duplicate.

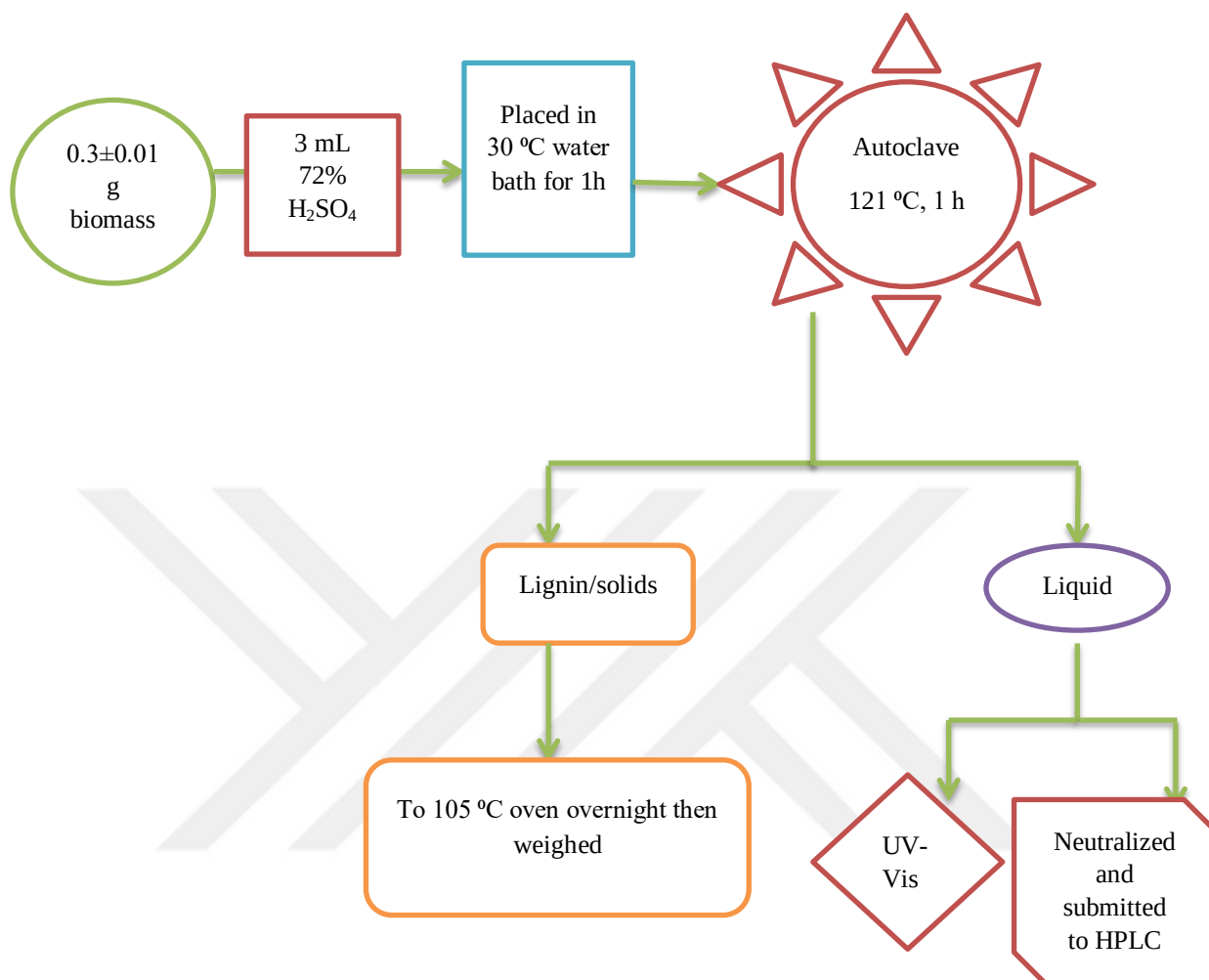


Figure 3.2 The schematic representation of compositional analysis protocol used by NREL

0.3±0.01 g of dry weight pulp was placed into long-necked tubes and 3 mL of 72% (w/w) sulfuric acid added to the tube through pipette. A glass stirring rod was inserted into each tube and the tubes were placed into (30 °C) beaker with water, then placed into 30 °C water bath for an hour with stirring occurring every 5-10 min. 84 mL of water was added every tube and transferred into Schott bottles, sealed, and then placed into autoclave at 121 °C for 60 min. Once the autoclave completed its cycle, the bottles were removed and allowed to cool. The contents of the filter crucibles were filtered through the filtration apparatus and collected in the flask. Roughly 15 mL of the filtered solution

was transferred to falcon tubes for ultraviolet-visible spectroscopy (UV-vis) determination of acid soluble lignin. Another 20 mL was transferred to another falcon tube for the High-Performance Liquid Chromatography (HPLC) for sugar determination.

The UV-vis was calibrated for readings at 205 nm in a 1 cm light path quartz cuvette. Target absorbance's was set between 0.500-1.000. Dilution of samples has been required to fall within that range. For dilution of samples and also the reference blank, 4% (w/w) sulfuric acid was used. Dilutions were noted and taken into account when calculating acid soluble lignin content. The following equation (3.4) was used to determine ASL (acid soluble lignin) content.

$$\% \text{ ASL} = \frac{UV_{\text{abs}} * \text{Volume}_{\text{filtrate}} * \text{Dilution}}{\epsilon * \text{ODW}_{\text{sample}} * \text{Pathlength}} * 100 \quad (3.4)$$

where UV_{abs} is the absorbance at 205 nm, $\text{Volume}_{\text{filtrate}}$ is the initial volume of 72% (w/w) of sulfuric acid solution (mL), Dilution is the dilution factor, ϵ is the absorptivity which is equal to 110 L/g.cm, $\text{ODW}_{\text{sample}}$ is the initial weight of the dry HB (g), pathlength is the cell path length which is 1 cm.

Samples submitted for Shidmadzu HPLC analysis were first neutralized to a pH between 5-6 with the addition of CaCO_3 . CaCO_3 was added under control because samples should never exceed a pH of 9, as this will result in a loss of sugars (Sluiter et al., 2008). And then, the samples were centrifuged at 6000 rpm for 5 min. The supernatant was decanted and filtered through a 0.2 μm PTFE syringe filter and approximately 1 ml of the aliquot is placed in a 1.5 ml HPLC glass vial for analysis in the HPLC. Also, previously prepared frozen mixed sugar standards were removed from the freezer, thawed and placed in the HPLC. Concentrations of prepared standards were 0.125, 0.25, 0.5, 1, and 2 g/L samples containing glucose, xylose. HPLC compositional analyses of samples have in average 15 min run time per sample.

The residues, after filtration, were obtained on the crucible and were washed with water, retaining all solid residues. The crucibles with solid residues incubated at 105 °C overnight. This insoluble part represented the acid insoluble lignin portion of the HB. The percentage acid insoluble lignin (AIL) was calculated with equation (3.5)

$$AIL (\%) = \frac{W_{(AIL+crucible)} - W_{(crucible\ tare)} - W_{ash}}{ODW_{sample}} \times 100 \quad (3.5)$$

where $W_{(AIL+crucible)}$ is the weight of the with AIL (g), $W_{(crucible\ tare)}$ is the empty crucible weight(g), W_{ash} is the weight of ash(g), ODW_{sample} is the initial weight of the dry HB (g).

For determination of ash weight, the crucibles were incubated in muffle furnace at 575 °C and recorded after they were cooled in a desiccator. The difference between the crucibles (empty and after incubated) is the ash weight.

3.2.4 Enzymatic hydrolysis

Enzymatic hydrolysis of UHB and PHB were conducted in 0.05 M sodium citrate buffer at pH 4.8 and 50 °C in JSR oven. While biomass was used at 3% (w/v) loading and Cellic Ctec2 with a cellulose activity of 225 FPU/ml was used at 2 % (v/v). The reaction was monitored for 72 h. To quench the enzymatic reaction, the samples were kept at 100 °C for 5 min. Later, the samples were centrifuged at 6000 rpm for 20 min before analysis of glucose. Prominence LC-20 A Modular HPLC System with RID detector was used to determine the concentration of glucose in the hydrolysates. Biorad Aminex HPX-87H column was employed at 55°C with 5mM H₂SO₄ as the mobile phase at a flow rate of 0.5 ml/min. Hydrolysates were filtered through 0.20 µm PTFE filter before their examination in HPLC. The sample volume injected was 20 µL and the retention time was 20 min.

Percentage glucose yields based on the cellulose content of the UHB was calculated according to the following formula (Equation 3.6):

$$\text{Glucose yield}(\%) = \frac{(SR(\%) \times C_G)}{(C_B \times \text{Cellulose}_U(\%) \times 1.11)} \times 100 \quad (3.6)$$

where SR(%) is the percentage solid recovery, where C_G is the glucose concentration in the hydrolysates (g/L), C_B is the initial concentration of the biomass in the hydrolysis buffer that is subjected to enzymatic hydrolysis (g/L) and $\text{Cellulose}_U(\%)$ is the cellulose

content of the pretreated biomass. 1.11 is the stoichiometric conversion factor of the cellulose to equivalent.

3.2.5 Detection of the sugars using HPLC analysis

HPLC was used to separate, detect and quantify sugars and their degradation products after the biomass pretreatment. Cellulose (%) and hemicellulose (%) content of the HB prior to pretreatments and after the pretreatments and enzymatic hydrolysis were determined with HPLC analysis.

The analysis was carried out with Shimadzu LC-20AD HPLC system (Kyoto, Japan) equipped with a BIORAD Aminex HPX-87H column (Hercules, CA, USA) operated at 55°C with a flow rate of 0.5 mL/min using 5 mM H₂SO₄ as the mobile phase. All samples were filtered through 0.2 µm filter before being analyzed, decanted to the HPLC vials and analyzed under the same operation conditions. Ultra-pure MilliQ deionized water, which had a conductivity of 18.2 MΩ.cm at room temperature, was utilized for dilution of the samples and also preparation of the standards. The calibration range of the standards; glucose and xylose were 0.125-2 g/L. The standard calibration curves were obtained by plotting peak areas as a function of standard concentrations (g/L) and given in Appendix A. Glucose's (g/L), and xylose's (g/L) concentrations were calculated with the standard calibration curves and the peaks' area attained on HPLC analysis of the samples. The glucose's concentration (g/L) (C_G) and the xylose's concentration (g/L) (C_X) were calculated using equations 3.7 and 3.8, respectively:

$$C_G = \frac{A_G - 48.25}{1980.8} \times DF \times \frac{\text{sample weight}}{\text{dry weight of sample}} \quad (3.7)$$

$$C_X = \frac{A_X - 71.958}{2398.1} \times DF \times \frac{\text{sample weight}}{\text{dry weight of sample}} \quad (3.8)$$

where A_G and A_X are the areas of the peaks obtained for glucose and xylose, respectively. DF is the dilution factor. Sample weight is the initial weight of HB and dry weight is the initial weight of the HB (g) subjected to compositional analysis. C_G and C_X are glucose and xylose concentrations (g/L).

The cellulose and hemicellulose content of the HB (%) were calculated using equations 3.9 and 3.10, respectively. The conversion factors, 1.11 and 1.14 are included in the equations due to the water gain during conversion of cellulose and hemicellulose to glucose and xylose, respectively.

$$\text{Cellulose (\%)} = \frac{C_G}{1.11} \times 100 \quad (3.9)$$

$$\text{Hemicellulose (\%)} = \frac{C_X}{1.14} \times 100 \quad (3.10)$$

3.2.6 Characterization of cellulose enriched biomass

Table 3.1 shows that various characterization techniques for screening cellulose enriched biomass.

