EFFECT OF BIOMASS PRETREATMENT CONDITIONS ON THE GLUCOSE AND BIODEGRADABLE FILM PRODUCTION FROM LIGNOCELLULOSIC WASTES

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

EFFECT OF BIOMASS PRETREATMENT CONDITIONS ON THE GLUCOSE AND BIODEGRADABLE FILM PRODUCTION FROM LIGNOCELLULOSIC WASTES

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Process parameters during the conversion of lignocellulosic agricultural wastes including corn cobs and cotton stalk into glucose and biodegradable polymeric materials were investigated. The investigated parameters were pretreatment type, pretreatment temperature, lignocellulosic biomass particle size prior to pretreatment, hemicellulose extraction temperature, salt type, salt concentration and polymer processing technique. Pretreatments applied to corn cobs including dilute acid, alkaline and ionic liquid pretreatments gave similar glucose yields (73 – 84%) upon enzymatic hydrolysis where the lowest and highest glucose yields were obtained for dilute acid and ionic liquid pretreatments, respectively. The ionic liquid pretreatment efficiency was shown to depend on the lignocellulosic biomass particle. 1-ethyl-3-methylimidazolium chloride functioned more efficiently at smaller biomass particle sizes (<0.15 mm and 0.15 – 0.5 mm) compared to larger ones (0.5 – 1.0 mm and 1.0 – 2.0 mm), while opposite was true for the ionic liquid 1-ethyl-3-methylimidazolium acetate in terms of glucose production. During the co-production of glucose and hemicellulose based films, the hemicellulosic part of the process, which is related to the film production, was shown to be much more sensitive to the changes in the alkaline pretreatment temperature compared to the cellulosic part of the process that considers glucose production. It was also shown that the salt potassium acetate, which can be found together with hemicelluloses, was beneficial for the films. Finally extrusion was used as a new processing technique for the hemicellulose based polymers where the produced materials showed an ultimate tensile strength of 76 MPa and an elongation at break of 35%.

Keywords: Lignocellulosic, cellulose, hemicellulose, pretreatment, glucose

ÖZ

BİYOKÜTLE ÖN İŞLEM KOŞULLARININ LİGNOSELÜLOZİK TARIMSAL ATIKLARDAN GLİKOZ VE BİYOBOZUNUR FİLM ÜRETİMİ ÜZERİNDEKİ ETKİSİ

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Mısır koçanı ve pamuk sapı gibi tarımsal atıkların glikoz ve biyobozunur malzemelere dönüştürülmesi sürecindeki parametreler incelenmiştir. İncelenen parametreler arasında ön işlem türü, ön işlem sıcaklığı, lignoselülozik biyokütlenin ön işlem öncesi parçacık boyutu, hemiselüloz özütleme sıcaklığı, tuz türü, tuz konsantrasyonu ve polimer işleme tekniği yer almaktadır. Mısır koçanlarına uygulanan seyreltik asit, alkali ve iyonik sıvı ön işlemlerini takiben uygulanan enzimatik hidroliz sonucunda benzer glikoz verimleri elde edilmiş (%73 – 84), en düşük ve en yüksek verimlere sırasıyla seyreltik asit ve iyonik sıvı ön işlemleri sonrasında ulaşılmıştır. İyonik sıvı ile ön işlem verimliliğinin lignoselülozik biyokütlenin ön işlem öncesi parçacık boyutuna bağlı olduğu gösterilmiştir. 1-etil-3-metilimidazolyum klorid küçük parçacık boyutlarında (<0.15 mm and 0.15 – 0.5 mm) büyük parçacık boyutlarına (0.5 – 1.0 mm and 1.0 – 2.0 mm) kıyasla glikoz üretimi açısından daha verimli çalışırken, 1-etil-3-metilimidazolyum asetat için tersinin geçerli olduğu belirlenmiştir. Glikoz ve hemiselüloz temelli filmlerin birlikte üretimi sürecinin film üretimiyle ilgili olan hemiselülozik kısmının, glikoz üretimiyle ilgili olan selülozik kısmına göre alkali ön işlem sıcaklığındaki değişimlere daha duyarlı olduğu gösterilmiştir. Buna ilaveten hemiselülozlarla bir arada bulunabilen potasyum asetat tuzunun filmler açısından faydalı olduğu belirlenmiştir. Son olarak hemiselüloz temelli polimerler işlenmesi için yeni bir teknik olan ektrüzyon yöntemi kullanılmış ve bu şekilde elde edilen malzemelerin 76MPa çekme mukavemeti ve %35 kopmada uzama değerlerine sahip oldukları belirlenmiştir.

Anahtar kelimeler: Lignoselülozik, selüloz, hemiselüloz, ön işlem, glikoz

To my mom and dad for their endless support

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BGM: Blood glucose monitor
E: Elastic modulus
e_b: Elongation at break
EMIMAc: 1-ethyl-3-methyl imidazolium acetate
EMIMCl: 1-ethyl-3-methyl imidazolium chloride
GDH-FAD: glucose dehydrogenase- flavin adenine dinucleotide
GDH-NAD: glucose dehydrogenase- nicotinamide adenine dinucleotide
GDH-PQQ: glucose dehydrogenase- pyrroloquinoline quinone
GOx: Glucose oxidase
KAc: Potassium acetate
UTS: Ultimate tensile strength

CHAPTER 1

INTRODUCTION

Petroleum has been and currently is a crucial resource, which serves as the main raw material for the production of a large variety products including fuels, polymers and numerous chemicals with a great number of application areas. However the problem with petroleum is that in addition to being a non-renewable resource, many of the countries in the world are not self-sufficient in terms of their petroleum needs, which make these countries dependent on a few other countries with large petroleum reserves. Furthermore the petroleum prices are rather volatile, with a serious potential to impact a nation's economy in an adverse manner. The undesired environmental impact of the petroleum based products is also another widely recognized aspect of a petroleum based economy. All of these undesired issues have eventually created the need for the development of processes and technologies that enable the utilization of renewable resources as raw materials for the production of similar range of products obtained from petroleum. Due to the direct and strong link of these efforts to the critical macro concepts including energy production, sustainable economy and sustainable environment, renewable resource based studies have become one of the hottest research areas of our time.

Lignocellulosic biomass, which is a composite biopolymeric structure mainly made up of three different types of biopolymers including cellulose, hemicellulose and lignin (Brandt et al., 2013), is considered as a serious alternative to fossil resources as a renewable bioresource (Dale and Ong, 2012). These biopolymers are capable of serving as raw or starting materials for the production of a large variety of products, which can substitute their petroleum based counterparts including fuels, polymeric materials and other chemical commodities. In addition to its very low economic value, lignocellulosic biomass is an excessively abundant bioresource and these two factors appear as the main drivers for the interest to the lignocellulosic biomass conversion research (Lynd et al., 1991; Ragauskas et al., 2006).

An important issue related to the utilization of lignocellulosic biomass for the production of value added products is that lignocellulosic feedstocks are not consumed as food by the society. The importance of this point becomes evident when it is considered that currently starch is being utilized for the production of various products, especially fuels (such as ethanol) and polymeric materials on an industrial scale. Compared to lignocellulosic biomass, conversion of starch to the previously mentioned products is relatively easier. However using starch for such a purpose

creates the quite famous “food vs. fuel” competition since crops that possess starch (such as corn) are consumed as food and fertile farming lands have to be dedicated for the production of these crops (Somerville et al., 2010). In other words, due to the nutritional qualities of starch possessing crops, the food function of these crops compete with their industrial function. This competition is a significant one since the target products (fuels and polymeric materials) have enormous demands in the market and thus they have to be produced in excessive quantities. Eventually, doing so will not only raise the prices of the starch rich crops, but since more and more farm lands will have to be allocated for these crops, an overall increase in the prices of other crops that are solely utilized as food will also take place as well. Due to the increased awareness on this issue, currently there is a shift from the starch based production processes to the lignocellulosic based ones (Waltz, 2008).

1.1. Lignocellulosic biomass

Lignocellulosic biomass is almost everywhere and it is a very abundant and easy to access renewable bioresource. Wood is one of the most common types of lignocellulosic biomass where numerous agricultural wastes also possess a lignocellulosic structure. Different parts of plants make up the lignocellulosic agricultural wastes. Some examples to this type lignocellulosic biomass would be corn cobs, corn stover, cotton and sunflower stalks, switchgrass, rice straw and wheat straw. The shells of many agricultural products such as hazelnut, walnut, peanut and sunflower seed also possess a lignocellulosic structure. From a different perspective, lignocellulosic biomass is also produced as a by-product of industrial processes. For instance corn cobs are a by-product of the glucose-fructose syrup production processes while brewers spent grain is a by-product of the beer production process where brewers spent grain is the portion that is left after the fermentation step. Wood industry also produces by products that have a lignocellulosic structure in the form of flour or shavings that can be utilized as lignocellulosic biomass resource for the production of value added products.

As mentioned previously, the lignocellulosic structure is composed of three different types of biopolymers, which are cellulose, hemicellulose and lignin. Although it can vary from one lignocellulosic biomass to the other, the distribution of these biopolymers in the lignocellulosic biomass network is roughly 40 – 50% cellulose, 20 – 40% hemicellulose and 18 – 35% lignin (Sun et al., 2011).

In the composite structure of lignocellulosic biomass, cellulose appears as rigid bundles, closely packed together into columns where each cellulose column is surrounded with hemicellulose and this structure is referred to as a microfibril (Sannigrahi et al., 2010). These microfibrils are held together with lignin and all

together this structure makes up the plant cell wall (Sannigrahi et al., 2010; Brandt et al., 2013).

Cellulose is a carbohydrate polymer and almost always it is the major component of a lignocellulosic biomass. It is considered as the most abundant biopolymer on earth. As shown in Figure 1.1, glucose is the monomer of the cellulose polymer while cellobiose is the name given to the dimer. As reviewed by Klemm et al. (2005), the monomer units of cellulose are connected to each other via the C1 and C4 atoms and this linkage is termed as the β-1→4 linkage. A key issue regarding cellulose structure is the hydroxyl groups where each monomer carries three of these groups, which are responsible for the characteristic properties of cellulose including its hydrophilicity and degradability in addition to the crystalline structure of cellulose that occurs due to the hydrogen bonding between these hydroxyl groups (Klemm et al., 2005). The degree of polymerization of a specific cellulose may vary dramatically from one source to another while the range for the degree of polymerization of cellulose is between 800 – 10000 (Klemm et al., 2005).