Table 3.1 Various characterization techniques for screening cellulose enriched biomass

Instrument	Manufacturer	Operation Conditions
SEM	QUANTA 400F Field Emission (Oregon, USA)	Operation at 20kV. Prior to analysis, all samples sputter coated with Au/Pd. Magnification rates: 250×, 500×, 1000×, 2000×
XRD	Rigaku Ultima-IV Diffractometer (Japan)	Analysis conducted between 2θ=10-30° Bragg angles using Cu radiation at room temperature with a step size of 0.02° and at a scanning speed of 1°/min.
ATR-FTIR	Bruker, Equinox 55 (Massachusetts, USA)	All samples, which were in powder form, were incubated in an oven at 60 °C overnight and analyzed in the absorption band mode in the range of 4000-700 cm ⁻¹ .

A standard Guassian function was applied to the recorded XRD patterns and the degree of crystallinity for HB and pretreated pulps were calculated using Segal method with equation 3.11.

$$\text{CrI}(\%) = \frac{I_{22.5} - I_{18.0}}{I_{18.0}} \times 100 \quad (3.11)$$

where $I_{22.5}$ is the intensity of the peak at position $2\theta = 22.5^\circ$. The peak at 22.5° is commonly assigned to the crystalline cellulose. $I_{18.0}$ is the intensity of the peak at position of $2\theta = 18^\circ$ which is attributes to the amorphous portion of biomass (i.e. hemicellulose, lignin, and amorphous cellulose).



4. RESULTS and DISCUSSION

Biomass material, hornbeam (HB) was used with some alterations of particle size regarding our published work (Semerci et al., 2019). The effects of temperature, biomass varieties such as HB and P, and time on TEAHSO₄ pretreatment were studied. Pretreatments were conducted at 120 °C, 150 °C, 170 °C and between 30 min and 8 h of time. More details can be found in this paper. As shown in Table 4.1, in addition to the removal of hemicellulose and lignin, cellulose content of HB increased from 34% to 84% with TEAHSO₄ pretreatment at 150 °C for 3h. As pretreatment temperature and time decreased, higher solid recoveries were attained because the process severity was reduced. According to Table 4.1, the solid recovery for HB after pretreatment at 120 °C and for 2 h was roughly 85% while the solid recovery for HB after pretreatment at 170 °C and for 4 h was 47%. 150 °C and 3h was decided as the optimum conditions for HB pretreatment according to the Table 4.1.

Table 4.1 Chemical compositions of HB and P before pretreatment and after pretreatment under all conditions applied

Pretreatment conditions	SR(%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	LE (%)	LPY (%)
UHB	-	34.2±0.6	17.0±1.7	26.3±0.0	-	-
UP	-	38.5±3.2	22.6±0.6	28.9±0.6	-	-
PHB-170°C-30	93.4±2.0	35.8±1.7	19.0±1.3	23.1±0.0	18.0± 1.6	3.5±0.1
PHB-170°C-1 h	53.6±0.6	64.6±1.5	0	15.0±0.1	69.5± 0.2	27.4±1.5
PHB-170°C-2 h	41.7±0.9	75.5±2.2	0	9.9±1.1	84.3± 2.1	52.3±0.5
PHB-170°C-3 h	39.3±0.2	76.3±1.0	0	7.8±0.3	88.3± 0.4	72.7±3.1
PHB-170°C-4 h	37.3±1.0	70.2±3.1	0	7.4±1.3	89.5±1.9	61.2±4.0
PP-170°C-30 min	84.6±0.2	44.3±1.0	15.9±0.5	28.8±0.0	15.8±0.1	1.4±0.2
PP-170°C-1 h	66.5±0.7	53.8±0.7	8.8±0.3	30.0±0.3	31.0±1.5	20.8±0.9
PP-170°C-2 h	56.7±4.6	64.4±5.1	7.2±0.2	25.6±0.4	49.7±3.3	33.7±1.6
PP-170°C-3 h	48.1±0.9	69.5±0.0	0	26.4±0.1	51.8±0.7	58.3±1.6
PP-170°C-4 h	47.4±1.6	64.7±0.2	0	31.5±0.6	43.2±0.7	55.7±0.3
PHB-150°C-30	95.2±0.3	37.5±0.4	22.1±0.2	23.9±0.3	13.5±0.9	0.5±0.1
PHB-150°C-1 h	78.0±2.9	44.4±1.3	16.4±0.6	23.0±0.6	31.6±4.4	10.1±0.5
PHB-150°C-2 h	52.6±0.2	77.3±0.7	0	13.4±0.7	73.2±1.4	43.7±5.0
PHB-150°C-3 h	40.3±0.7	84.5±3.5	0	8.5±0.2	86.9±0.1	78.0±7.9

Table 4.1 Chemical compositions of HB and P before pretreatment and after pretreatment under all conditions applied (continued)

PHB-150°C-4 h	43.0±0.2	79.8±5.8	0	11.8±2.0	80.7±3.3	71.7±1.4
PP-150°C-30 min	95.0±0.7	44.0±0.7	19.6±0.3	25.7±0.1	15.4±0.2	4.9±0.5
PP-150°C-1 h	84.5±0.4	49.1±1.3	17.9±0.2	27.0±0.8	21.2±2.0	11.4±0.1
PP-150°C-2 h	74.1±0.3	53.1±1.4	12.8±0.8	26.8±0.6	31.2±1.8	19.2±1.1
PP-150°C-3 h	58.8±1.8	56.3±4.2	11.2±0.1	24.1±1.4	46.1±1.8	27.0±2.0
PP-150°C-4 h	57.8±0.0	59.1±0.4	10.0±0.1	25.7±0.1	41.5±0.3	35.9±2.4
PHB-120°C-2 h	85.3±1.7	45.8±0.9	20.8±0.5	25.5±0.4	17.3±0.3	4.4±0.2
PHB-120°C-3 h	76.8±1.1	46.1±1.6	14.7±0.3	25.2±0.0	26.5±0.9	7.0±0.5
PHB-120°C-4 h	68.1±3.2	53.9±0.2	13.2±2.0	23.1±1.0	40.1±5.3	11.8±2.5
PHB-120°C-6 h	44.1±2.6	76.6±4.8	0	16.9±1.7	71.6±2.9	19.4±0.7
PHB-120°C-8 h	45.6±0.5	75.4±1.5	0	18.0±2.2	68.8±4.5	36.5±7.0
PP-120°C-2 h	78.6±0.1	48.2±0.3	12.5±0.3	29.3±0.1	20.2±0.2	8.3±0.2
PP-120°C-3 h	76.4±0.6	44.3±1.0	10.0±0.2	29.9±0.1	21.0±0.3	10.8±0.8
PP-120°C-4 h	76.4±0.1	41.4±3.7	9.9±0.9	29.2±0.7	22.8±2.1	8.8±0.2
PP-120°C-6 h	72.7±0.3	42.3±6.6	8.4±1.4	29.3±0.2	19.0±0.1	14.2±2.5
PP-120°C-8 h	67.3±0.8	48.4±2.1	7.2±1.1	29.3±0.5	25.0±0.4	15.8±0.1

In this study, the effects of different at particle sizes, PILs, biomass loading, reuse of PILs on the pretreatment of HB were investigated. The studied operation conditions are detailed in Table 4.2.

Table 4.2 Conditions used for pretreatment of HB via PILs

Parameter	Range
Particle sizes	< 0.15 mm, 0.15 mm- 1.18 mm, 1.18-2.00 mm, >2.00 mm
PILs	TEAHSO ₄ , HBIMHSO ₄ , HMMorpHSO ₄
Biomass Loading	10%, 20%, 30%, 40%, 50%
IL Recycle	up to 10-fold

Below are how HB looks like after separated according to certain particle sizes (Figure 4.1).

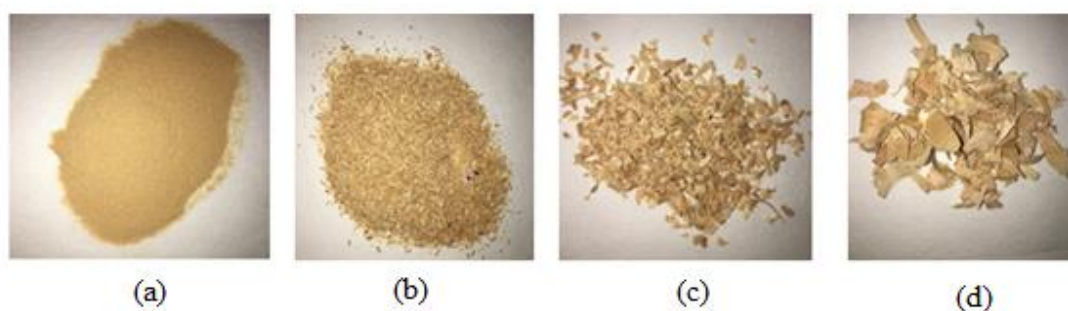


Figure 4.1 Particle sizes of <0.15 mm (a), 0.15 mm- 1.18 mm (b), 1.18-2.00 mm (c), >2.00 mm (d)

This is how UHB, PHB and extracted lignin shown visually (Figure 4.2).



Figure 4.2 UHB, PHB and extracted lignin

The general process flowchart is shown in Figure 4.3. The process started with UHB and ended up with the analysis of sugars from enzymatic hydrolysis of HB by HPLC. All pretreatments were conducted at 150 °C and 3 h in duplicate. After the pretreatments, two phases of products were obtained; liquid and solid phases through several separation and purification steps such as washing, filtration and centrifugation. Lignin was precipitated from the liquid phase and cellulose enriched biomass was derived from the solid phase.

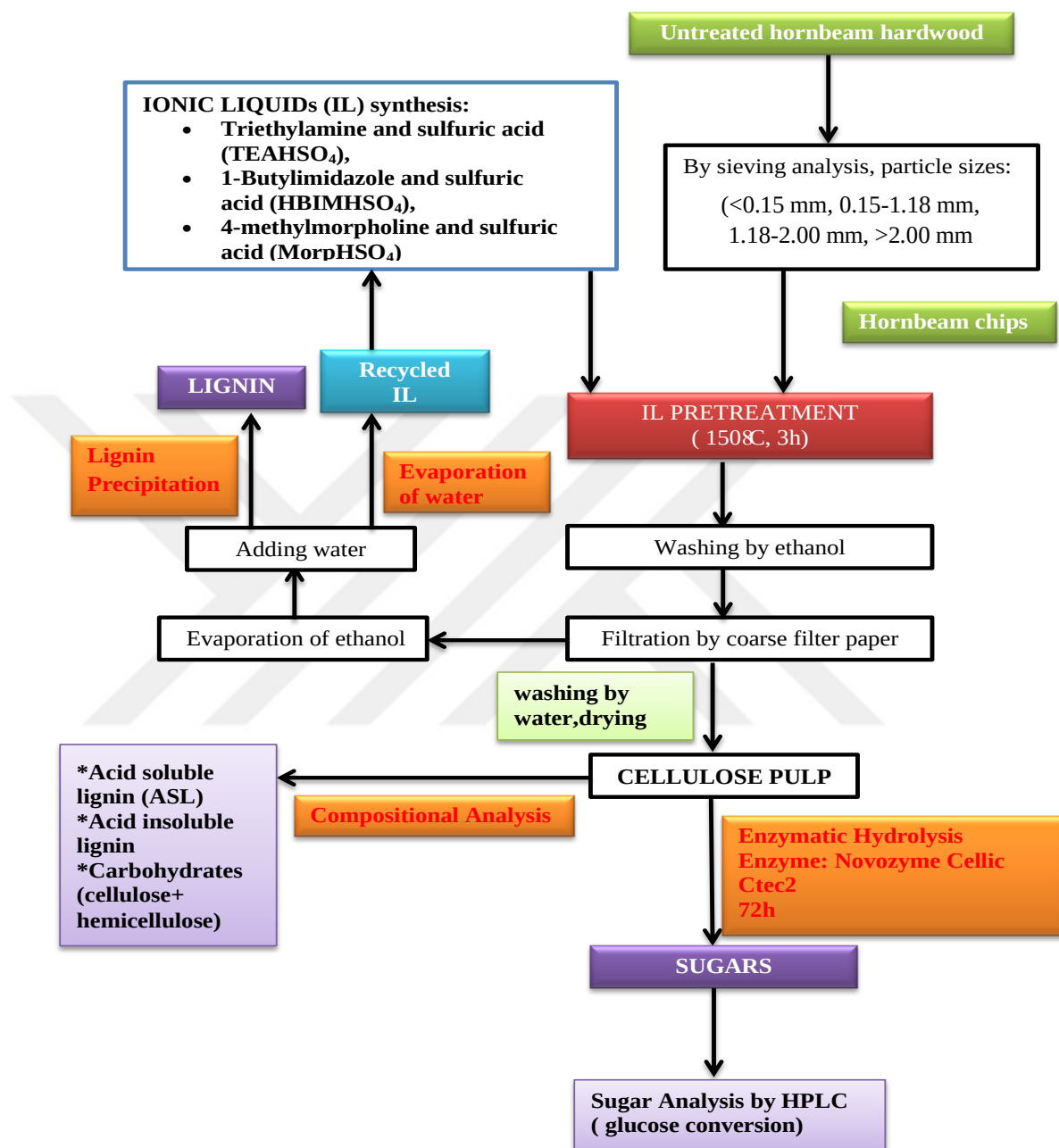


Figure 4.3 Process flowchart

4.1 Effects of PIL Type and Particle Size on Biomass Composition

Biomass composition analysis is important to demonstrate biomass' value, valorize the major constituents of biomass, and understand the structural modifications in the biomass. Table 4.3 shows the composition of untreated biomass samples and solid recovery and constituents of cellulose, hemicellulose, and lignin for the HB pretreated at 150 °C and for 3 h.

Table 4.3 Chemical compositions of HB before pretreatment and after pretreatment under certain conditions

PIL	Particle Sizes	Solid Recovery (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	LE (%)	LPY (%)
Untreated			34.2 ±0.6	17.0 ±1.7	26.3 ±0.0		
TEAHSO ₄	<0.15 mm	41.1±1.3	62.6±0.5	4.7±1.1	18.6±0.4	70.9±0.0	47.3±3.2
	0.15-1.18 mm	45.6±2.0	72.4±2.2	3.8±0.5	8.9±1.5	84.5±3.2	41.6±3.6
	1.18-2 mm	48.2±2.1	66.9±10.3	5.2±0.4	13.0±1.7	76.0±4.1	40.7±0.4
	>2 mm	51.4±3.6	65.8±5.5	5.5±0.4	13.2±1.9	74.0±5.5	24.4±4.9
HBIMHSO ₄	<0.15 mm	54.8±0.2	75.1±3.9	2.4±0.2	7.5±1.6	89.5±2.2	54.7±1.6
	0.15-1.18 mm	57.2±0.1	82.7±2.1	3.4±0.3	5.6±0.5	91.2±1.1	44.7±1.5
	1.18-2 mm	55.7±0.6	78.4±2.2	3.7±0.0	10.4±0.9	83.2±1.3	42.6±2.5
	>2 mm	59.6±0.0	77.9±8.7	3.1±0.4	9.5±1.8	84.5±2.9	46.0±4.9
HMMorpHSO ₄	<0.15 mm	37.0±1.0	52.6±2.9	2.2±0.4	31.4±1.1	34.5±2.6	16.6±3.9
	0.15-1.18 mm	41.0±0.3	59.8±2.3	2.3±0.6	30.7±1.2	33.2±2.8	19.3±2.6
	1.18-2 mm	42.5±0.2	55.6±0.9	1.8±0.1	29.8±0.0	36.9±0.5	26.5±1.3
	>2 mm	43.0±0.4	43.0±4.5	3.7±0.6	30.5±3.9	31.0±8.7	20.7±0.4

Biomass' particle size influenced the solid recovery. Since IL diffusion to the interior parts of the biomass and ILs' capability to have an effective interaction with the biomass have been related to the particle size of the biomass (Kilpeläinen et al., 2007, Bahcegul et al., 2012b). However, utilizing larger biomass particle sizes is more favorable because particle size reduction increases energy requirements in the process (Yang and Wyman, 2008, Zhu and Pan, 2010).

As shown in Table 4.3, the solid recovery changed from 41% to 51%, from 55% to 60%, from 37% to 43% for HB with an increase in particle sizes subjected to TEAHSO₄ pretreatment, HBIMHSO₄ pretreatment, HMMorphHSO₄ pretreatment, respectively. This implies that solid recoveries increase with an increase in particle size. Due to a decrease in surface area, PILs did not easily interact with the interior structure of HB.

The highest cellulose contents were obtained at a particle size between 0.15- 1.18 mm. Pretreatments conducted with TEAHSO₄, HBIMHSO₄, and HMMorphHSO₄, respectively increased cellulose content of biomass from 34% to 72%, 83%, and 60%.

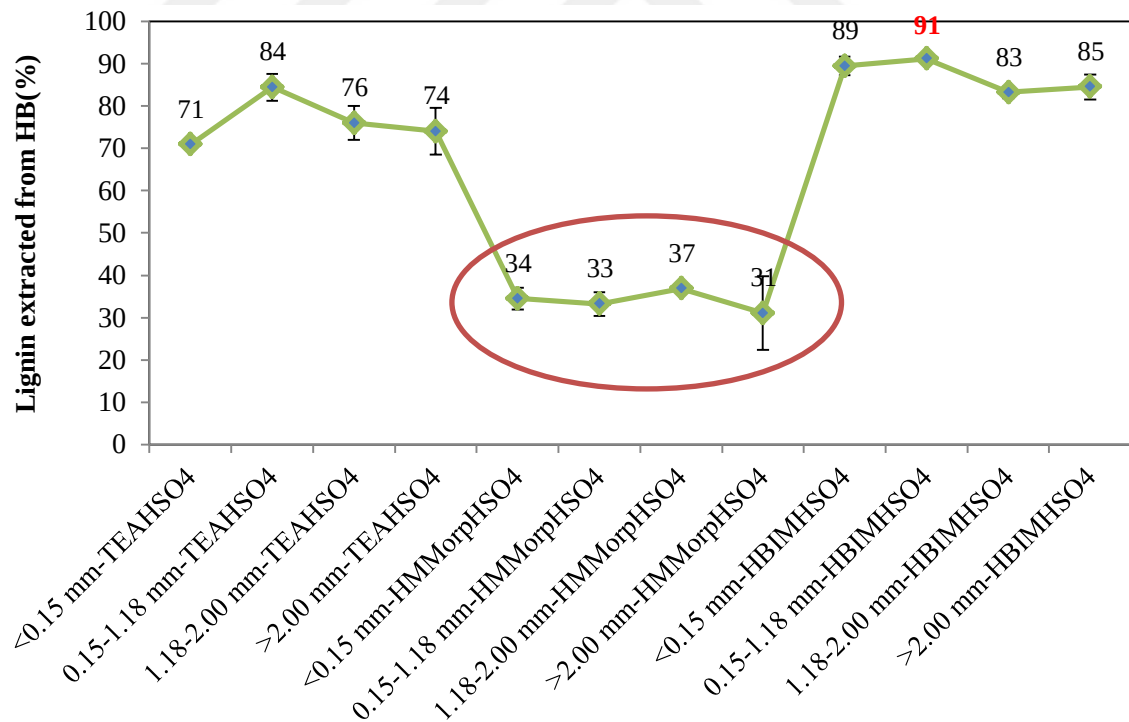


Figure 4.4 The variation of lignin extracted from HB with PIL type and biomass particle size

The lignin extraction with respect to IL type and particle size was evaluated because the lignin extraction capacity of IL is a significant parameter. As seen in Figure 4.4 HBIMHSO₄ and TEAHSO₄ showed superior performances compared to HMMorpHSO₄ (demonstrated via the red circle) considering the compositional changes in the biomass.

Concerning the literature, Verdia et al. (2014) studied using HBIMHSO₄ with C₄im : H₂SO₄ = [1.00 : 1.01]_{80%} pretreatment for *Miscanthus giganteus* at 120 °C. They found 91% lignin removal after the 24 h pretreatment. In another study, Brandt-Talbot and her co-workers (2017) investigated TEAHSO₄ pretreatment in the presence of 20% of water on the same biomass at 120 °C and 10% loading and found more than 80% extraction lignin from biomass. George et al. (2014) studied for TEAHSO₄ pretreatment of switchgrass and obtained roughly 80% extraction lignin from biomass.

Current results have been consistent with previously reported findings (Verdia et al., 2014; George et al., 2014; Brandt-Talbot et al., 2017).

4.2 Effects of Biomass Loading on Biomass Composition

Previous work on PIL pretreatments were generally conducted at lower biomass loadings. Achinivu et al. (2014) studied PyrAc pretreatment of corn stover at 5% biomass loading. In the study performed by Rocha et al. (2017), sugarcane bagasse was subjected to four different PILs pretreatment at 5% biomass loading. They showed that of the four PILs, 2-HEAA gave the best performance. In another study, Reis et al. (2017) studied pretreatments of cashew apple bagasse using 2-HEAA at 5% loading. Uju et al., (2013) validated the potential of 1-hexylpyridinium chloride (HPyCl) for the pretreatment of bagasse at 5% loading.

Unlike the aforementioned loadings, Weigand et al. (2017) investigated TEAHSO₄ pretreatment of willow at 20% biomass loading. In another study, Semerci and Güler (2018) investigated various PILs' performances on cotton stalks at 15% loading.

Less is known about the effects of biomass loading on PIL pretreatment and biomass loading as high as 20% has not been used in the literature yet. Therefore, biomass loading between 10% and 50% was used for HB subjected to TEAHSO₄ and

HBIMHSO₄ pretreatments for 3 h at 150 °C. Related results are shown in Table 4.4. The highest cellulose content was obtained at 20% biomass loading for HBIMHSO₄ pretreated biomass. Also, solid recoveries increased as the biomass loading increased from 10% to 50%. Since PILs interact with biomass effectively at lower biomass loadings.