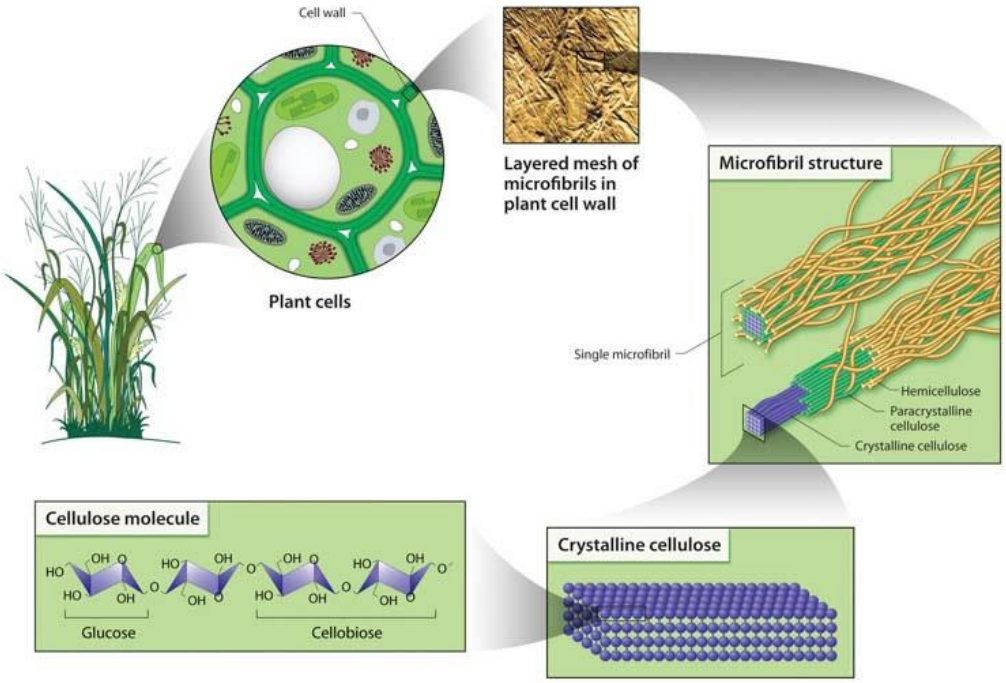

Figure 1.1. Structure of cellulose, its arrangement into microfibrils and its relation to the plant. (U.S. Department of Energy Genomic Science Program; http://genomicscience.energy.gov)

Compared to cellulose, hemicellulose has a lower degree of polymerization and a rather more complicated structure, since it has different types depending both on its monomer units as well as on the side groups a hemicellulose possesses (Brandt et al., 2013). Xylan and mannan are the two most common types of hemicelluloses and the main difference between these is that while xylose constitutes the backbone of xylan, it is replaced with mannose for mannan (Ebringerova et al., 2005). In addition to the monomer units that make up the hemicellulose backbone, hemicelluloses almost always possess side groups which can be hexoses and pentoses such as glucose, galactose and arabinose in addition to glucuronic acid residues (Ebringerova et al., 2005; Brandt et al., 2013). The presence of the side groups arabinose and glucuronic acid is especially important while determining the sub type of xylans since xylans are further categorized as glucuronoxylans, arabinoxylans and arabinoglucuronoxylans depending on the presence, absence or co-presence of these side groups (Ebringerova and Heinze, 2000). Two different hemicelluloses, which were obtained from cotton stalks and corn cobs, were utilized during the studies performed within the context of the present thesis. The hemicelluloses found in the corn cobs is previously reported to be of arabinoglucuronoxylan type since backbone made up of xylose monomers contains both of the arabinose and glucuronic acid side groups (Ebringerova et al., 1992). On the other hand, due to the absence of the arabinose side group and the presence of glucuronic acid residues, hemicelluloses found in cotton stalks are categorized as glucuronoxylans (Akpınar et al., 2011).

The third polymer to mention within the structure of lignocellulosic biomass is lignin. Lignin is considered to hold the entire lignocellulosic structure together, by acting as some kind of a paste filled between cellulose blocks surrounded with hemicellulose (Sannigrahi et al., 2010). Unlike the cellulose and hemicellulose, lignin is not a carbohydrate polymer and its structure is considered to be much more complicated while it is not yet fully understood (Yuan et al., 2013). Lignin is mainly constituted of three different types of monomers, which are coumaryl, coniferyl, and sinapyl alcohols and there are several different types of linkages between these three units as well as the linkage between the lignin molecule itself and the carbohydrate molecules (Yuan et al., 2013). The main difference between the lignin molecules found in different plants is the different amounts of the three monomer units or their ratio to each other (Sannigrahi et al., 2010).

1.2. Lignocellulosic biorefinery concept

In a similar manner to the petroleum refineries, the biorefinery concept is considered to be the framework that includes the processes and technologies required for the conversion of renewable resources into fuels, polymers and other chemicals that can replace those produced from non-renewable resources (Bozell and Petersen, 2010). In

a very significant majority of today's biorefineries, in which ethanol production is the core objective, starch serves as the main renewable resource where agricultural products rich in starch such as corn are used as raw materials. These biorefineries are generally referred to as 1st generation biorefineries and due to the problems mentioned above related to the utilization of starch as an industrial raw material, there is a serious shift to the 2nd generation biorefineries, which are called as lignocellulosic biorefineries (Waltz, 2008).

A very important notion regarding the lignocellulosic biorefinery concept is the multi-product perspective (Zhang, 2008). A rather self-explanatory term, multi-product perspective means the production of multiple products, especially products that accompany ethanol or another type of biofuel, in the product range of a lignocellulosic biorefinery (Zhang, 2008; Bozell and Petersen, 2010). Such an approach makes lignocellulosic biorefineries more feasible, since the revenues are increased as compared to producing just a single type of product (such as ethanol) while also providing flexibility to the biorefinery concept by increasing the variety of the products (Zhang, 2008). The value of the products is very crucial at this stage since low value products that has to be produced in huge volumes such as ethanol should be balanced with high value chemicals co-produced with ethanol in order to increase the feasibility of a lignocellulosic biorefinery (Bozell and Petersen, 2010). The choice of the products to be produced depends on a variety of factors including the type of the lignocellulosic biomass used and the type of the biorefinery and the processes taking place inside this biorefinery (Fernando et al., 2006; FitzPatrick et al., 2010).

In terms of the products and the three components that make up the lignocellulosic biomass, cellulose is mostly considered to serve as the resource for glucose production, since as mentioned earlier cellulose is made up of glucose monomers. Glucose can be considered as a key intermediate product since once you have glucose, you can obtain a large variety of products from it via fermentation. The most notable of these products are biofuels including ethanol. In addition to the biofuels, glucose can be utilized by the microorganisms for the production of valuable organic acids, enzymes as well as vitamins. Starting from glucose, it is also possible to obtain polymers and polymeric materials. The most popular example of this is the production of lactic acid from glucose via fermentation, followed by the polymerization of lactic acid into polylactic acid. Overall glucose provides a lot of flexibility and options in terms of the final product choices.

The case with hemicellulose is a little different and hemicellulose based products can be considered by thinking on the basis of the degree of polymerization of hemicellulose (Zhang, 2008). At high degrees of polymerization or in other words when the hemicellulose is obtained from the biomass without much degradation, hemicellulose can be used for the production of emulsifiers, thickeners and adhesives

(Zhang, 2008) as well as for the production of biodegradable polymeric films as also demonstrated in the present thesis study. Hemicelluloses can also be used for the production of hydrogels (Gabrielii and Gatenholm, 1998; Gabrielii et al., 2000; Li and Pan, 2010). If the hemicellulose is degraded to the monomer form the xylose obtained can be used for the production of organic acids and ethanol via microorganisms capable of fermenting xylose while the production of the sweetener xylitol is also made from the fermentation of xylose (Zhang, 2008). Further degradation of the xylose molecules yields furfural, which is used as a solvent and which can also be used for production of Nylon-6 (Zhang, 2008).

Lignin is also considered as a bioresource that can be utilized for the production of a large variety of products depending on its modification and depolymerization during its extraction from the lignocellulosic biomass (Sannigrahi et al., 2010). Products that can be obtained from lignin include adhesives, adsorbers, dispersants, carbon fibers as well as solvents such as benzene, toluene and xylene (Zhang, 2008; Yuan et al., 2013).

1.3. Lignocellulosic biomass pretreatment

1.3.1. General aspects of pretreatment

Pretreatment is the name given to a series of operations that can involve chemicals, microorganisms, heat and pressure applied to a lignocellulosic biomass in order to make the production of the above mentioned products possible or to increase the yield of these products, which would be irrationally lower without pretreatment. Pretreatment operations aim to alter or to deconstruct the native organization of the lignocellulosic structure in order to achieve higher sugar yields by increasing the working efficiency of the enzymes (Chang and Holtzapple, 2000; Mosier et al., 2005; Samayam et al., 2011) and/or to enable the separation of the three major components of biomass to a certain extent (Mosier et al., 2005). A conventional and widely studied route for the conversion of lignocellulosic biomass into value added products, particularly to ethanol, is the pretreatment of lignocellulosic biomass followed by the enzymatic hydrolysis of the pretreated material in order to obtain sugars, particularly glucose, where glucose is finally converted to ethanol via fermentation (Hendriks and Zeeman, 2009). In this sequence of processes, pretreatment is considered to be one of the most costly steps (Mosier et al., 2005). Since this cost is eventually reflected to the final products including ethanol in which achieving lower prices is of prime importance and since pretreatment has significant impacts on the processes that should be conducted before and after it, pretreatment is considered as the “key to unlocking low cost cellulosic ethanol” (Yang and Wyman, 2008).

A given pretreatment process might be evaluated on the basis of a large number of considerations with respect to the changes taking place in the lignocellulosic structure

where such changes are important from the enzymatic hydrolysis point of view (Mosier et al., 2005). Increase in the surface areas accessible to enzymes, reduction in the crystallinity of cellulose, removal of hemicellulose and lignin and the alteration of the native lignin structure at the end of a given type of pretreatment are desired aspects since these improve the performance of the enzymes that will be used to break down the cellulose in the lignocellulosic biomass (Mosier et al., 2005). In addition to the glucose production via enzymatic hydrolysis, another important consideration for a pretreatment is its effect on the fermentation step conducted by microorganisms for the production of ethanol or other fermentative products (Hendriks and Zeeman, 2009). A pretreatment might adversely affect the fermentation step if it results in the formation of inhibitory compounds, which are toxic to the microorganisms taking place in the fermentation step where these inhibitory compounds can arise either from the cellulose or the hemicellulose fractions of the lignocellulosic biomass (Hendriks and Zeeman, 2009). Furfural, hydroxymethyl furfural and phenolic compounds are the three major inhibitory compounds that might occur as a result of a specific type of pretreatment (Hendriks and Zeeman, 2009). The compound furfural occurs as a result of the dehydration of xylose and it is thus related to the hemicellulose component while hydroxymethyl furfural occurs due to the dehydration of glucose, which makes up the cellulose fraction of lignocellulosic biomass. On the other hand phenolic compounds are not of carbohydrate origin and they arise from the depolymerization of the lignin component of lignocellulosic biomass.

The large number of different pretreatments techniques available increases the importance of the above mentioned evaluation criteria since different pretreatment techniques have advantages and disadvantages regarding the issues described in the previous paragraph. Liquid hot water, hot water flow through, steam explosion, ammonia fiber expansion (AFEX), biological, mechanical (size reduction due to grinding), dilute acid, alkaline and ionic liquid pretreatments are examples to different pretreatment types (Mosier et al., 2005; Hendriks and Zeeman, 2009; Dale and Ong, 2012). For example both the hot water flow through and the steam explosion pretreatments increase the surface area of lignocellulosic biomass accessible to enzymes and both techniques are good at hemicellulose solubilization (Hendriks and Zeeman, 2009). However water flow through pretreatment is better at solubilizing (and thus removing) lignin and it also results less furfural and hydroxymethyl furfural to occur compared to the steam explosion pretreatment, making is more advantegous in terms of its effect on the post-pretreatment operations (Hendriks and Zeeman, 2009).

In addition to the post-pretreatment related factors mentioned above, more general considerations are also important while evaluating a given type of pretreatment. Such general considerations can be the pretreatment time, water consumed during pretreatment, co-product potential, fermentation compatibility, feedstock flexibility,

toxicity of the pretreatment, severity of temperature and pressure applied during pretreatment and the relative ease of the process control during the pretreatment in addition to the economic aspects including the costs of energy, chemicals and capital costs (Dale and Ong, 2012). For example, the biological pretreatment has significant advantages compared to most other pretreatment types in terms of the low costs associated with the water, energy and chemical consumption as well as a low capital cost, operation at atmospheric conditions, fermentation compatibility and non-toxicity (Dale and Ong, 2012). However this type of pretreatment has two important drawbacks. First it requires days to be completed while all other types of pretreatments require minutes or hours and additionally it works well only with certain types of lignocellulosic biomass and not with the others, giving it a low degree of feedstock flexibility (Dale and Ong, 2012).