Table 4.4 Effects of biomass loading on compositional analysis

PIL	Particle Sizes	Biomass Loading (%)	Solid Recovery (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	LE (%)	LPY (%)
Untreated			34.2 ±0.6	17.0 ±1.7	26.3 ±0.0			
TEAHSO ₄	0.15	10	45.6±2.0	72.4±2.2	3.8±0.5	8.9±1.5	84±3.2	41.6±3.6
		20	45.5±0.4	63.5±0.9	4.2±0.9	13.4±1.0	76.9±1.5	48.2±2.1
		30	48.1±0.4	62.7±0.5	4.0±0.8	16.5±0.6	69.8±0.7	45.9±2.0
		40	53.0±0.9	61.8±0.6	5.7±1.7	21.6±1.6	56.6±2.4	29.6±3.1
		50	62.3±3.4	53.4±1.8	6.3±0.1	20.1±1.1	52.5±0.0	28.0±0.1
HBIMHSO ₄	1.18 mm	10	41.0±1.0	82.7±2.1	3.4±0.3	30.7±1.2	91.2±1.1	44.7±1.5
		20	43.3±0.1	74.7±0.0	1.9±0.0	13.5±1.0	77.8±1.8	50.1±0.4
		30	47.4±0.1	70.3±0.0	2.3±0.0	16.8±2.2	69.7±4.0	42.7±0.1
		40	49.7±1.0	71.7±2.5	2.6±0.8	22.0±1.7	58.3±4.1	37.6±3.1
		50	53.6±1.0	76.2±12.3	2.8±0.4	25.4±3.3	48.0±7.7	33.3±1.4

Interestingly, both TEAHSO₄ and HBIMHSO₄ can remove at least 77 % lignin from biomass at 20% loading (Figure 4.5). Therefore, the study was continued with this loading for recycling experiments.

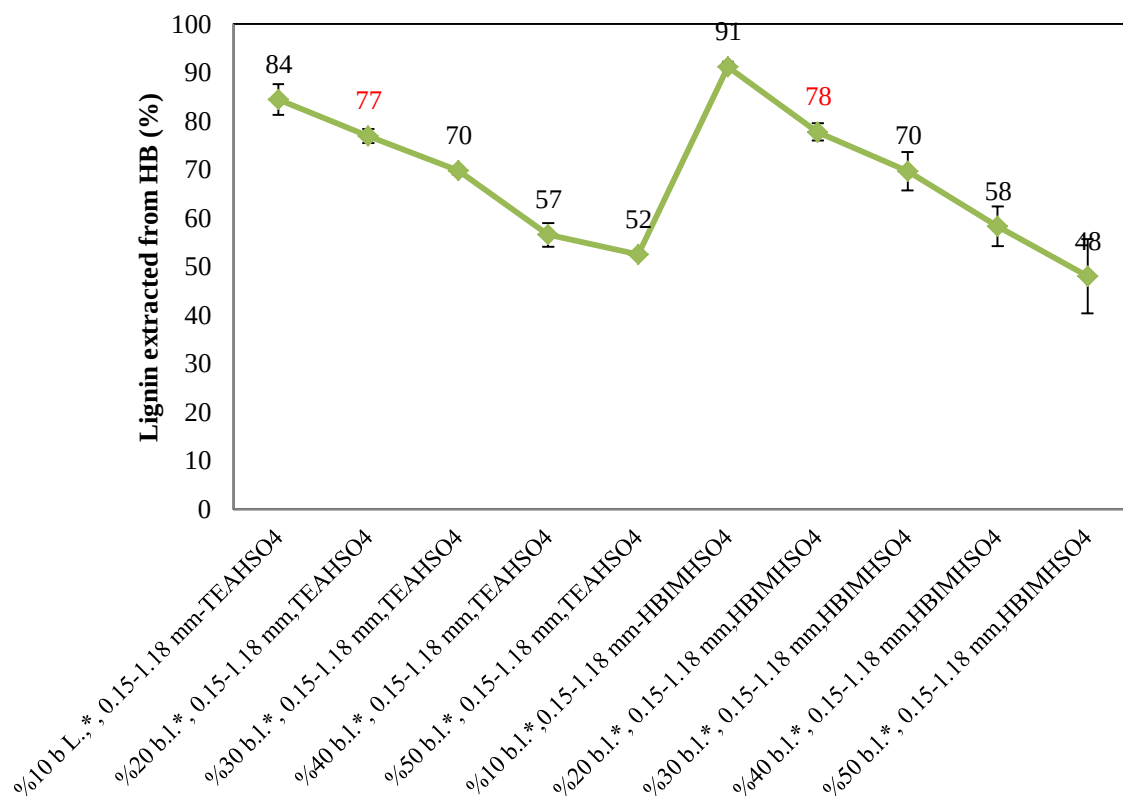


Figure 4.5 The variation of lignin extracted from HB with PIL type and biomass loading

4.3 Effects of the IL Recycling on the Biomass Composition

Both the recycled TEAHSO₄ and HBIMHSO₄ were generated from pretreatments using 20% loading of HB at 150 °C for 3h. Recycled PILs were treated with rotary evaporator after lignin water washes. Recycled TEAHSO₄ experiments were conducted in duplicate whereas HBIMHSO₄ experiments were at once due to a pair tube was broken unfortunately. Recycling of PILs were conducted for ten times. The effects of PIL type and recycle number on the biomass composition of HB were studied and the results are presented in Table 4.5. As shown in Table 4.5, more than 50% of solid recovered upon TEAHSO₄ pretreatment, whereas at least 45% solid recovery were obtained on HBIMHSO₄ pretreatment when both ILs recycled for 10 times. It was noted that the lignin extraction from HB decreased with the increase of reuse cycles. This likely arose from the partial deterioration of delignification capacity of recycled PILs. Percent cellulose and hemicellulose content decreased with an increase in reuse number of both

PILs. Cellulose, hemicellulose, and lignin percentage contents were obtained up to seven-fold recycle of TEAHSO₄ and up to six-fold recycle of HBIMHSO₄.

Table 4.5 Effects of TEAHSO₄ and HBIMHSO₄ recycling on the biomass composition

PIL	Particle Sizes & Biomass Loading	Reuse	Solid Recovery (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	LE (%)	LPY (%)
Untreated				34.2 ±0.6	17.0 ±1.7	26.3 ±0.0		
TEAHSO ₄	0.15-1.18 mm & 20% b.l.	0.	45.5±0.4	63.5±0.9	4.2±0.9	13.4±1.0	76.9±1.5	48.2±2.1
		1.	53.3±0.8	86.7±1.7	8.8±0.5	17.4±0.2	64.7±0.8	54.5±2.0
		2.	60.3±4.7	79.8±9.0	12.8±3.2	23.1±0.2	47.0±3.7	36.9±7.6
		3.	54.0±0.2	82.5±1.8	8.8±1.4	22.5±0.6	53.9±1.0	51.7±0.2
		4.	56.2±1.4	81.9±0.2	8.6±1.0	22.9±0.6	51.1±2.4	46.1±2.5
		5.	54.2±0.5	84.5±2.6	7.6±0.8	24.5±0.0	49.6±0.5	45.5±0.7
		6.	50.7±0.1	83.5±1.8	9.1±1.2	21.9±0.1	57.8±0.0	36.5±0.5
		7.	50.2±0.4	81.3±0.0	8.7±0.0	21.9±0.0	58.5±0.0	31.0±0.8
		8.	55.0±0.7	-	-	-	-	27.9±0.3
		9.	51.9±0.8	-	-	-	-	24.7±0.8
HBIMHSO ₄		10.	52.8±1.0	-	-	-	-	43.8
		0.	43.3	74.7	1.9	13.0	77.8	50.1
		1.	47.3	98.5	4.1	14.8	73.5	74.4
		2.	49.5	95.0	3.1	20.0	62.4	67.5
		3.	52.3	90.8	2.7	22.2	55.7	49.6
		4.	50.4	88.6	2.9	24.5	53.2	57.8
		5.	46.1	90.5	3.9	20.4	64.3	48.5
		6.	49.6	86.3	3.3	22.8	57.1	44.0
		7.	49.6	-	-	-	-	35.8
		8.	46.5	-	-	-	-	40.8
		9.	47.5	-	-	-	-	21.7
		10.	58.4	-	-	-	-	

4.4 Effects of PIL Pretreatment on the Structure of Recovered Solids

The structural changes of recovered solids were interpreted through SEM, XRD, and ATR-FTIR analysis. Firstly, the structure of HB upon pretreatment via different PILs with four varieties of particle size was analyzed. Secondly, the structure of HB upon pretreatment through different PILs type at a particle size of 0.15-1.18 mm was analyzed and was compared to the untreated HB. Thirdly, the effects of high biomass loading on the structure were analyzed, respectively. Afterwards, the structural changes for the HB pretreated via recycled-TEAHSO₄ and via recycled- HBIMHSO₄ were analyzed.

Initially, the structural modifications in HB upon four different PILs pretreatment was monitored by SEM analysis. HB pretreatment via TEAHSO₄, HMMorpHSO₄, and HBIMHSO₄ with four-particle sizes (<0.15 mm, 0.15-1.18 mm, 1.18-2.00 mm, > 2.00 mm) was demonstrated in Figure 4.6. According to the images, the most significant modifications were obtained at a particle size between 0.15-1.18. So, the findings obtained via SEM images supported the compositional changes in HB.

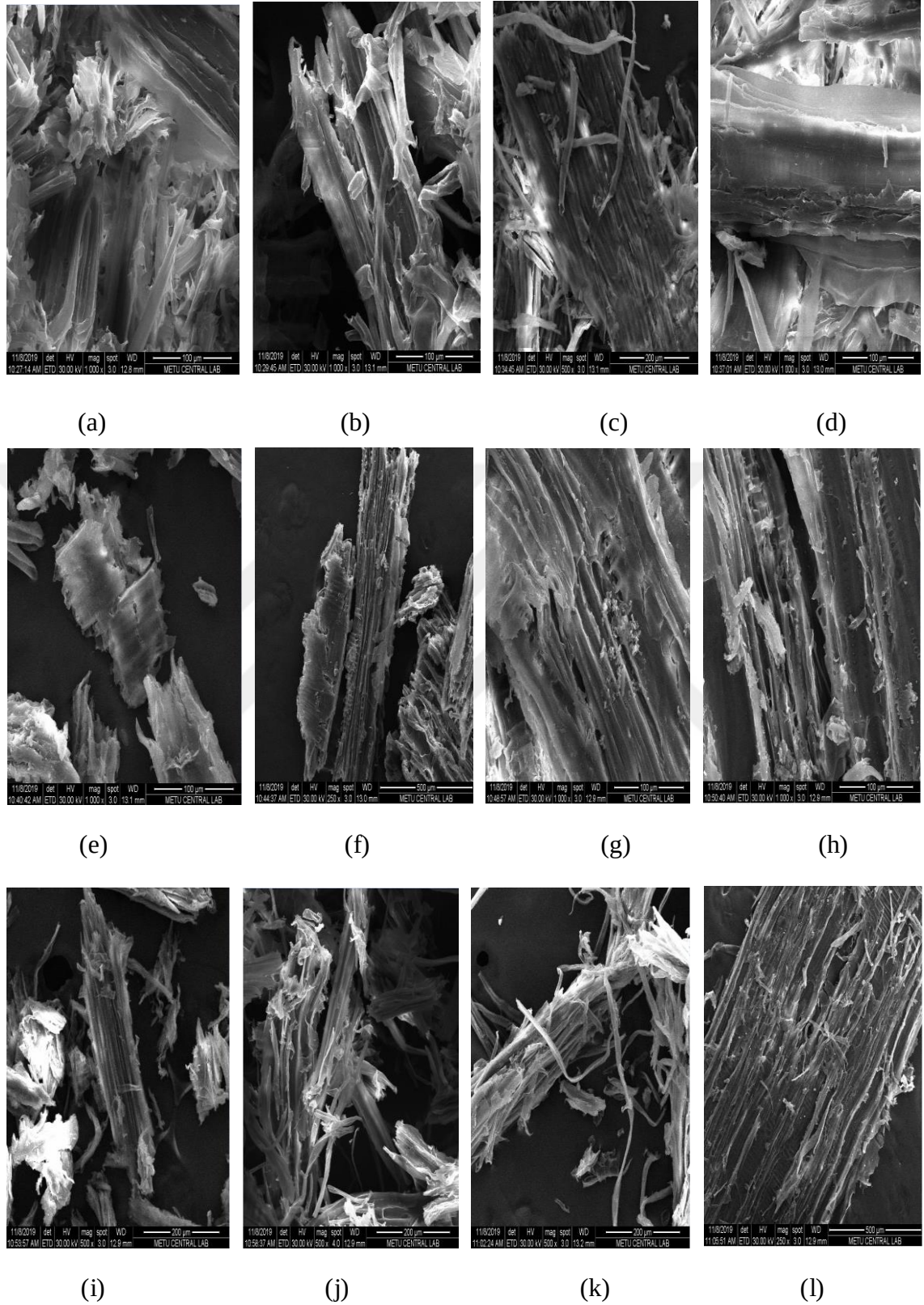


Figure 4.6 SEM images of pretreated HB via TEAHSO₄ with particle size of less than 0.15 mm (a), 0.15-1.18 mm(b), 1.18-2.00 mm(c), more than 2.00 mm (d);