Wyman et al. (2005) compared the efficiency of seven different types of pretreatments applied to corn stover with respect to the sugar yields obtained. All the seven methods gave similar glucose yields at the end of the enzymatic hydrolysis performed following the pretreatments where the glucose yields were between 82 – 96% on the basis of the theoretical maximum amount of glucose that could be obtained from native corn stover based on its cellulose content (Wyman et al., 2005). The rather narrow range of glucose yields indicates that for corn stover, the choice of pretreatment did not have a major impact on the glucose production efficiency via enzymatic hydrolysis. In another study where poplar wood was used instead of corn stover as the lignocellulosic feedstock, different results regarding the glucose yields were observed (Wyman et al., 2009). In the poplar wood case, the range of glucose yields was much broader (48 – 97%) where the lowest glucose yield obtained from enzymatic hydrolysis was observed for the aqueous ammonia recycle pretreatment while sulfur dioxide catalyzed steam explosion technique gave the highest glucose yields followed by the rather simple lime pretreatment (Wyman et al., 2009). When these two studies are considered together it appears that the choice of lignocellulosic feedstock can have a major impact on the pretreatment efficiencies where some feedstocks are suitable for a large range of pretreatments while others can only be pretreated efficiently with certain specific pretreatment types.

Within the scope of the present thesis study, three types of pretreatments were studied, which are dilute acid, alkaline and ionic liquid pretreatments. The dilute acid pretreatment is one of the oldest and most studied pretreatment techniques and it is easy to apply on the lab scale. During dilute acid pretreatment, the pretreatment reagent is an aqueous solution of acid where the concentration of the acid is generally between 0.5 – 2%. Sulfuric acid is considered to be the most popular choice of acid used in dilute acid pretreatments (Mosier et al., 2005). The acidic solution is incubated together with the biomass generally at a temperature around 100°C. At the end of the pretreatment, the solids are removed from the suspension, washed with water,

neutralized and finally dried prior to the enzymatic hydrolysis. Dilute acid pretreatment is effective at increasing the surface area accessible to enzymes and removing hemicelluloses from the lignocellulosic structure while it is not effective for lignin removal and for reducing the crystallinity of cellulose (Mosier et al., 2005). Dilute acid pretreatment mainly owns its effect to the depolymerization it induces on the carbohydrates found in the lignocellulosic structure and thus fermentation inhibitory furfural and hydroxymethyl furfural are accumulated during this type of pretreatment (Mosier et al., 2005; Hendriks and Zeeman, 2009).

Alkaline pretreatment is on the opposite end of the pH scale compared to dilute acd pretreatment in terms of the pretreatment reagents it utilizes, which are aqueous solutions of different bases such as KOH or NaOH at a concentration between 5 – 30% w/v. Conventional alkaline pretreatment is a batch operation and the pretreatment temperatures are generally lower compared to acid pretreatment where pretreatment durations can be a few times higher. Alkaline pretreatment's major effect is the partial solubilization of hemicellulose together with lignin in the alkaline solution (Hendriks and Zeeman, 2009). Alkaline pretreatment increases the accessible surface area and results in the formation of the inhibitory compounds in lower amounts as compared to dilute acid pretreatment.

Silverstein et al. (2007) have compared the dilute acid and alkaline pretreatments applied to cotton stalks for their effect on the enzymatic hydrolysis of the pretreated biomass. H_2SO_4 and NaOH were used at a concentration of 2% (w/v) for the dilute acid and alkaline pretreatments, respectively, where the pretreatment temperature was 121°C for both of the pretreatments for a duration of 1 h. Alkaline pretreatment was shown to be much more efficient compared to dilute acid pretreatment where 61% of the cellulose found in the cotton stalks was converted to glucose while this conversion was only 24% for dilute acid pretreatment at the end of 72 h of enzymatic hydrolysis. The better performance of the alkaline pretreatment under identical conditions was attributed to the higher lignin removal achieved by alkaline pretreatment compared to dilute acid retreatment (Silverstein et al., 2007).

Due to its dissolution capacity towards hemicellulose, alkaline pretreatment can also be considered as a way to isolate hemicellulose from lignocellulosic biomass (Figure 1.2). In other words, the hemicelluloses dissolved in the basic liquid phase during alkaline pretreatment already form an alkaline solution of hemicellulose and lignin where these polymers can be recovered from the solution via precipitation by adding ethanol and acetic acid (Zilliox and Debeire, 1998) or via spray drying (Gabrielii et al., 2000). This issue regarding the alkaline pretreatment is worth mentioning since hemicelluloses used in the present study were isolated with this technique.

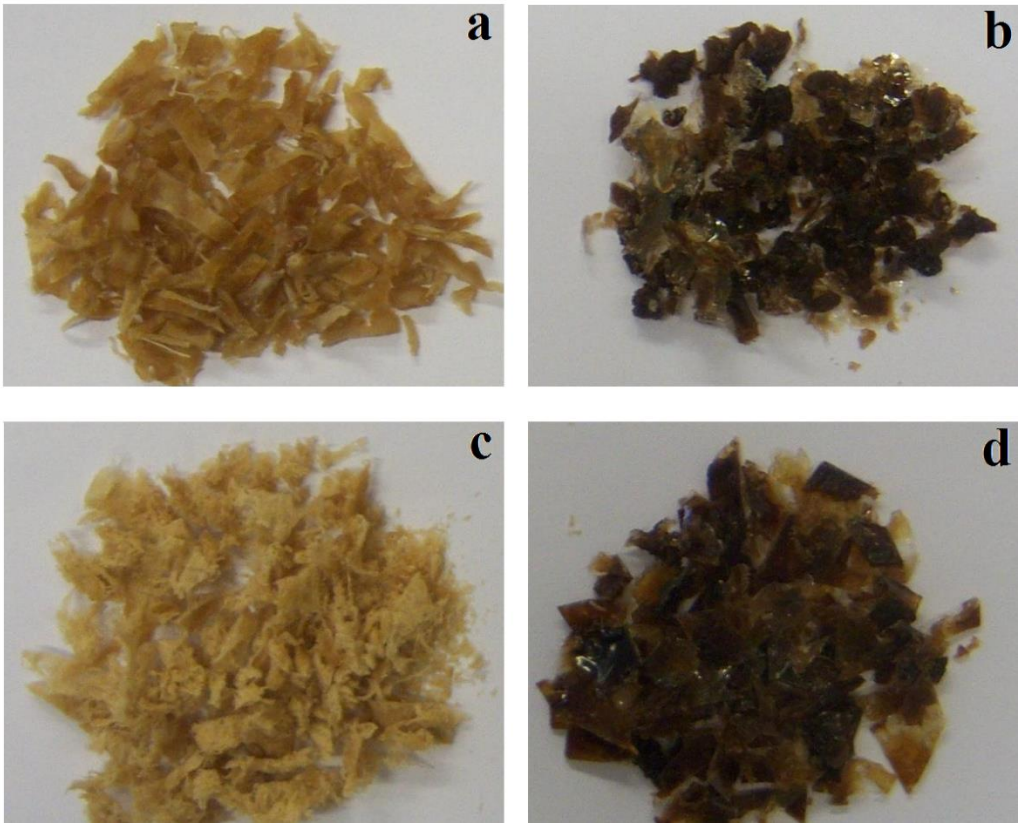

Figure 1.2. Appearance of hemicelluloses isolated from wheat bran (a), sunflower stalk (b), corn cob (c) and cotton stalk (d) via alkaline extraction (Bahçegül, 2009).

The work by Lawther et al. (1996) is a very informative experimental study that examines a large set of parameters regarding the alkaline extraction of hemicelluloses from lignocellulosic biomass, namely wheat straw. In this work, the authors examine the effect of alkaline concentration, alkaline type, extraction time, extraction temperature and boric acid concentration on the hemicellulose and cellulose fraction yield in addition to the molecular weight of the isolated hemicelluloses and together with their sugar compositions. Lawther et al. (2006) showed that increasing the pretreatment time beyond 5 h did not cause any significant increase in the hemicellulose yield while increasing the KOH concentration above 10% also did not cause any significant improvements in the hemicellulose yield, which was roughly 30%. Among the different alkali types studied including $Ca(OH)_2$, NaOH, KOH and LiOH, the last three gave similar hemicellulose yields where KOH was suggested as the ideal alkali for hemicellulose extraction. The reason for this suggestion was that the salt KOH forms with acetic acid during the neutralization step, which is potassium

acetate, is more soluble in ethanol compared to the other salts that could form from the reaction of acetic acid with the other bases. This higher solubility was perceived as an advantage since the removal of the salts from the recovered hemicelluloses was thought to be necessary as the salts were accepted as undesired impurities. The authors have also examined the effect of extraction temperature and found that no important change in the hemicellulose yield takes place above room temperature up to a temperature of 50°C. The molecular weight of the extracted hemicelluloses were found to decrease with increasing KOH concentration used during extraction, while the sugar composition of the hemicelluloses in terms of xylose and arabinose contents varied depending on the extraction conditions employed where this was most evident for the arabinose content of the polymers that changed by a factor of two depending on the extraction conditions.

Celebioglu et al. (2012) studied the extraction of hemicellulose from three different lignocellulosic feedstocks including corn peel, corn stalk and sugar beet pulp where different alkaline extraction parameters were evaluated in terms of their effect on the hemicellulose yield and purity. It was found that using lower NaOH concentrations (10%) and lower temperatures (30°C) resulted in hemicelluloses with higher purities (65%). Removal of fat and proteins prior to alkaline extraction was also shown to increase the purity of the isolated hemicelluloses. The effect of lignocellulosic biomass particle size during the extraction on the hemicellulose yields obtained from corn peel and sugar beet pulp were also investigated. It was found that increasing the particle size of corn peels from 0.8 mm to 1.1 mm increased the hemicellulose yields from roughly 30% to 45%, while no statistically significant change could be observed for the case where sugar beet pulp was used as the lignocellulosic feedstock (Celebioglu et al., 2012).

1.3.2. Ionic liquid pretreatment

Compared to dilute acid and alkaline pretreatments, ionic liquid pretreatment is a much recent technique and it can be considered as the most popular pretreatment technique in the literature within the last few years. Ionic liquids are salts, which are at liquid phase at room temperature or at moderate temperatures less than 100°C (Mora-Pale et al., 2011). A key property of ionic liquids is their negligibly low vapor pressure, making them green solvents especially when compared to the conventional volatile solvents (Lopes et al., 2013). Ionic liquids are composed of a cation and an anion where the properties of the ionic liquids such as melting temperature, degradation temperature, toxicity, polarity, viscosity, solubilization power towards a given solute, thermal and electrical conductivity are all determined by the combinations of the cations and anions, which implies that ionic liquids with desired specific properties can be synthesized by choosing the necessary combination (Mora-Pale et al., 2011; Tadesse and Luque, 2011).

The trick that made ionic liquids so popular for lignocellulosic biomass pretreatment is their capability to dissolve cellulose (Swatloski et al., 2002; Brandt et al., 2013). Currently, the dissolution capability of ionic liquids towards cellulose is explained on the basis of the hydrogen bonding between the ionic liquid anion and the hydroxyl groups found on the cellulose backbone (Remsing et al., 2006; Sun et al., 2011). The hydrogen bond basicity of the anion, which is defined as the Kamlet-Taft parameter β, is the determining factor for the dissolution capability of ionic liquids where β values greater than 0.8 are required for the dissolution of 10% or more cellulose in an ionic liquid (Doherty et al., 2010; Brandt et al., 2013). Although not yet fully understood, the cation is also accepted to have a role in the cellulose dissolution, which appears to be less significant compared to the anion where increase in the length of the cation is considered to be undesired for efficient cellulose dissolution (Sun et al., 2011; Brandt et al., 2013).

Swatloski et al., (2002) have shown that the ionic liquid 1-butyl-3-methylimidazolium chloride (BMIMCl) was capable dissolving cellulose up to a concentration of 25% depending on the solubilization procedure. While the dissolution of cellulose in BMIMCl could not be observed at room temperature, heating BMIMCl to 70°C gave a solution of 3% cellulose concentration where further heating BMIMCl to 100°C gave a 10% cellulose solution in BMIMCl. The authors have also noted that by using microwaves at 3 – 5 second pulses for heating, cellulose solutions of 25% with high viscosity could be obtained.