HMMorPHSO₄ with particle size of less than 0.15 mm (e), 0.15-1.18 mm(f), 1.18-2.00 mm(g), more than 2.00 mm (h); HBIMHSO₄ with particle size of less than 0.15 mm (i), 0.15-1.18 mm(j), 1.18-2.00 mm(k), more than 2.00 mm (l)

The structural morphology of HB upon pretreatment via three different PILs and untreated HB was visualized by SEM as shown in Figure 4.7. Images were taken at magnifications, 500X. According to the images, untreated hornbeam showed an intact and stiff macrofibrillar structure. TEAHSO₄ pretreated biomass presented neat cellulose macro fibers as clearly seen whereas for HMMorPHSO₄ pretreated biomass, it is not the case. In HBIMHSO₄, cellulose disruption was presented. SEM images appropriately verified the compositional changes in the HB.

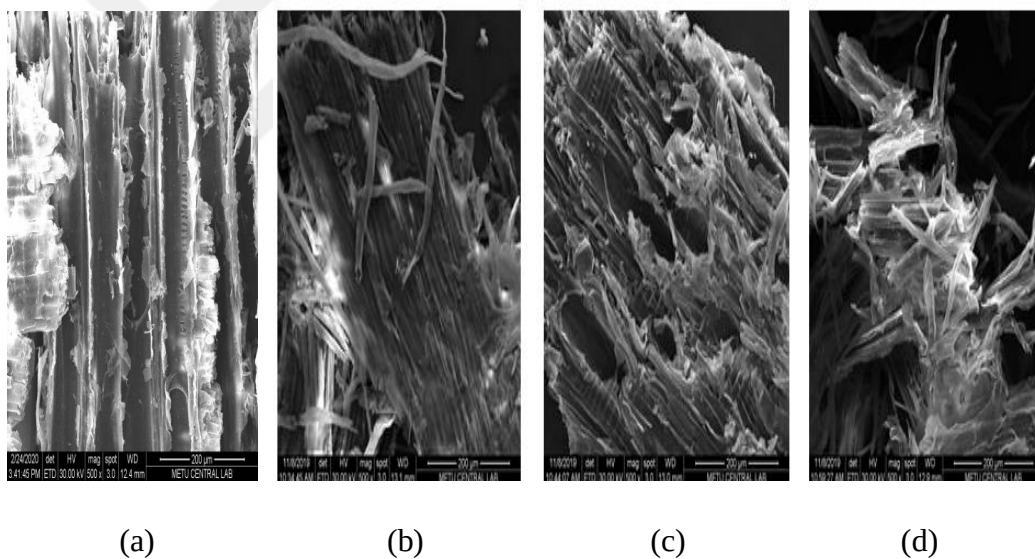


Figure 4.7 SEM images of untreated HB (a), pretreated HB via TEAHSO₄ (b), HMMorPHSO₄ (c), HBIMHSO₄ (d) with particle size of 0.15-1.18 mm at magnification of 500X

SEM images in Figure 4.8 presented the effects of TEAHSO₄ and HBIMHSO₄ at high biomass loading. As revealed in Figure 4.8(a) and (b), minimal modifications observed

with increasing biomass loading for TEAHSO₄ pretreated samples, whereas in Figures 4.8 (c) and (d), cellulose fibers were more clearly separated from each other.

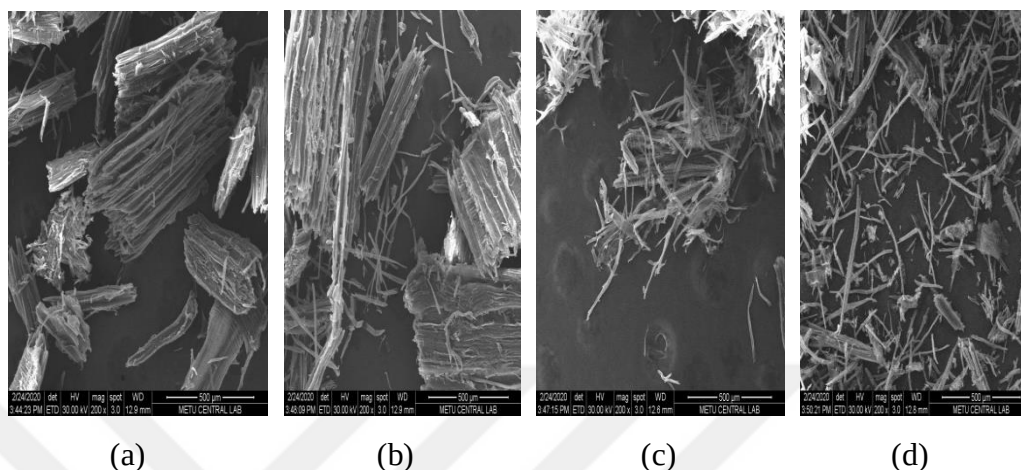


Figure 4.8 SEM images of pretreated HB with TEAHSO₄ at 20% and 50% biomass loading, respectively, (a), (b); pretreated HB with HBIMHSO₄ at 20% and 50% biomass loading, respectively, (c), (d)

As shown in Figure 4.9, untreated HB, pretreated HB via non-recycled and recycled TEAHSO₄, and HBIMHSO₄ were visualized by SEM analysis. According to the SEM images, pretreated HB via 10-fold recycled-TEAHSO₄ (Figure 4.9c) resembled to untreated HB (Figure 4.9a). However, SEM image of HB pretreated with 10-fold recycled-HBIMHSO₄ was similar to that of non-recycled HBIMHSO₄.

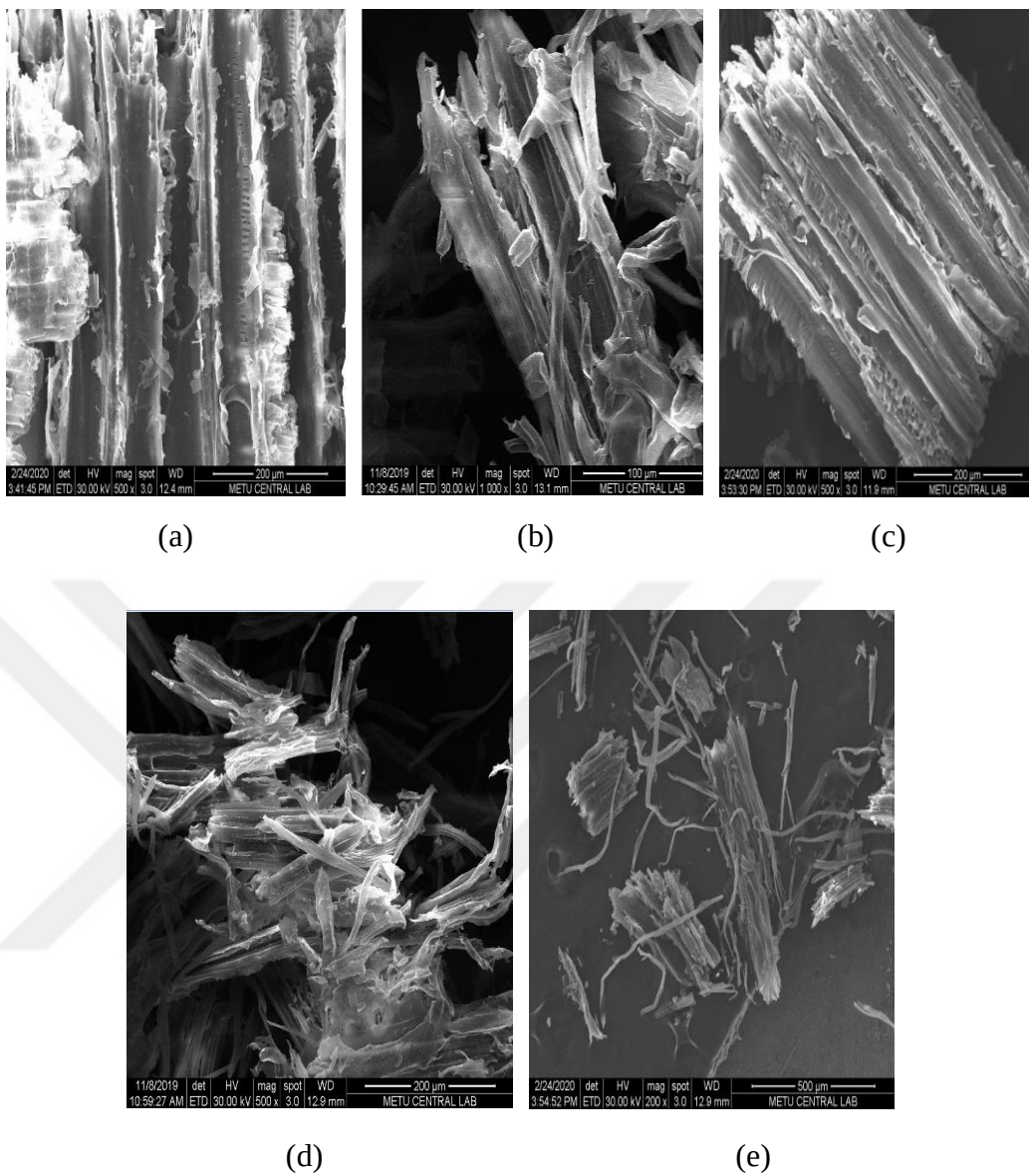


Figure 4.9 SEM images of untreated HB (a), pretreated HB via non-recycled TEAHSO₄ (b), 10-fold recycled TEAHSO₄ (c), non-recycled HBIMHSO₄ (d), and 10-fold recycled HBIMHSO₄ (e)

The crystalline structure of the biomass is another significant parameter that should be evaluated to acknowledge the changes in the biomass structure. Due to this reason, untreated and pretreated HB was assessed with respect to their crystalline structure via XRD analysis. As revealed in Figures from 4.10 to 4.13, crystalline structure of HB was especially represented with the changes in two diffraction peaks at around $2\theta=18^\circ$ and 22.5 , respectively. As shown in Figure 4.10, XRD patterns of UHB with various particle sizes were exhibited. According to Figure 4.10, biomass with particle size of 0.15-1.18 mm displayed utterly a different diffraction pattern compared to other particle sizes based on the peaks at $2\theta=18^\circ$ and 22.5 . Figures 4.11 and 4.12 demonstrated the effect of various particle sizes for TEAHSO₄ and HBIMHSO₄ pretreated HB. Figure 4.13 exhibited the variations in XRD patterns with different biomass loadings during TEAHSO₄ and HBIMHSO₄ pretreatments.

As shown in Table 4.6, CrI indices of the UHB, pretreated TEAHSO₄ and HBIMHSO₄ HB with various biomass particle sizes (<0.15 mm, 0.15-1.18mm, 1.18 -2.00 mm, >2.00 mm) were demonstrated. Besides, CrI indices of HB at high biomass loadings (20% and 50%) were shown in Table 4.7. Accordingly, CrI (%) indices increased from 60.6 to 63.0 and 72.7 for the biomass (0.15-1.18 mm) pretreated with TEAHSO₄ and HBIMHSO₄ pretreatment, respectively. Because of the effective removal of amorphous fractions of the structure with PIL, CrI (%) indices of the samples increased. Even for HB pretreatment at %50 biomass loading, CrI (%) indices were quite high.

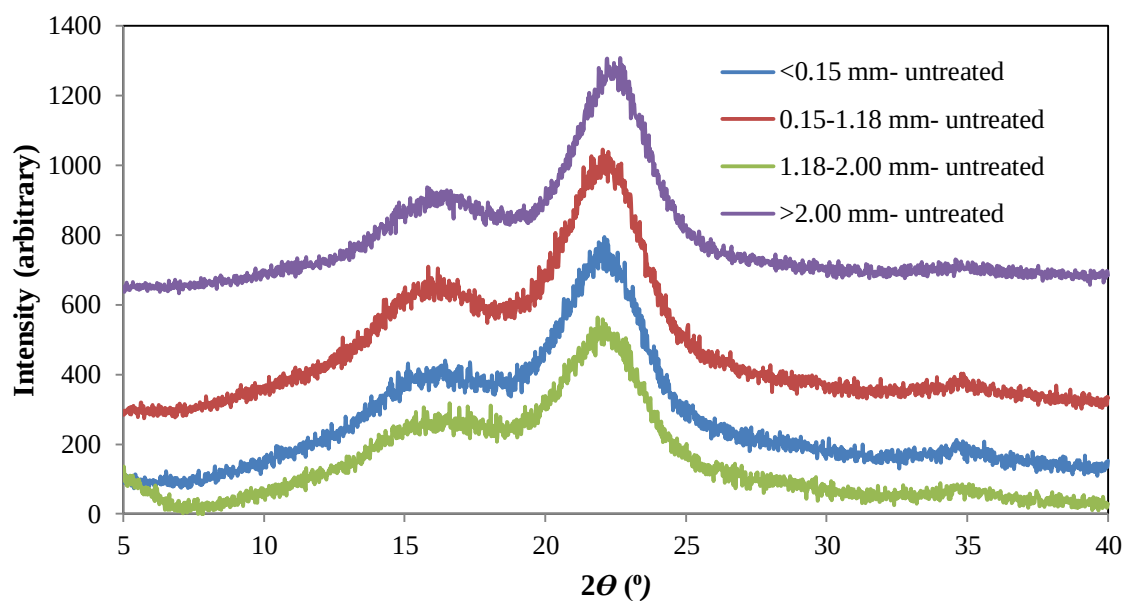


Figure 4.10 XRD pattern for HB before pretreatment with four different particle sizes

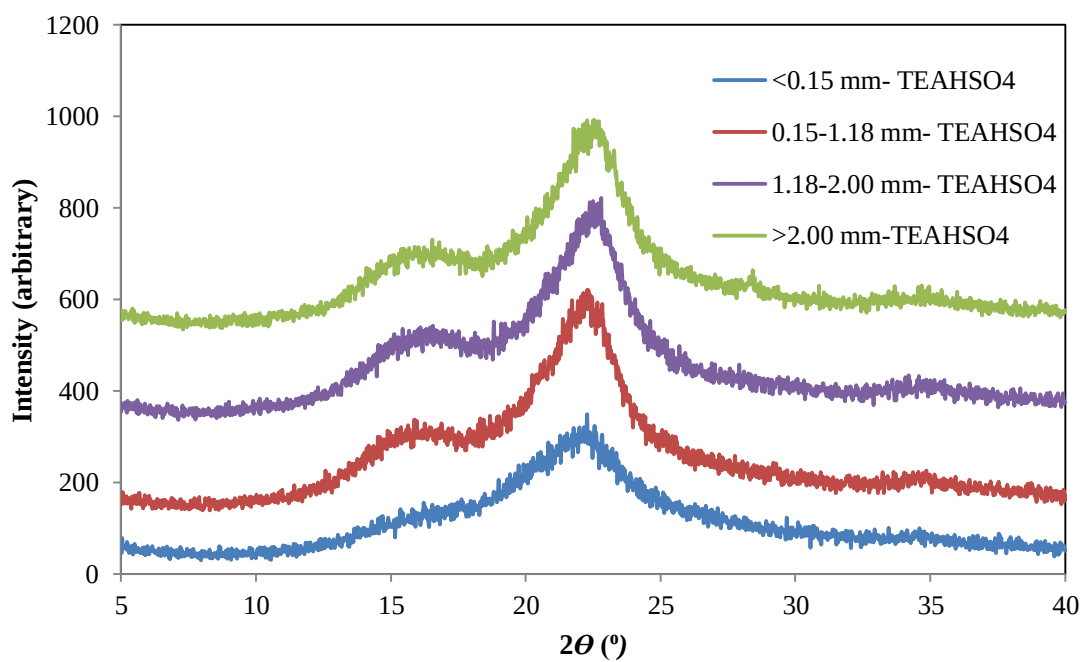


Figure 4.11 XRD pattern for HB pretreated via TEAHSO₄ with different particle sizes

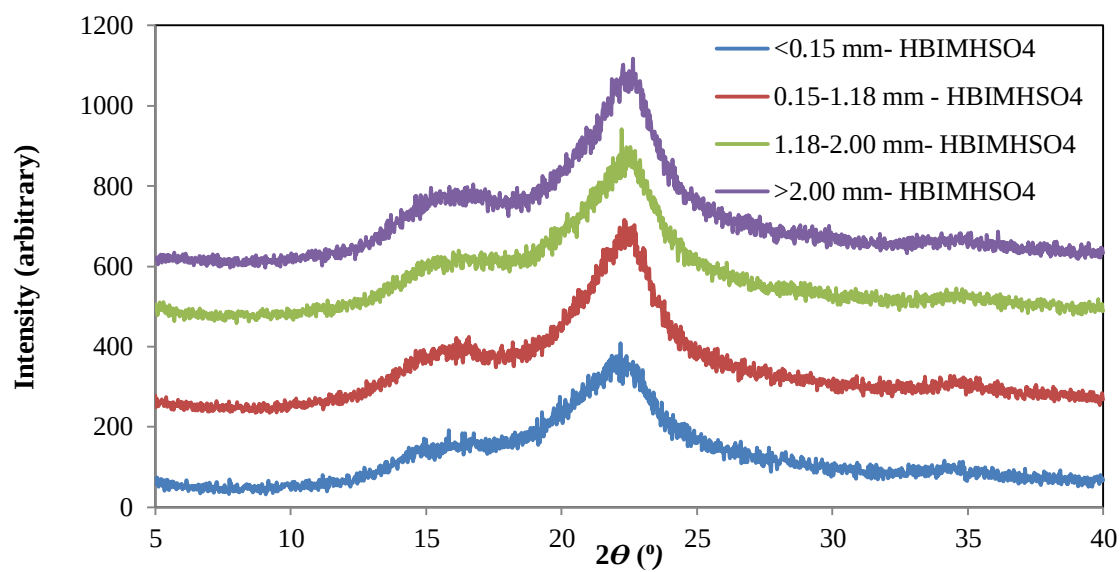
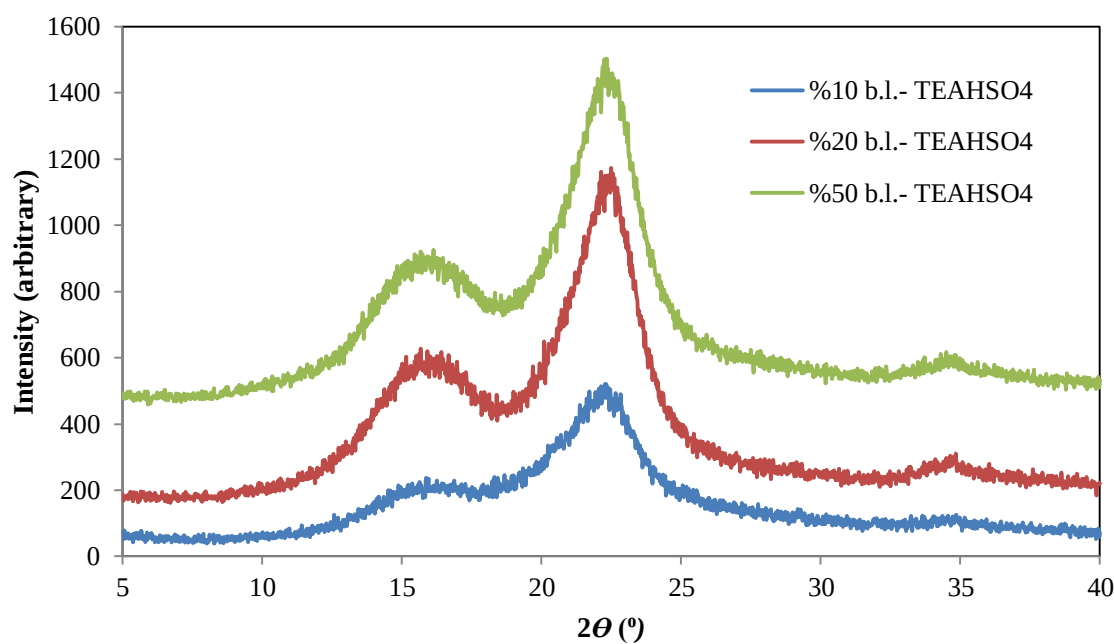


Figure 4.12 XRD pattern for HB pretreated via HBIMHSO₄ with different particle sizes



(a)

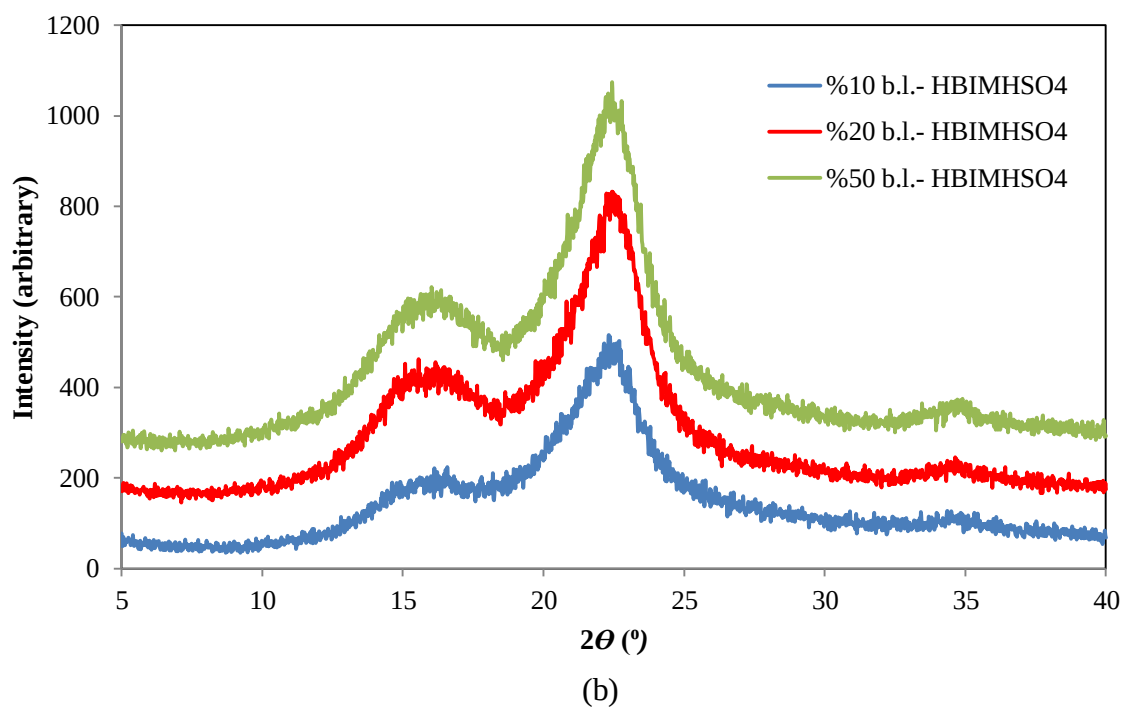


Figure 4.13 XRD pattern for HB pretreated via TEAHSO₄ (a), HBIMHSO₄ (b) with particle sizes of 0.15-1.18 mm at high biomass loading