Zavrel et al., (2009) employed a high-throughput screening method in order to investigate the cellulose dissolution capabilities of 21 different ionic liquids where most of the ionic liquids tested composed of 1-butyl-3-methylimidazolium (BMIM) or 1-ethyl-3-methylimidazolium (EMIM) cations including 1-allyl-3-methylimidazolium (AMIM) while chloride (Cl), acetate (Ac), bromide (Br) and iodide (I) constituted the majority of the anions. 6 of these ionic liquids were found to completely dissolve 5% cellulose at 90°C, which were AMIMCl, EMIMCl, EMIMAc, BMIMCl, 1,3-dimethylimidazolium-dimethylphosphate (ECOENG) and 1-butyl-3-methylpyridinium-chloride (BMPYCl) (Zavrel et al., 2009).

The dissolution of cellulose in ionic liquids is important since the dissolution of cellulose followed by its precipitation, which is referred as cellulose regeneration, from the solution with an anti-solvent (such as water) modifies the closely packed, organized and rigid structure of crystalline cellulose in to a more amorphous form, which is loosely packed and thus much more accessible by the enzymes (Dadi et al., 2006; Kuo and Lee, 2009). The appearance of a regenerated (precipitated) cellulose sample pictured while the cellulose was in the ionic liquid-water solution is shown in Figure 1.3.

Figure 1.3. Picture of regenerated cellulose right after its precipitation by the addition of water to the ionic liquid cellulose solution (Haykir, 2013).

As shown by Dadi et al. (2006), microcrystalline cellulose subjected to BMIMCl pretreatment at temperatures between 130°C – 150°C for 10 – 180 min showed a glucose conversion of around 90% at the end of 48 h of enzymatic hydrolysis while this value was 60% for the untreated microcrystalline cellulose. The authors have also investigated the effect of the anti-solvent used to precipitate the cellulose from the cellulose-BMIMCl solution where the precipitation step is rather typical for ionic liquid dissolution processes and it is conducted to recover the solubilized polymers in the solution. Cellulose samples dissolved in BMIMCl and precipitated in three different anti-solvents (water, ethanol and methanol) gave similar initial reaction rates in terms of reducing sugar formation during enzymatic hydrolysis indicating the choice of anti-solvent did not have a major effect on the initial rate of enzymatic digestibility. At this point it is important to mention that the initial reaction rate of reducing sugar formation was enhanced for roughly 50 fold for all the BMIMCl processes samples compared to the native microcrystalline cellulose, which demonstrates another important feature of cellulose dissolution and subsequent precipitation in ionic liquids (Dadi et al., 2006).

Fort et al. (2007) used the cellulose dissolution capability of BMIMCl for the extraction of cellulose from the lignocellulosic structure of wood where partial dissolution of wood took place. Depending on the type of wood, around 40 – 50% of the initial cellulose in the woods was extracted via partial BMIMCl dissolution at 100°C for 24 h (Fort et al., 2007).

On the other hand, Kilpelainen et al., (2007) reported complete dissolution of wood in ionic liquids including BMIMCl and AMIMCl and pointed out the importance of particle size of lignocellulosic biomass for ionic liquid dissolution. The authors have stated that while big wood particles (chips) were only partially soluble, smaller wood particles including wood powder, wood sawdust or wood fibers were completely soluble at a lignocellulosic biomass concentration of 2 – 8% depending on the ionic liquid type, dissolution temperature and time. The dissolution of the wood particles and their subsequent regeneration (precipitation) was shown to reduce the crystallinity of the lignocellulosic biomass. Kilpelainen et al. (2007) have also subjected the regenerated wood particles to enzymatic hydrolysis and showed that around 60% glucose yield was obtained from AMIMCl pretreated wood particles while the glucose yield was only 10% for the native wood particles, which was a result that demonstrated the effectiveness of ionic liquids as lignocellulosic biomass pretreatment agents.

As opposed the complete solubilization approach, Lee et al., (2009) have demonstrated that highly efficient ionic liquid pretreatments could be made despite of the partial dissolution of the biomass in the ionic liquid EMIMAc, which is one of the two ionic liquids used in the present thesis study together with EMIMCl. Lee et al.

have attributed to the increases in the glucose yield to lignin extraction performed by EMIMAc, which was also capable of dramatically reducing the crystallinity of cellulose in wood without completely solubilizing it. The authors have shown that at a biomass loading of 5% ($w_{biomass}/w_{ionic\ liquid}$) increasing the pretreatment temperature from 50°C to 130°C also increased the amount of lignin extracted (removed) from lignocellulosic biomass, resulting in higher digestibility of cellulose during the subsequent enzymatic hydrolysis step where this was also true for increasing EMIMAc pretreatment time. Removal of roughly 50% of the initial lignin in the lignocellulosic biomass was determined to be sufficient for achieving cellulose digestibility values of around 90% since the reduction of lignin was accompanied by the reduction of cellulose crystallinity. Lee at al. (2009) have also shown that EMIMAc could be reused at least up to 4 times without falling below the 90% cellulose digestibility limit, which is a critical point taking into account the high prices of ionic liquids. Similar results regarding the lignin extraction performed by EMIMAc pretreatment, which resulted in increased enzymatic hydrolysis efficiency were also reported by Fu et al. (2010).

Singh et al. (2009) studied the dissolution of switchgrass in EMIMAc and observed that prior to dissolution observed at 120°C, swelling of the plant cell walls took place, which was recognized as the disruption of the hydrogen bond network between and within the cellulose and lignin polymers by EMIMAc. On the basis of the reducing sugars obtained, which were determined via the dinitrosalicylic acid (DNS) assay, the authors have reported an enzymatic digestibility of around 73% for EMIMAc pretreated switchgrass where only 17% digestibility could be achieved for the untreated samples (Singh et al., 2009).

Arora et al., (2010) have studied the effects of pretreatment time and temperature for the EMIMAc pretreatment of switchgrass. The authors reported that pretreatment temperature had a dramatic impact on the surface area and pore volume of the pretreated biomass where increasing the EMIMAc pretreatment temperature from 120°C to 160°C resulted in an increase of around 23 and 57 folds for the surface area and pore volume, respectively. These increases were correlated with the improvements that took place for the initial enzymatic hydrolysis rate, which successively increased as the EMIMAc pretreatment temperature raised from 110°C to 160°C in 10°C increments. The authors observed similar digestibility values between the samples that were treated at 160°C for 3 h and at 120°C for 3 h, 24 h and 5 days while very low digestibility values (similar to that of untreated samples) were obtained for the pretreatments conducted at 160°C for 24 h. Based on their results, the authors suggested that pretreatment temperature was more important than pretreatment time in terms of its influence on the enzymatic hydrolysis (Arora et al., 2010).

Li et al., (2010) have shown that the density of the lignocellulosic biomass subjected to ionic liquid pretreatment via AMIMCl could influence the pretreatment efficiency. The authors suggested the pulverization of biomass particles for 2 – 5 days prior to ionic liquid pretreatment, since this was shown to increase the enzymatic hydrolysis efficiency compared to the samples pretreated without pulverization. Unlike the results reported by Lee et al. (2009), Li et al. (2010) determined that notable decreases in the pretreatment efficiency took place upon the reuse of the recycled AMIMCl during pretreatments due to the presence of various lignocellulosic biomass components left in AMIMCl from previous pretreatment runs (Li et al., 2010).

As mentioned previously, in the beginning, there appears to be an emphasis on the complete dissolution of lignocellulosic biomass for an efficient pretreatment where later on this complete dissolution view was challenged with the partial dissolution approach, which is advantageous simply because it is not restricted with the solubility limit of the biomass in the ionic liquid. The work of Wu et al., (2011), fully exploits this strategy since very high solid loadings of up to 50% ($w_{biomass}/w_{ionic\ liquid}$) were pretreated with EMIMAc and shown to result in improved enzymatic hydrolysis efficiency. The authors have worked with solid loading between 5 – 50% during the pretreatment corn stover with EMIMAc at 125°C for 1 h. Glucose yields of around 80% were reported regardless of the increase in the solid loading while increasing the solid loading during pretreatment to 41% and 50% reduced the glucose yield to approximately 70% and 60%, respectively, which are still good values taking into account how high the solid loading is. The authors have shown that increasing the solid loadings resulted in a decrease in the lignin extraction efficiency of EMIMAc. However this did not adversely affect the enzymatic hydrolysis up to the 33% solid loading threshold since all the samples experienced similar reductions in their crystallinity. The crystallinity of the samples for 41% and 50% solid loadings were higher compared to cases where EMIMAc pretreatment was applied at lower solid loadings, which resulted in decreased glucose yields a t the end of enzymatic hydrolysis. Based on the correlations between lignin extraction, crystallinity reduction and glucose yields, the authors suggested that the crystallinity of the samples was a more important issue compared to lignin removal from the samples in terms of pretreatment efficiency (Wu et al., 2011).

Ninomiya et al., (2012) have used a range of ionic liquids, including EMIMCl and EMIMAc for the pretreatment of kenaf powder where it was shown that EMIMAc pretreatment gave better results in terms of the cellulose digestibility compared to EMIMCl, BMIMCl and AMIMCl. Ultrasonication following the ionic liquid pretreatment was shown to dramatically increase the cellulose digestibility during the enzymatic hydrolysis where an increase of more than 4 fold in cellulose digestibility was reported following the ultrasonication assisted EMIMCl pretreatment of lignocellulosic biomass compared to EMIMCl pretreatment alone. On the other hand,

no significant increase in the cellulose digestibility due to ultrasonication took place since the digestibility values following the EMIMAc pretreatment were already high except for the case where EMIMAc pretreatment was conducted for only 15 min.

Pezoa et al., (2010) used EMIMCl for the pretreatment of corn, wheat, eucalyptus and lenga residues at three different pretreatment temperatures (80 – 121 – 150°C). Increasing pretreatment temperature also increased the glucose yields upon enzymatic hydrolysis and this was most evident for the pretreatment temperature of 150°C where similar glucose yields to that of untreated samples were obtained from eucalyptus residues pretreated at 80°C and 121°C with EMIMCl, indicating the ineffectiveness of EMIMCl pretreatment at relatively low temperatures (Pezoa et al., 2010).

Li et al. (2010) compared the performance of EMIMAc pretreatment applied to switchgrass with that of the conventional dilute acid pretreatment technique where both types of pretreatments were conducted at 160°C with a solid loading of 3%. EMIMAc was much more effective compared to dilute acid in terms of reducing the crystallinity and increasing the enzymatic hydrolysis rate, in which over a 16 fold increase was observed when EMIMAc pretreatment was applied as compared to dilute acid pretreatment. The authors have also reported that while the lignin content of dilute acid pretreated switchgrass was 29%, this was reduced to 14% for the EMIMAc pretreated switchgrass, indicating that EMIMAc pretreatment was much better in terms of lignin removal. As it can be expected from the results regarding the lignin content and crystallinity, EMIMAc pretreated samples had a much higher cellulose digestibility of 96% compared to dilute acid pretreated samples, which were only 48%.

Haykir et al., (2013) have compared the pretreatment efficiencies of several different ionic liquids including 2-hydroxy ethyl ammonium formate (HEAF) in addition to EMIMCl, EMIMAc, BMIMCl and AMIMCl. The authors showed that HEAF performed in a similar efficiency as a pretreatment agent compared to the chloride containing ionic liquids while EMIMAc pretreatment resulted in higher biomass digestibility (around 2.5 times higher) compared to other ionic. The deconstruction of the native lignocellulosic structure of cotton stalks by EMIMAc pretreatment was clearly demonstrated via scanning electron microscopy where it was also shown that the deconstruction power was retained upon recycling of EMIMAc (Figure 1.4).