Table 4.6 CrI indices of untreated, TEAHSO₄ and HBIMHSO₄ pretreated biomass with different particle sizes

Particle size	PIL	CrI (%)
<0.15 mm	Untreated	55.0
	TEAHSO ₄	61.3
	HBIMHSO ₄	65.5
0.15-1.18 mm	Untreated	60.6
	TEAHSO ₄	63.0
	HBIMHSO ₄	72.7
1.18-2.00 mm	Untreated	49.5
	TEAHSO ₄	66.2
	HBIMHSO ₄	60.9
>2.00 mm	Untreated	66.9
	TEAHSO ₄	72.3
	HBIMHSO ₄	66.7

Table 4.7 CrI indices of TEAHSO₄ and HBIMHSO₄ pretreated biomass at different biomass loadings

PIL	Biomass loading (%)	CrI (%)
Untreated	-	60.6
TEAHSO ₄	10	63.0
	20	74.8
	50	69.8
	10	72.7
HBIMHSO ₄	20	70.8
	50	70.4

The structural modifications in the untreated and pretreated samples were also interpreted through ATR-FTIR analysis. ATR-FTIR spectra for UHB and HB pretreated via TEAHSO₄, HMMorpHSO₄ and HBIMHSO₄ at particle sizes of 0.15-1.18 is given Figure 4.14. As indicated in Figure 4.14, the signals assigned to the presence of lignin (Xu et al., 2013) were found to decrease for PIL pretreated samples compared to UHB (in the bands 1200-1300 cm⁻¹). Specifically, peak intensities got lower and peaks got broadened for HBIMHSO₄ and TEAHSO₄ pretreated samples. Besides, the bands between 896–1200 cm⁻¹ due to crystalline cellulose (Gaur et al., 2016), which was preserved during PIL pretreatments, got more intense for PIL pretreated biomass. Regarding the decrease in the bands at around 1260 and 1510 cm⁻¹ predicted the disruption of lignin.

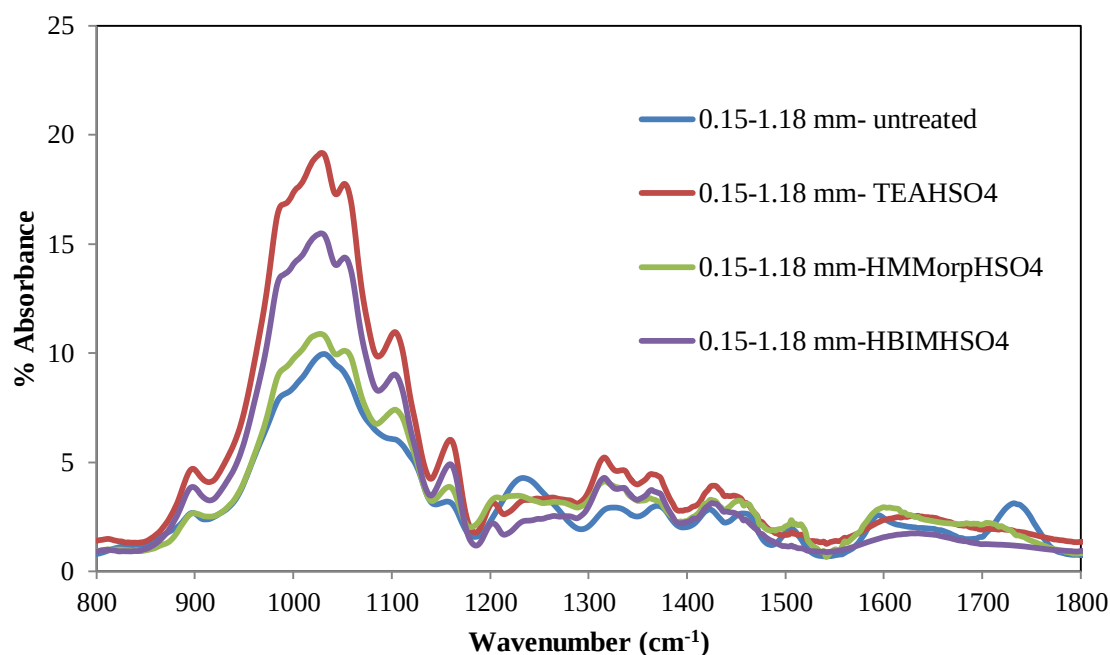


Figure 4.14 ATR-FTIR spectra for UHB and HB pretreated via TEAHSO₄, HMMorPHSO₄ and HBIMHSO₄ at particle sizes of 0.15-1.18

4.5 Effects of PILs Pretreatment on the Enzymatic Hydrolysis of Biomass

After pretreatments, pretreated biomass was examined for 72 h regarding its enzymatic conversion. Glucose yields (%) were shown in Figures 4.15-4.18 in which the effects of particle sizes, biomass loading, and IL recycling on the conversion of the cellulose into glucose was presented. According to the Figure 4.15, glucose yields decreased with increasing particle sizes. Figure 4.16 demonstrated the highest glucose yield was obtained roughly 88% for HBIMHSO₄ pretreatment. As shown in Figure 4.17, the highest glucose yield was attained at 20% biomass loading for both TEAHSO₄ and HBIMHSO₄ pretreatments. Figure 4.18 indicated glucose yield remained quite high up to 5-fold recycling for both the PILs. These findings were also consistent with the composition of the HB.

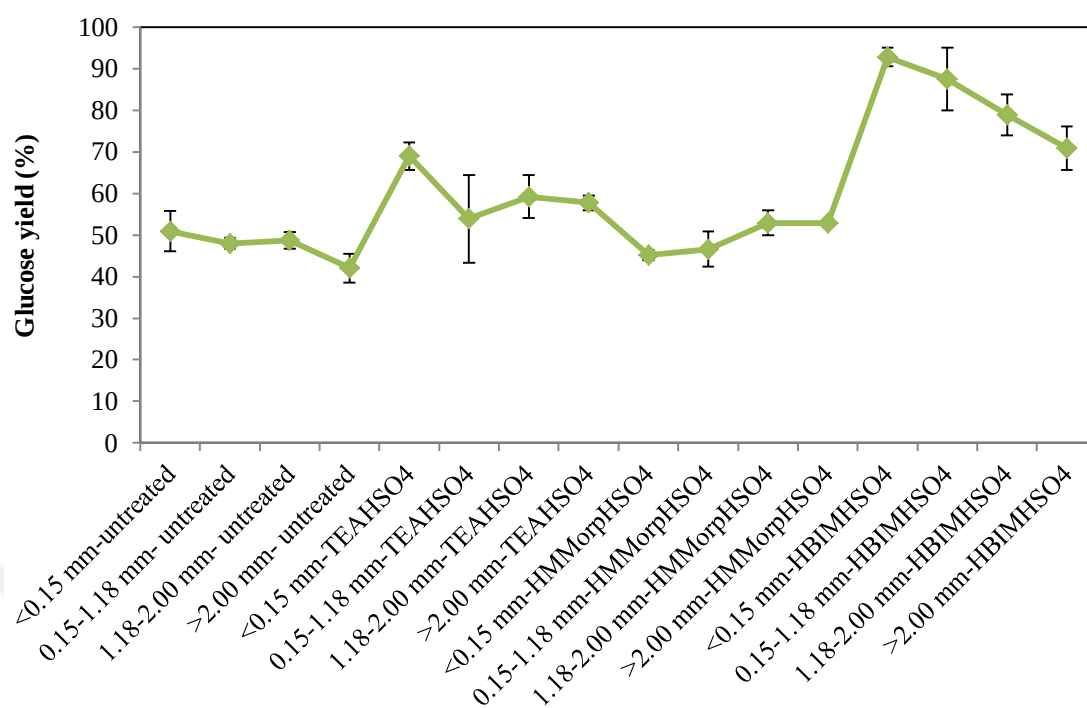


Figure 4.15 Glucose yields obtained of enzymatic hydrolysis of UHB and HB subjected to TEAHSO₄, HMMorPHSO₄, and HBIMHSO₄ pretreatments at various particle sizes

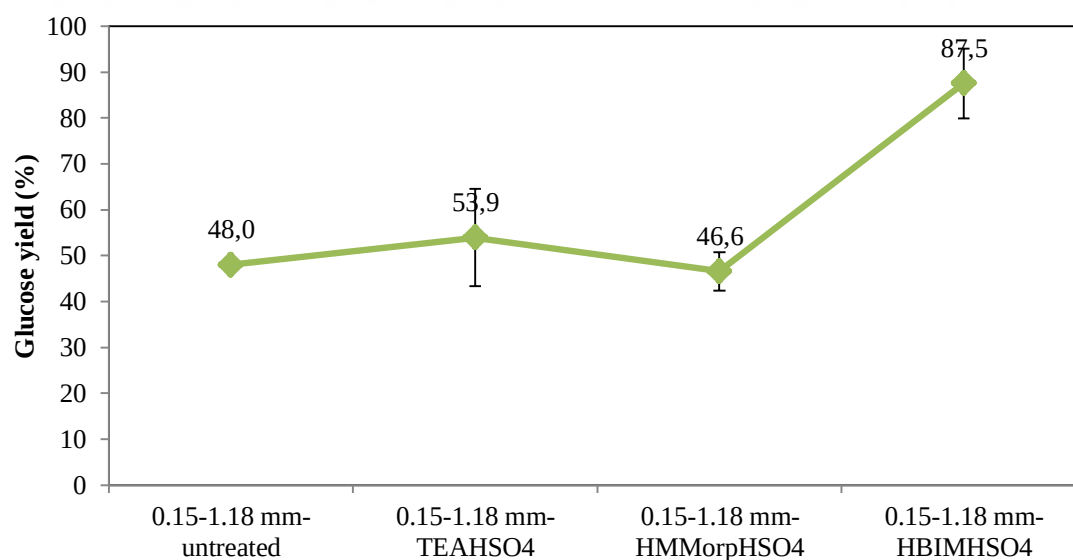


Figure 4.16 The variations of glucose yield of enzymatic hydrolysis of UHB and HB subjected to TEAHSO₄, HMMorPHSO₄, and HBIMHSO₄ pretreatments at particle sizes of 0.15-1.18 mm

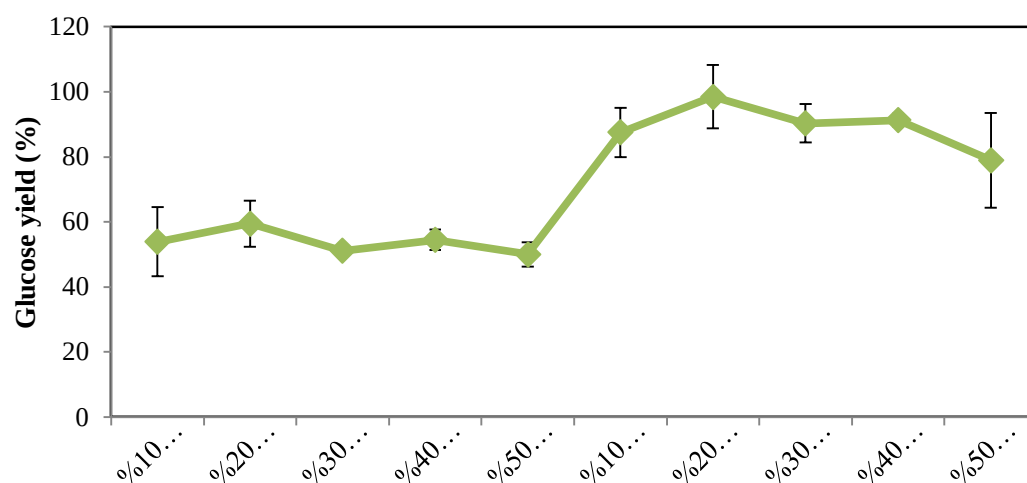


Figure 4.17 Glucose yields obtained of enzymatic hydrolysis of HB subjected to TEAHSO₄ and HBIMHSO₄ pretreatments with biomass loading

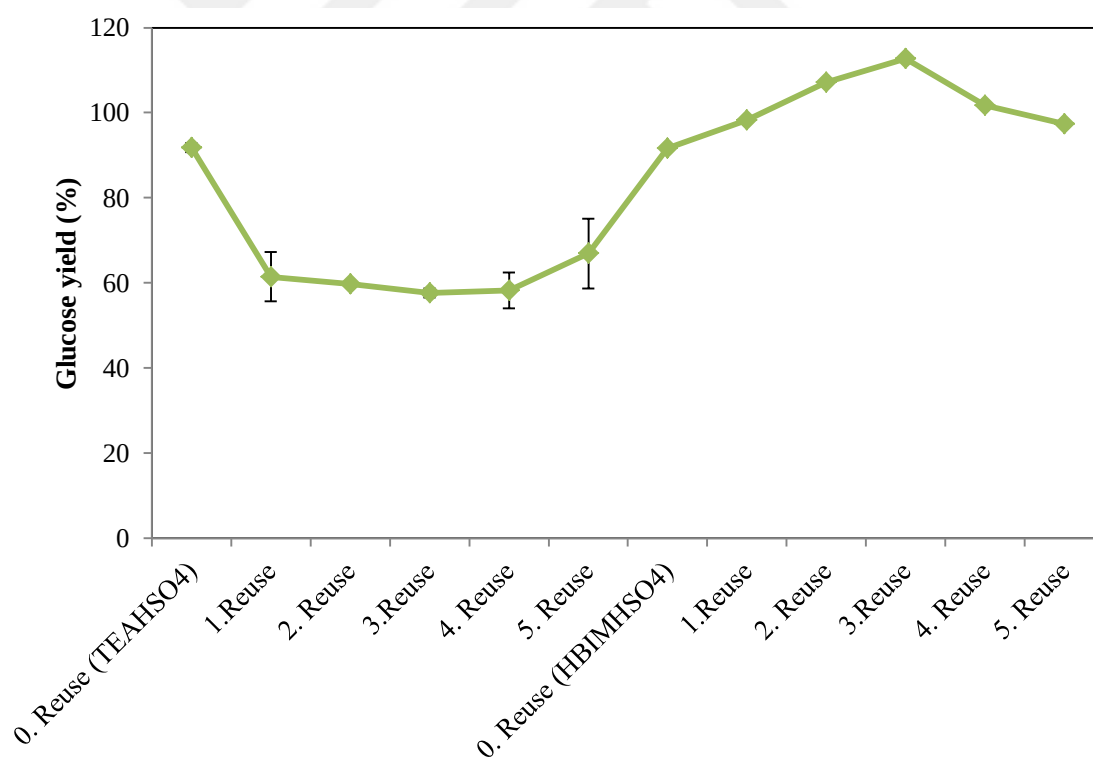


Figure 4.18 Glucose yields obtained of enzymatic hydrolysis of HB pretreated via non-recycled TEAHSO₄, HBIMHSO₄ and recycled TEAHSO₄, HBIMHSO₄

4.6 Cost Analysis

ILs' cost has been recognized as one of the important obstacles to IL employment in the biomass pretreatment processes. For this reason, use of inexpensive starting chemicals such as the bases, triethylamine, 1- butylimidazole and 1-methylimidazole and the acids, acetic acid and sulfuric acid promise significant advantages for the economics of biomass processing. As shown in Table 4.8, the synthesis costs for the typical PILs and the APIL, EMIMAc per gram of biomass are given. In the cost calculation, Sigma-Aldrich website is referred (SigmaAldrich, 2020).

Table 4.8 The results of the cost analysis of TEAHSO₄ and HBIMHSO₄

PIL/APIIL	Cost (€/g biomass)
TEAHSO ₄	0.83
HBIMHSO ₄	4.00
EMIMAc	240

As shown, there is a remarkable difference between the ILs' costs calculated per gram biomass. Cost of HBIMHSO₄ is roughly 5-fold greater than that of TEAHSO₄. On the other hand, EMIMAc as a powerful agent in this field constitutes a challenge for the process economics with its current price. Furthermore, performing PIL pretreatment at a higher biomass loading as high as 50% (w/w) can alleviate the costs without a doubt as verified in the present thesis; price of each PILs can decrease by one fifth. The last but not the least, PILs' reuse also matter to render their use economically competitive.

5. CONCLUSION AND RECOMMENDATIONS

This thesis focused on the pretreatment of hardwood, hornbeam with the protic ionic liquids (PILs), triethylammonium hydrogen sulfate (TEAHSO_4), 1-butylimidazolium hydrogen sulfate (HBIMHSO_4) and 4-methylmorpholinium hydrogen sulfate (HMMorPHSO_4). The effects of different biomass particle sizes, biomass loadings as high as 50% (w/w) and PIL recycling were investigated with respect to the modifications in the biomass composition, digestibility and structure which will further contribute to the realization of PIL processing of lignocellulosic biomass in larger scales.

Success of PIL pretreatment on different biomass types have been shown in the literature during the last decade; dissolution and recovery of the lignin and hemicellulose components was focused mainly in joint with the generation of a cellulose enriched fraction. From the time that the cost effective PILs were synthesized and confirmed for their use in the bio refinery context, studies on the deconstruction of abundant forestry and crop residues, and related mechanisms are under consideration.

Still, an entire work constituting the evaluation of elevated biomass loadings together with larger particle sizes and reuse of PIL has not been introduced in the literature, up to date. Hornbeam as an abundant forest biomass that can be grown in any region of Turkey was used for this purpose.

In this study, PILs, TEAHSO_4 and HBIMHSO_4 successfully fractionated hornbeam into cellulose enriched biomass and lignin precipitate as confirmed by the compositional, SEM, XRD and FTIR analysis. Although mid-range sized hornbeam samples gave the most promising findings regarding enzymatic hydrolysis and biomass fractionation, HBIMHSO_4 was shown to be capable of extracting almost 85% lignin even from the biomass with particle size >2 mm.

Both the PILs extracted lignin as high as 70% at 30% biomass loading. Besides, no significant changes were observed between the pretreatment performances of pristine PILs and recycled PILs. As a conclusion, this study shed more light on biomass fraction with PILs under cost effective conditions.

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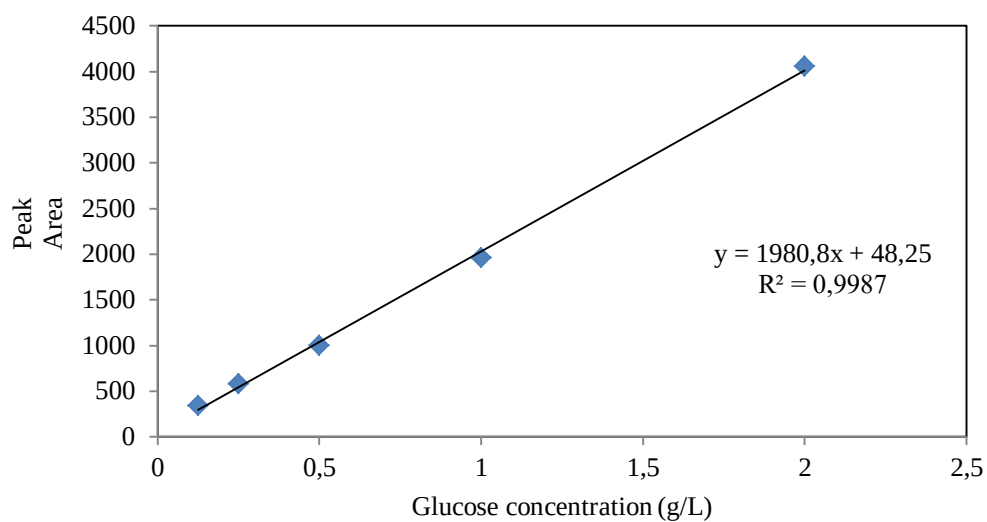
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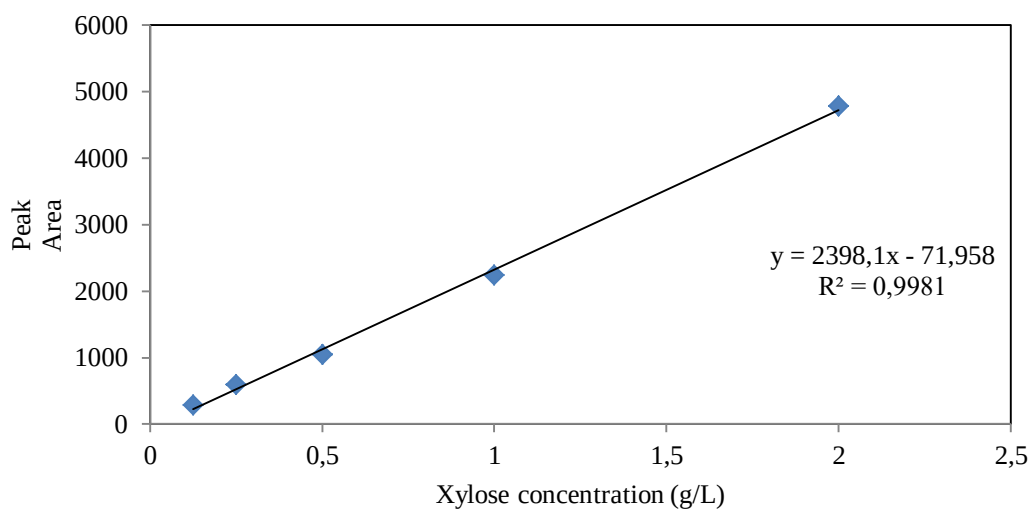
APPENDIX A CALIBRATION CURVES OF THE STANDARDS USED IN HPLC ANALYSIS

Calibration curve for glucose



Glucose calibration curve

Calibration curve for xylose



Xylose calibration curve

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PUBLICATIONS

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