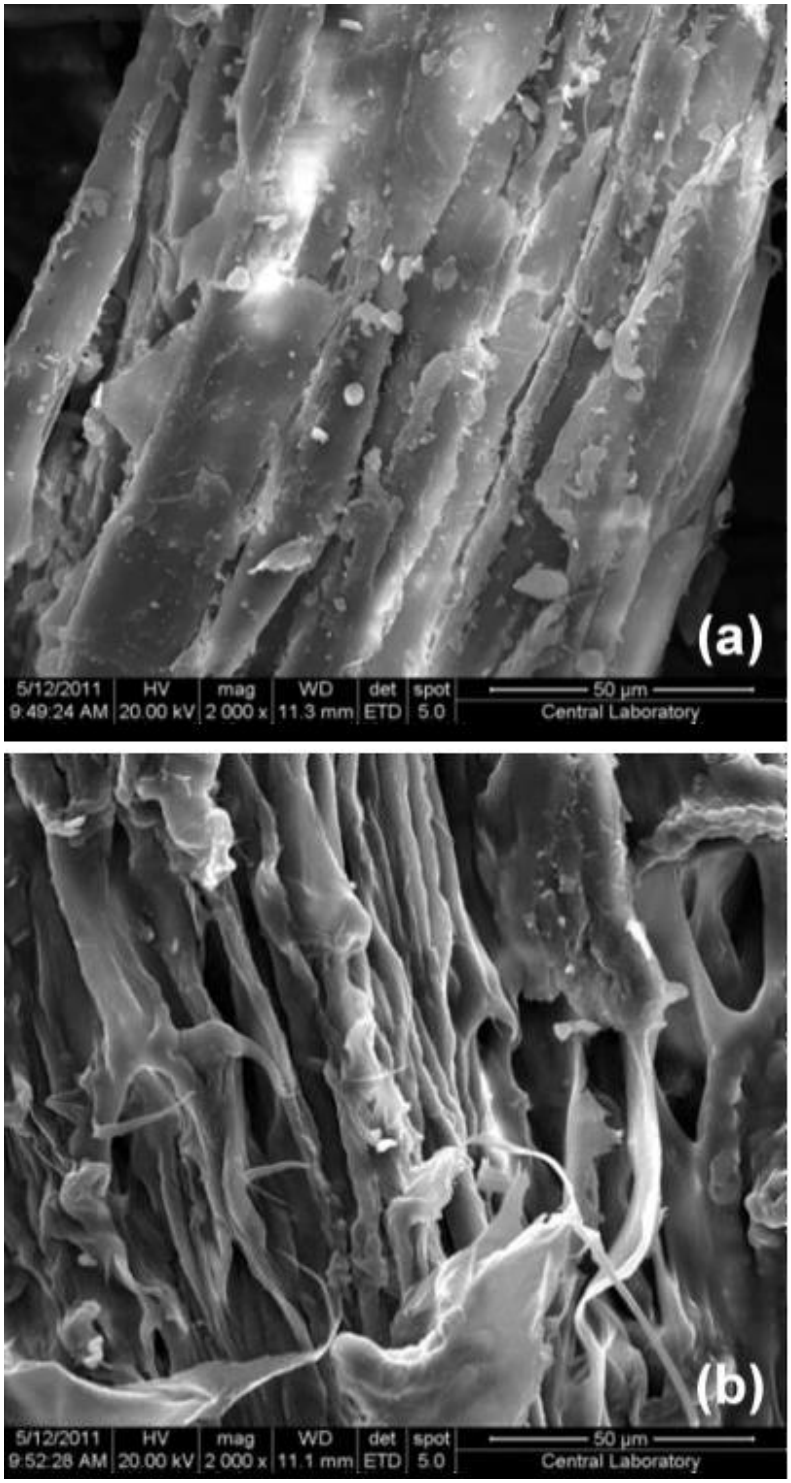

Figure 1.4. Scanning electron microscopy images of untreated cotton stalks (a) and cotton stalks following EMIMAc pretreatment (b) (Haykir, 2013).

Tatlı (2013) used peanut shells as a lignocellulosic resource for the production of sugars and lignin from lignocellulosic biomass with the aim of using lignin as an additive to concrete preparations in order to increase the flowing properties of concrete. EMIMAc and EMIMCl ionic liquids were used for the pretreatment of peanut shells where lignin was obtained as the residue remaining after the enzymatic hydrolysis of the pretreated samples. Both the reducing sugar and the lignin yields obtained upon EMIMAc pretreatment were higher compared to the EMIMCl pretreatment.

1.4. Enzymatic hydrolysis

So far, one of the most frequently addressed notions in the present thesis study has been enzymatic hydrolysis, which is an essential step for the production of glucose from lignocellulosic biomass within the described lignocellulosic biomass conversion framework. The role of the enzymes during the glucose production via enzymatic hydrolysis is to breakdown the cellulose polymer, preferably down to its monomer, which is glucose. This is accomplished by the utilization of cellulase enzymes during enzymatic hydrolysis.

Cellulases are a class of hydrolytic enzymes that are composed of two domains or two task specific components, which are the binding domain that enables the enzymes to bind to the cellulose molecules and the catalytic domain in which the hydrolytic reactions related to the breakdown of cellulose take place (Henrissat, 1994). The word cellulases actually refers to an enzyme system composed of three different hydrolytic enzymes, each capable of acting on different parts or forms of cellulose (Bubner et al., 2013). These enzymes are endoglucanases, exoglucanases and β-glucosidases where endo and exoglucanases act on interior and exterior (chain ends of the cellulose) portions of cellulose, respectively, while β-glucosidases act on smaller cellulose molecules in order to convert them into glucose (Zhang and Lynd, 2004; Bubner et al., 2013).

The first event during the enzymatic hydrolysis of cellulose by the cellulase enzymes is the binding of cellulases to the cellulose molecules, which is defined as cellulase adsorption. At this point it should be remembered that cellulose molecules are insoluble in aqueous medium, in which the enzymatic reactions are conducted, but they are solubilized as their degree of polymerization (DP) is reduced to the oligomeric level. As described by Zhang and Lynd (2004), following the cellulase adsorption, three different reactions take place at the same time since the three types of cellulases work together in a synergistic manner. Upon cellulase adsorption to cellulose, changes related to the chemical and physical properties of the cellulose takes place including the reduction of the DP of cellulose and the increase of surface area accessible to enzymes. The second step that takes place is the liberation soluble

cellulose fragments in to the hydrolysis medium from the larger insoluble cellulose molecules where this step is called as the primary hydrolysis. The third step as described by Zhang and Lynd (2004) is the breakdown of the soluble fragments released in the second process to smaller molecules, down to the monomer level, which is glucose where this third process is referred to as secondary hydrolysis and it proceeds faster compared to the primary hydrolysis. As it can be understood from this description, the reduction in the DP of cellulose is especially important since it starts the rather complicated simultaneous events by detaching cellulose fragments from the intact cellulose molecule. The reduction of DP occurs due to the action of both the endo- and exo-glucanases but since the endoglucanases act on the interior portions of the cellulose molecules as compared to the exoglucanases that act only on the chain end, the effect of endoglucanases on the reduction of DP more prominent compared to the incremental effect of exoglucanases (Zhang and Lynd, 2004). In relation to the reduction of cellulose DP, an important point to mention is that unlike the endo and exoglucanases, β-glucosidases can only act during the liquid phase of the hydrolysis or in other words once the DP of the cellulose is reduced to the point where it becomes soluble in the aqueous enzymatic hydrolysis medium (Zhang and Lynd, 2004). A schematic representation of the description provided here is given in Figure 1.5.

Thinking the cellulose hydrolysis within the context of lignocellulosic biomass conversion complicates the issue of enzymatic hydrolysis since the lignocellulosic biomass is not solely composed of cellulose. An important component of the lignocellulosic structure to consider related to the enzymatic hydrolysis is lignin, since it adversely effects the working efficiency of cellulases due to various reasons (Chang and Holtzapple, 2000; Eriksson et al., 2002; Studer et al., 2011).

As shown by Eriksson et al., (2002) cellulase enzymes have a problem of unproductive binding to lignin where unproductive means no products occur as a result of this binding in which only the binding domain (not the catalytic domain) of the cellulases participate. As a result of unproductive binding, the bound enzymes cannot be utilized efficiently during the enzymatic hydrolysis of cellulose, resulting in slower rates and lower glucose yields. Eriksson et al. (2002) have explained the unproductive lignin binding of cellulases on the basis of the hydrophobic amino acids that take place in the primary structure of the cellulase enzymes where the interactions between these hydrophobic groups and those on the lignin was proposed to result in this unproductive binding. It can be illuminating to mention at this point that like all enzymes, cellulases are proteins, which are biopolymers made up of amino acid monomers where the sequence and the nature of these monomers determine the properties of the protein molecule and thus the enzyme.

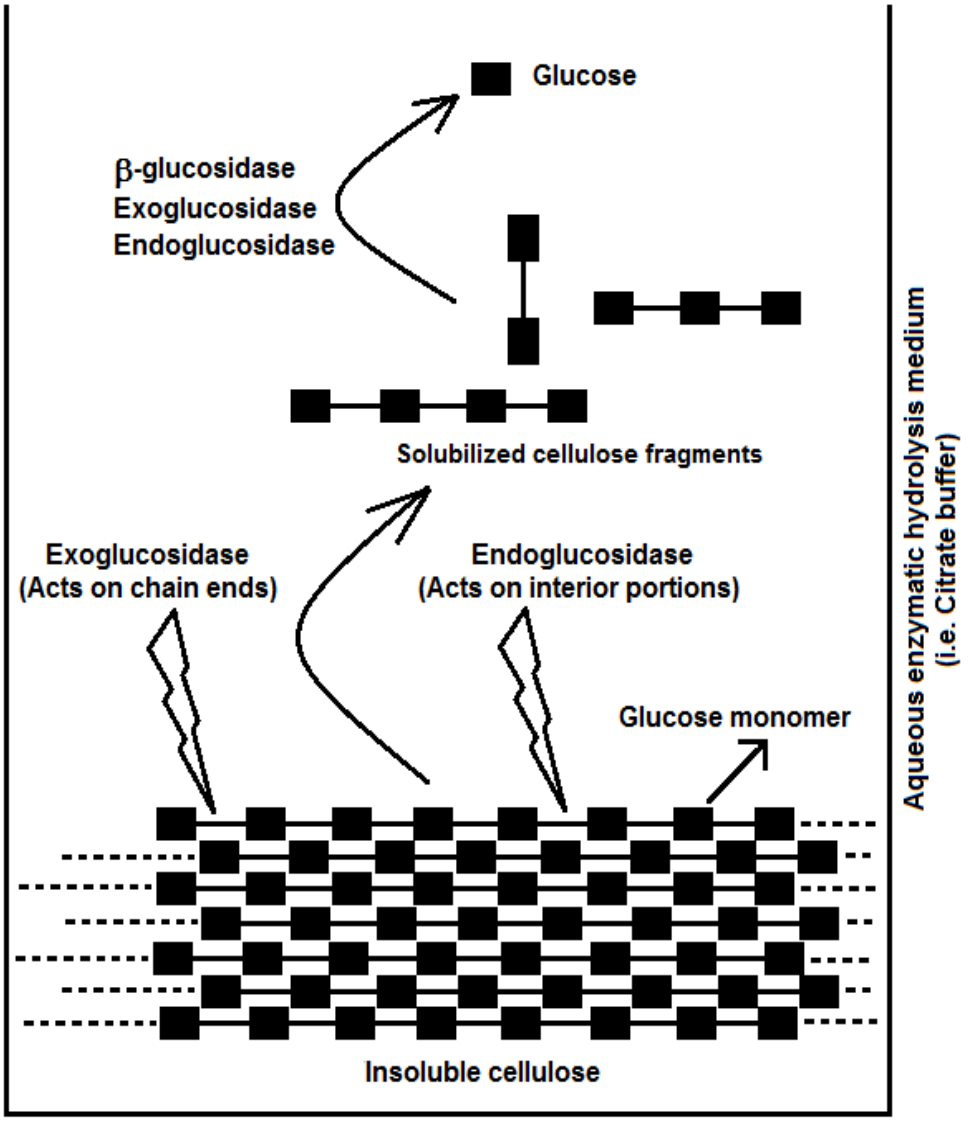

Figure 1.5. A schematic representation of primary and secondary hydrolysis of cellulose via cellulase enzymes.

A rather less complicated adverse effect of lignin on the performance of cellulase enzymes is that lignin simply acts as a physical barrier between the cellulose molecules and the cellulase enzymes, preventing the enzymes binding to cellulose simply because the enzymes cannot reach their substrates (Chang and Holtzapple, 2000). By analyzing the enzymatic hydrolysis of 147 different lignocellulosic biomass samples (poplar wood), each pretreated with different pretreatment parameters in

order obtain samples with different lignin contents (between 0.7 – 26%) and crystallinity, Chang and Holtzapple (2000) have shown that lower lignin contents (or higher lignin removal from the lignocellulosic biomass samples) leads to more efficient enzymatic hydrolysis where the reduction in the crystallinity of the samples also led to similar results.

The adverse effects reducing the performance of the cellulase enzymes not only occurs due to the factors associated with the structure of lignocellulosic biomass but pretreatment agents, particularly ionic liquids, can also adversely affect the cellulase enzymes, reducing the efficiency of enzymatic hydrolysis as well as decreasing the sugar yields (Turner et al., 2003; Zhao et al., 2009; Li et al., 2009). The negative effect of ionic liquids on cellulases was reported to be irreversible, occurring due to the denaturation of the proteins, which eventually led to their inactivation (Turner et al., 2003). Protein denaturation is a process that affects the 3D structure of the protein and it is related to the disruption of the way in which a protein is folded in 3D space where the original folding patterns of a protein are important simply because they make up the certain sites related to the specific function of a given protein. Zhao et al., (2009) have attributed this denaturation to the interactions between the ionic liquid anion and the cellulase enzymes since ionic liquids with higher anion hydrogen bond basicity (those with acetate anions) reduced the cellulase activity much more than BMIMCl, which has chloride anion of lower basicity compared to acetate anion. The implication of these findings during application can be seen in the work of Li et al. (2009) where the authors showed that washing the ionic liquid pretreated wheat straw samples with water prior to enzymatic hydrolysis was vital to achieve high sugar yields since residual ionic liquid left on the samples due to poor washing led to an almost 50% decrease in the sugar yields.

1.5. Hemicellulose based films

Just like the production of glucose from lignocellulosic biomass followed by the utilization of this glucose for the production of biofuels such as ethanol, production of environmentally friendly, biodegradable materials is also a subject that draws a lot of attention. Although the durability of synthetic plastics against environmental effects is a desired property for many applications, such durability is generally more than necessary considering the excessively long degradation periods of plastics in nature once their useful periods are over. On the other hand materials produced from biodegradable polymers degrade in nature within a few years provided that the adequate environmental conditions are met. Plastics are a daily part of our lives and they are produced in very large quantities where their disposal is problematic due to their resistance against environmental factors, which results in environmental pollution. The research and product development regarding biodegradable polymers with a broad range of application areas is thus crucial for a sustainable environment.

Biodegradable polymers can be defined as polymers that can be degraded due to biological effects related to the attack of microorganisms to the polymer structure followed by its consumption, which results in the conversion of the polymers to CO_2 + H_2O or CH_4 + H_2 under aerobic and anaerobic conditions, respectively (Chiellini and Solaro, 1996). The number of polymers that can be referred as biodegradable is very high including cellulose, hemicellulose, lignin, proteins, chitin, chitosan (Gandini, 2011; Johansson et al., 2012). However in terms commercial availability in the form of polymeric materials such as films, bags, cups and bottles, starch and polylactic acid (PLA) can be considered as the two major biodegradable polymers each with roughly dozens of different producers around the world (Vroman and Tighzert, 2009).

In a similar context with that of the starch based biofuel production, production of starch and PLA also create a competition between the nutritional function of starch containing (or sugar containing) crops and their function as raw materials for the biodegradable polymer production. A competition for the farm lands also takes place. Starch based polymers contain the starch directly where the starch is mostly obtained from corn. PLA is synthesized from lactic acid, which is produced via the fermentation of sugars where these sugars are obtained from the hydrolysis of starch found in corn or directly from sugar cane or sugar bagasse (Chiellini and Solaro, 1996). This is one of the major points where the rationale of producing biodegradable polymers and materials from hemicelluloses is based upon since these polymers can be obtained from lignocellulosic biomass, which does not have any function as a food.

As described previously, hemicellulose is one of the major components of lignocellulosic structure and it accounts for around 20 – 40% of a lignocellulosic biomass depending on the type of the biomass. The form of the polymeric materials that are produced from hemicelluloses is generally polymer films or coatings (Hansen and Plackett, 2008; Mikkonen and Tenkanen, 2012). In addition to being biodegradable and renewable, an important property of hemicellulose based films is their barrier property (or low permeability) against oxygen, aroma and grease, making them suitable for food packaging applications and thus increasing their economic value (Hansen and Plackett, 2008; Mikkonen and Tenkanen, 2012; www.xylophane.com).

So far, a large variety of lignocellulosic resources were utilized for the production of hemicellulose based films including aspen wood (Gabrielii et al., 2000; Gröndahl et al., 2004), maize bran (Peroval et al., 2002; Fredon et al., 2002), corn hull (Zhang and Whistler, 2004), barley husks (Hoije et al., 2005), cotton stalks (Göksu et al., 2007; Bahçegül, 2009), sunflower stalks (Bahçegül, 2009), corn cobs (Bahçegül, 2009; Toraman, 2012), rye (Hoije et al., 2008) and oat spelts (Mikkonen et al., 2009). Other than directly obtaining hemicelluloses from lignocellulosic biomass, hemicelluloses that are found in the by-product streams of wood processing operations can also be

recovered and utilized for the production of hemicellulose based films (Hartman et al., 2006a; Hartman et al., 2006b; Edlund et al., 2010; Ryberg et al., 2011; Ibn Yaich et al., 2012). Appearance of a hemicellulose based film is shown in Figure 1.6.

Figure 1.6. A hemicellulose based polymeric film pictured in front of the cotton stalks it was obtained from.

Properties of hemicellulose based films are dependent on the properties of the hemicelluloses they are made from where the extraction procedure used for the isolation of the hemicelluloses from lignocellulosic biomass can have dramatic effects on the properties of the isolated hemicelluloses. Glasser et al. (2000) have shown that using alkaline extraction enabled the isolation of hemicelluloses at higher yields and

higher molecular weights compared to hemicellulose extraction via steam explosion. The authors have used poplar and barley husks as lignocellulosic biomass where it was shown that the steam explosion technique prevented the hemicelluloses to be isolated in polymeric form while opposite was true for alkaline extraction. For instance hemicelluloses isolated from barley husks had 10 times higher DP compared to those isolated via steam explosion (Glasser et al., 2000). These findings are especially important from the hemicellulose based film production point of view since the DP and thus the molecular weight of the polymers significantly affect the material properties including mechanical properties.

Hoije et al. (2005) have also investigated the effects of isolation conditions where different pretreatments prior to alkaline extraction of hemicelluloses were applied to barley husks followed by the preparation of hemicellulose based films from the isolated fractions via solvent casting. The authors reported that the best films were obtained from hemicelluloses that were isolated from delignified barleyhusks where the delignification was accomplished by chlorite treatment. These films were homogeneous and transparent and their UTS and e_b values were 50 MPa and 2.5% (Hoije et al., 2005).

Film formation, which is observed upon solvent casting, is a crucial point that should be mentioned regarding the hemicellulose based films since utilization of plasticizers and/or polymers other than hemicelluloses in order to aid in the intact film formation in addition to improving the mechanical properties is a quite common approach. Gabrielii and Gatenholm (1998) observed that film formation could not be obtained for the xylan type hemicelluloses isolated from birchwood while addition of 10% chitosan to the film forming solution on a dry weight basis was needed in order to obtain continuous and intact films. Similar results were also reported by Gabrielii et al. (2000) for the hemicelluloses isolated from aspen wood where it was reported that while pure hemicelluloses could only form very small fragments of film like structures with roughly 1 × 1 mm dimensions, 10% chitosan was again necessary to induce complete film formation upon solvent casting. The authors have also reported that increasing the hemicellulose to chitosan ratio of the composition of the films resulted in more brittle films (Gabrielii et al., 2000). Kayserilioglu et al. (2003) followed a different approach compared to these two studies where hemicelluloses from birchwood were used as additives in wheat gluten films. It was shown that wheat gluten based films could tolerate up to 40% birchwood xylan in terms of film formation while further increases in the hemicellulose content impaired the film formation. Mikkonen et al. (2008) used another biodegradable polymer, polyvinyl alcohol (PVA) in order to improve the mechanical properties of the mannan type hemicellulose based films and reported that despite of the significantly positive effects of the PVA addition at increasing concentrations, the two polymers were not totally compatible to each other since phase separations were observed in the films.

Carboxymethyl cellulose, a water soluble cellulose derivative, was used in a series of studies between a concentration of 30 – 50% where mannan type hemicelluloses were used as the base polymer matrix for the production of oxygen barrier films and coatings (Hartman et al., 2006a; Hartman et al., 2006b; Edlund et al., 2010; Ryberg et al., 2011; Ibn Yaich et al., 2012). The reason for the utilization of CMC was explained on the basis that films composed of solely hemicelluloses were fragile and CMC addition improved the mechanical properties as well as the moisture resistance of the films where CMC was identified to be compatible with the hemicellulose since no phase separation was observed in the films (Hartman et al., 2006a). Hartman et al. (2006a) have also shown that compared to the addition of the plasticizers sorbitol and xylitol, CMC addition resulted in lower oxygen permeability, which is a desired result since it means the enhancement of the oxygen barrier properties of the films.

Edlund et al. (2010) used CMC and chitosan separately as additives during the preparation of mannan type hemicellulose based films and coatings with oxygen barrier properties where the hemicelluloses were obtained from spruce wood hydrolysate. Films composed of hemicellulose and chitosan at a 1:1 ratio had an oxygen transmission rate of 10.8 cm^3/m^2 day, which was reduced to 0.3 cm^3/m^2 day when CMC was used instead of chitosan, showing that CMC was a better option compared to chitosan if oxygen barrier performance is the crucial criteria. The authors also provided the oxygen transmission rate of polyethylene terephthalate (PET) as 38.9 cm^3/m^2 day, which emphasized that films containing hemicellulose together with CMC are really good barriers against oxygen when compared to the conventional synthetic plastics. Edlund et al. (2010) have also demonstrated that the solutions of these polymers can be coated on to PET where a coating of approximately 6 μm in thickness resulted in the oxygen transmission rate of PET to decrease from 38.9 cm^3/m^2 day to 4.2 cm^3/m^2 day in the case of hemicellulose blended with CMC. In addition to oxygen barrier properties of the films the authors have also investigated the effect of preferring CMC over chitosan in terms of the tensile properties of the films where it was found that unlike the oxygen transmission rate case, blends of hemicellulose and chitosan have better mechanical properties compared to the films prepared with CMC. However the differences in the oxygen transmission rates due to the preference of CMC over chitosan were much higher compared to the differences taking place in the mechanical properties of the films and thus CMC can be considered as a better option compared to chitosan (Edlund et al., 2010).

Grondahl et al. (2004) added the plasticizers xylitol and sorbitol at concentrations of 20, 35 and 50% to xylan type hemicelluloses obtained from aspen wood where complete film formation could not be obtained when hemicellulose was used alone. In this case, instead of intact films, very brittle fragments were observed where the authors associated this with the high glass transition temperature of the hemicellulose and added plasticizers in order to lower it. Of the tested plasticizers, sorbitol showed a

better performance compared to xylitol since slightly higher elongation at break values were obtained with this plasticizer. The poorer performance of xylitol compared to sorbitol was explained on the basis of the crystallization of this plasticizer at high loadings. Films containing 35% sorbitol as plasticizer were also tested for their oxygen barrier properties and it was found that the oxygen permeability of hemicellulose based films were similar to that of ethylene vinyl alcohol (EVOH) film, which is a conventional oxygen barrier film widely utilized for food packaging purposes in the market.

A different application of hemicelluloses in terms of food packaging was demonstrated by Zhang and Whistler (2004) where grapes were coated with film forming solutions composed of hemicellulose and sorbitol. The idea that hemicellulose based polymers could be used as edible coatings on foods was emphasized. The effect of hemicellulose based edible coatings on the water loss of the grapes was compared to uncoated grapes where it was found that the hemicellulose based coatings caused a significant reduction in the water loss of the grapes at the end of seven days of storage from 25% water loss for the uncoated grapes to 15% for the grapes coated with hemicellulose and sorbitol solution. The authors have also prepared films other than coatings and inspected the effect of the plasticizers propylene glycol (PG), glycerol and sorbitol. Among the three plasticizers glycerol was most effective in increasing the elongation at break values but it also severely reduced the tensile strength of the films (Zhang and Whistler, 2004).

The mechanical properties of a polymeric material are of major importance for their utilization in a given application area since a material is expected to maintain its integrity against forces that it might be exposed to. The mechanical properties of different hemicellulose based films containing various additives and other polymers in terms of ultimate tensile strength (UTS), elongation at break (e_b) and elastic modulus (E) are given in Table 1.1 in order to provide an overall picture of the issue. Based on the literature values shown in Table 1.1, it can be said that UTS values of hemicellulose based films can reach around 50 MPA or slightly higher, which can be considered as satisfactory values. However a rather typical problem of the hemicellulose based films appears to be their very low elongation at break values or their ductility.

In terms of industrial production, a Swedish company named Xylophane (www.xylophane.com) is currently conducting pilot scale production of xylan type hemicellulose based films where the application will be in the form of dispersion coating of the hemicellulose solution as an oxygen barrier layer applied on to other packaging materials made of paper, cardboard or synthetic plastics.

Table 1.1. Mechanical properties of hemicellulose based films produced from different types of lignocellulosic biomass (Adapted from Toraman, 2012).

Biomass	**Additives**		**E (MPa)**	**UTS (MPa)**	**e_b (%)**	**Reference**
	Polymer	**Plasticizer**				
Birch wood	Wheat gluten (60-90%)	Glycerol (2%)	~20-200	~2-8	~20-380	Kayserilioğlu et al., 2003
Corn cob	Wheat gluten (80%)	-	~10	~1	~620	
Grass	Wheat gluten (80%)	-	~35	~3	~40	
Aspen wood	-	Xylitol (20-50%)	~100-900	5-40	2-8	Gröndahl et al., 2004
		Sorbitol (20-50%)	200-5000	6-40	2-11	
Corn hull	-	-	1316	54	6	Zhang and Whistler, 2004
		Glycerol (0-22%)	365-1148	10-47	6-12	
		Propylene Glycol (0-22%)	1225-1320	53-61	6-8	
		Sorbitol (0-22%)	463-1161	20-48	6-9	
Barley husk	-	-	2930	50	3	Höije et al., 2005
Rye	-	-	630-1750	37-58	5-10	Höije et al., 2008
Oat spelt	-	Glycerol (10-40%)	~100-1100	~4-28	~5-11	Mikkonen et al., 2009
		Sorbitol (10-40%)	~300-700	~9-17	~4-9	
Softwood hydrolysate	CMC (20-50%)	-	1624-2432	10-51	1-3	Edlund et al., 2010
	Chitosan (40-50%)	-	1915-2365	48-62	6-7	
Norway spruce	-	-	2735	55	3	Escalante et al., 2012
	-	Sorbitol (5-25%)	1163-2612	26-57	3-4	

The multilayered packaging materials produced in this manner are proposed for the packaging of oxygen sensitive products (such as dairy products) or aroma rich products including spices and coffee.

1.6. Objective of the study

Biorefineries capable of utilizing lignocellulosic biomass as raw materials for the production of fuels, chemicals and materials are considered as central facilities for the transformation of petroleum based products to renewable and environmentally friendly products. As mentioned in the previous sections, production of multiple products is essential for developing an economically realistic lignocellulosic biorefinery strategy. Based on the importance of this multi-product approach, the present thesis study aims the investigation of the effects of various process parameters during the conversion of lignocellulosic agricultural wastes to glucose and hemicellulose based polymeric materials. Different types of biomass pretreatments and pretreatment parameters were studied in addition to different hemicellulose extraction strategies and two types of polymer processing techniques. The studied process parameters were mainly evaluated with respect to their effect on the glucose yield and on the mechanical properties of hemicellulose based films.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Corn cobs and cotton stalks were obtained from Hatay and Adıyaman (Turkey), respectively. Ionic liquids 1-ethyl-3-methylimidazolium chloride (EMIMCl, purity ≥ 95%) and 1-ethyl-3-methylimidazolium acetate (EMIMAc, purity ≥ 90%), which were produced by BASF, were purchased from Sigma-Aldrich. The enzymes Celluclast 1.5L (Cellulase mixture) and Novozyme 188 (β-glucosidase) were obtained from Novozymez (Denmark). Sulfuric acid, sodium hydroxide, potassium hydroxide, tri-sodium citrate dihydrate, citric acid monohydrate, sodium azide, D-glucose and D-xylose were purchased from Merck (Germany). Potassium sodium tartarate, 3,5-dinitrosalicylic acid, phenol, sodium sulfate, cellobiose, potassium acetate, sodium chloride, sorbitol and alkali lignin were purchased from Sigma–Aldrich (USA).

2.2. Pretreatments

2.2.1. Dilute acid pretreatment

Dilute acid pretreatment was applied to corn cobs. Dilute acid pretreatment was based on a previously reported procedure (Silverstein et al., 2007). 5 grams of corncob at a solid loading 10% (w/v) was incubated with 2% (w/v) H_2SO_4 in an autoclave at 121°C for 1 h. At the end of pretreatment, the recovered biomass was washed with 200 ml deionized water for 3 times. During the final wash, the pH of the suspension was adjusted to 4.8 via NaOH and the biomass was rinsed with deionized water. The pretreated biomass was dried in an incubator at 60°C for 16 h and weighted prior to enzymatic hydrolysis.

2.2.2. Alkaline pretreatment

2.2.2.1. Alkaline pretreatment of corn cobs

Alkaline pretreatment applied to corn cobs was conducted identically to dilute acid pretreatment with the exception that NaOH was used instead of H_2SO_4 during pretreatment and the pH adjustment was made with acetic acid instead of NaOH (Silverstein et al., 2007).

2.2.2.2. Alkaline pretreatment of cotton stalks

The alkaline pretreatment applied to cotton stalks had different parameters compared to that applied to corn cobs. Alkaline pretreatment procedure applied to cotton stalks was mainly derived from the previously reported procedures by Lawther et al. (1996) and Zilliox and Debeire (1998). 5 grams of cotton stalk was swelled in 100 ml water for 1 h at room temperature with magnetic stirring. The suspension was filtered and the cotton stalks were incubated with 50 ml of 10% (w/v) KOH solution at three different temperatures (25, 60 and 90°C) for 3 h. Pretreatment vessels were placed in a silicon oil bath, which was placed on a magnetic stirrer equipped with a PT 1000.60 temperature sensor (IKA RCT Basic Safety control, Germany) in order to provide magnetic stirring at 400 rpm and to maintain desired pretreatment temperatures. At the end of pretreatment, the biomass was obtained by filtration. The pretreated samples were washed with 200 ml of deionized water for 3 times, where during the final wash the pH of the suspension was adjusted to 4.8 via the addition of acetic acid. Finally, the pretreated biomass was rinsed with de-ionized water and dried in an incubator at 60°C overnight and weighted. Cellulosic portion recovery is defined as the ratio of alkaline insoluble biomass recovered after pretreatment to untreated cotton stalks subjected to pretreatment.

2.2.3. Ionic liquid pretreatment

2.2.3.1. Ionic liquid pretreatment of corn cobs

EMIMAc was used for the ionic liquid pretreatment of corncobs. 1.5 g of corncob was incubated with EMIMAc at a solid loading of 10% (w biomass / w ionic liquid) in an open atmosphere. The beakers containing corncob and ionic liquid were placed in a silicon oil bath placed on a magnetic stirrer equipped with a temperature sensor in order to control the temperature of the oil bath. The pretreatment was conducted at 150°C for 1 h with magnetic stirring at 400 rpm. At the end of 1 h, 75 ml of deionized water was added to the pretreatment system and the suspension was filtered to recover the pretreated biomass. The recovered biomass was washed extensively with 75 ml of deionized water for 5 times in order to prevent cellulase deactivation due to the ionic liquid, which may adversely affect the subsequent enzymatic hydrolysis (Turner et al., 2003; Li et al., 2009; Zhao et al., 2009) and dried at 60°C for 16 h prior to enzymatic hydrolysis.

2.2.3.2. Ionic liquid pretreatment of cotton stalks

Cotton stalks with four different particles sizes were subjected to pretreatment via the EMIMAc and EMIMCl ionic liquids. Cotton stalks were milled with a Thomas-Wiley mill (Arthur Thomas Co., USA) and separated via sieves into four different particles

sizes (<0.15 mm, 0.15–0.5 mm, 0.5–1.0 mm and 1.0–2.0 mm). Prior to pretreatments cotton stalks were dried at 105°C for 2 h followed by their incubation with the ionic liquids at a solid loading of 5% (w biomass/w ionic liquid). The pretreatments were conducted at open atmosphere in glass vessels immersed in a silicon oil bath placed on a magnetic stirrer equipped with a temperature sensor to control the temperature of the oil bath while providing magnetic stirring at 400 rpm. All pretreatments were conducted at 150°C for 30 minutes. Complete dissolution of cotton stalks in the ionic liquids was not observed for any of the pretreatments. Identical steps to those reported for corn cobs were followed during the washing and drying steps conducted at the end of 30 minutes of pretreatment. All kinds of pretreatments in the present study were conducted in duplicates.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of biomass samples was conducted via the Celluclast 1.5L and Novozyme 188 enzyme preparations where the cellulase activity was determined as 75 filter paper units (FPU)/ml for the Celluclast 1.5L (Haykir, 2013) and the β-glucosidase activity for Novozyme 188 was reported as 480 cellobiose units (CBU)/ml. Enzymatic hydrolysis was performed in glass bottles with screw caps. Untreated and pretreated lignocellulosic biomass samples was subjected to enzymatic hydrolysis at a biomass loading of 3% (w/v) in 0.05 M sodium citrate buffer at pH 4.8. Enzyme loadings were 50 FPU/g biomass and 60 CBU/g biomass for the cellulase and β-glucosidase enzymes, respectively. Enzymatic hydrolysis suspensions also contained sodium azide at a concentration of 0.02 w/v in order to prevent the growth of microorganisms thoroughout the course of enzymatic hydrolysis, which lasted for 72 h in some cases (Selig et al., 2008). The enzymatic hydrolysis systems were placed in a shaking incubator (Infors Minitron, Switzerland) at 50°C and 150 rpm and at the required time intervals. Samples were pipetted from the bottles into 1.5 ml centrifuge tubes. Following the collection of the samples from the enzymatic hydrolysis systems, the tubes were immersed into boiling water for 5 minutes in order to stop the enzymatic reaction. Following this the samples in the tubes were centrifuged at 10000 × g for 5 minutes and the supernatants were collected for reducing sugar and glucose analysis. Each enzymatic hydrolysis was conducted in duplicates.

2.4. Compositional analysis

The compositional analysis of corn cobs were conducted according to the National Renewable Energy Laboratory (NREL) analytical procedure (Sluiter et al., 2008). This procedure is based on the digestion of the lignocellulosic biomass via sulfuric acid in two steps. In the first step 300 mg of biomass was added to 3 ml of 72% H_2SO_4 in glass tubes. The tubes were placed in a water bath at 30°C for 1 h where at every 5 minutes the suspensions in the tubes were stirred with glass rods. At the end of 1 h, the

H_2SO_4 was diluted to 4% with the addition of deionized water and the suspensions were transferred to autoclave bottles with screw caps. The bottles were placed in an autoclave for 1h at 121°C. The bottles were removed from the autoclave, cooled to room temperature and the suspensions were filtered through crucibles in order to recover the insoluble material in the suspensions, which was the acid insoluble lignin content. The crucibles were dried and the lignin content was calculated as the difference between the initial and final weights of the crucibles. On the other hand, the filtrate, which contains the sugar monomers, was subjected to HPLC analysis for the determination of the cellulose and hemicellulose content of the samples following the neutralization step performed by the addition of calcium carbonate to the samples until the samples pH reached 6.

2.5. HPLC analysis

A Shimadzu LC-20A HPLC system (Japan) was used for the HPLC analysis of the samples by using a refractive index detector. BIORAD Aminex HPX-87H column was installed to the HPLC system where the analysis conditions were 55°C with a flow rate of 0.6 ml/min. 5 mM H_2SO_4 was used as the mobile phase. D-glucose and D-xylose were used as standards. All samples to be analyzed in the HPLC system were syringe filtered through 0.22 μm filters into the HPLC auto sampler vials.

2.6. DNS assay

The reducing sugar contents of the enzymatic hydrolysates were determined via dinitrosalicyclic acid (DNS) assay (Miller, 1959). This assay relies on the reaction of the reducing ends of the sugars with the DNS reagent where increasing reducing sugar concentration results in a stronger color development upon reaction, which is quantified by using a spectrophotometer at 540 nm wavelength. Appropriately diluted samples were reacted with the DNS reagent in tubes submerged into boiling water for 5 minutes. The tubes were cooled to room temperature and the absorbance of the samples was determined at 540 nm. D-glucose was used as the standard.

2.7. Glucose determination via blood glucose monitors (BGMs)

4 different BGMs were used in order to measure the glucose concentrations in the enzymatic hydrolysates of biomass samples. BGMs and their corresponding strips were purchased from a local pharmacy. According to the manufacturer's specifications, Optium Xceed (Abbott Diabetes Care, UK) uses test strips (Optium Plus) containing glucose dehydrogenase with nicotinamide adenine dinucleotide cofactor (GDH-NAD) while OneTouch Select (LifeScan Inc., USA) uses test strips with glucose oxidase (GOx). The test strips used with Bayer Contour Ts (Bayer Consumer Care AG, Switzerland) contain glucose dehydrogenase with flavin adenine

dinucleotide cofactor (GDH-FAD). All three BGMs are amperometric biosensors, where the sample is automatically withdrawn into the test strip upon the contact of the strip's tip with the sample. Accu-Chek Active (Roche Diagnostics GmbH, Germany) is a colorimetric device where the sample is applied on the designated area on the strip, which results in the development of a certain color in a circular area on the other side of the strip where this color is read by the BGM and converted to a glucose concentration. This measurement is conducted via the pyrroloquinoline quinone dependent glucose dehydrogenase (GDH-PQQ) mediator reaction. All the BGMs used in the study were capable of performing measurements within 5 to 8 seconds with sample volumes of 0.6 – 1 μl according the manufacturer's specifications. Prior to the measurements, each device was first coded according to the directions of the manufacturers for its own LOT# of strips via inserting a coding bar (Optium Xceed), entering the code manually to the device (One Touch Select) or inserting a coding chip to the side of device (Accu-Chek Active) where no coding was required for Contour Ts. For each BGM, strips with the same LOT# were used throughout the study.

Standard solutions of glucose and cellobiose in 0.05 M sodium citrate buffer at pH 4.8 were prepared in order to measure the response of the BGMs to glucose and cellobiose at a concentration range of 0.5 – 4.0 mg/ml and to obtain the calibration curves and equations used to calculate glucose concentrations in the enzymatic hydrolysates of biomass samples. The samples from the enzymatic hydrolysis systems were diluted in order to obtain readings within the glucose concentration range used to obtain the calibration curves. The dilutions were made with the same buffer used to obtain the calibration equations in order to eliminate the effect of pH, which might influence the readings made with the BGMs to a certain extend depending on the type of the BGM used (Choy et al., 2007, FitzGerald and Vermerris, 2005). Each measurement was conducted in duplicates. The % error made by each BGM was calculated based on the following equation:

$$\%\ \text{Error} = \frac{|C_{BGM} - C_{HPLC}|}{C_{HPLC}} \times 100$$

C_{BGM} is the glucose concentration obtained with BGM and C_{HPLC} is the glucose concentration obtained via HPLC. Each measurement with a given BGM was conducted twice.

2.8. Hemicellulose extraction

2.8.1. Hemicellulose extraction from cotton stalks

Hemicellulose extraction from cotton stalks is actually performed during the pretreatment applied to cotton stalks as described in section 2.2.2.2 where the isolation

of hemicelluloses is conducted following the removal of the removal of the alkaline insoluble portion from the pretreatment suspension. The filtrate was centrifuged at 8000 × g for 10 min in order to obtain a clear solution free of any remaining small alkaline insoluble particles. Following the centrifugation, 125 ml of precipitation solution, which is composed of 1:10 volumetric ratio of acetic acid to ethanol, was added to the solution as suggested by Zilliox and Debeire (1998). The precipitated material, which is regarded as the hemicellulosic portion, was collected by filtration and dried at ambient conditions and weighted upon drying. Hemicellulosic portion recovery is defined as the ratio of precipitated biomass recovered after pretreatment to untreated cotton stalks subjected to pretreatment. Extractions were conducted in duplicates.

2.8.2. Hemicellulose extraction from corn cobs for the production of films via solvent casting

The procedure of hemicellulose isolation from corn cobs was adapted from previously reported procedures (Lawther et al., 1996; Zilliox and Debeire, 1998). Prior to the alkaline extraction, corn cobs were milled with a Thomas-Wiley mill to a particle size of less than 2 mm. 20 g of ground corn cobs was swelled in water and filtered with a filtering cloth. The wet corn cob particles were suspended in 170 ml of 10% KOH solution at room temperature with magnetic stirring. At the end of 3 hours the suspension was filtered and centrifuged at 8000 × g for 10 minutes. The hemicellulose in the alkaline solution was precipitated with 500 ml of ethanol-acetic acid solution, which had an ethanol:acetic acid volumetric ratio of 10:1, and the precipitated polymers were recovered via filtration. In order to remove the salts formed during precipitation due to the acid-base reaction between KOH and acetic acid, the recovered polymers were partially dissolved in 100 ml of water followed by their precipitation by adding 300 ml of ethanol into the medium. This cycle was repeated for three times prior to the final filtration and recovery of the polymers. This last step was not conducted for the case in which KAc was intentionally retained together with the hemicellulose. KAc content of the isolated polymers was determined via inductively coupled plasma optical emission spectrometry (ICP-OES). The polymers were dissolved in ultra-purified water and the solutions, which were syringe filtered through a 0.22 μm filter, were analyzed with a Perkin Elmer Optima 4300DV ICP-OES instrument to determine the potassium content of the samples. Extractions were conducted in duplicates.

2.8.3. Hemicellulose extraction from corn cobs for the production of strips via extrusion

The isolation of xylan from corn cobs was conducted according to a previously reported procedure by Zilliox and Debeire (1998) with some modifications. Corn cobs

were milled to a particle size of less than 2 mm and 100 g of milled corn cobs were swelled in water at room temperature for 15 minutes. Following filtration, swelled corn cobs were suspended in 850 ml of 24% KOH solution at room temperature with magnetic stirring for two hours. The suspension was filtered with a filtering cloth and centrifuged at 5000 × g in order to obtain an alkaline xylan solution. The xylan polymers in the alkaline solution were precipitated by the addition of a 2.5 L of acetic acid and ethanol solution (1:10 volumetric ratio of acetic acid to ethanol). The precipitated polymers were recovered from the suspension via a filtering cloth. The recovered polymers were partially solubilized in 200 ml of water and re-precipitated by the addition of 600 ml of ethanol. This step was repeated 3 times and finally the precipitated polymers were recovered and left to dry at room temperature. Extractions were conducted in duplicates.

2.9. Solvent casting

2.9.1. Solvent casting of hemicelluloses extracted from cotton stalks

0.5 grams of hemicellulosic portion was dissolved in 15 ml of de-ionized water at 85°C in a period of 45 minutes on a magnetic stirrer equipped with a temperature sensor. After the dissolution, the solutions were poured into petri dishes with a diameter of 9 cm. Films were dried and aged for 2 days upon drying in ambient conditions with a temperature of 21 ± 2°C at 42 ± 3% relative humidity as determined by a thermo-hygrometer with data logging function (TFA Dostmann Klima Logger, Germany). The water content of the films was determined by drying the films at 105°C in an oven until they reached a constant weight. The lignin content of the films was analyzed by dissolving the films in de-ionized water and measuring the absorbance at 280 nm with a UV-Vis spectrophotometer as suggested by Westbye et al. (2007). Alkali lignin was used as the standard during the determination of the lignin content of the films.

2.9.2. Solvent casting of hemicelluloses extracted from corn cobs

A stock solution of the desalted hemicelluloses in water was prepared with a concentration of 1 g of hemicellulose/37.5 ml of water. The solution was divided equally into separate beakers where the amount of hemicellulose in each beaker was 0.4 g on a dry basis. Separate solutions of each additive (Potassium acetate, sodium chloride and sorbitol) were prepared and the corresponding volumes of these solutions were transferred to the hemicellulose solutions in order to obtain the film forming solutions containing the specified amount of the additives (10% or 25% w/w on a dry basis). For the films obtained from hemicelluloses containing potassium acetate, the film forming solution was obtained by dissolving 0.4 g of hemicellulose in 15 ml water. The film forming solutions were poured into plastic petri dishes with a diameter

of 9 cm and left to dry at ambient conditions where the temperature and the relative humidity of the surroundings were recorded as 22 ± 1°C and 47 ± 2% throughout the drying period with a thermo-hygrometer. The films were detached from the petri dishes and used in further characterizations. Each type of film was prepared in duplicates.

2.10. Extrusion

A twin-screw, co-rotating extruder (Thermo HAAKE MiniCTW) with conical screws (Screw diameter: 4–15 mm, screw length: 109.4 mm) and two heating zones was used for the extrusion of xylan polymers (Figure 2.1). An important feature of this extruder is its capability to work with small amounts of material where 5 grams of polymer is sufficient to obtain the desired extrudate. A ribbon die plate with a rectangular opening of 5×0.5 mm (width × length) was attached to the extruder in order to obtain the extruded material in the form of a strip. The strip coming out of the extruder was collected on a mini moving belt. The extrusion parameters, including extrusion temperature and screw speed, were controlled via a PC connected to the extruder by using the software supplied together with the extruder.

Prior to extrusion, xylans were first conditioned at three different relative humidities (10%, 55% and 90%) for 24 h in order to obtain polymers with different water contents. For each batch of conditioned polymers, 3 extrusion temperatures (60, 90 and 120°C) were studied. The extrusions were performed at a screw speed of 50 rpm and 5 g of xylan polymers were manually fed to the extruder for each run. Each extrusion trial was conducted twice by using two different batches of xylan polymers. All parts of the extruder including the barrel, screws and the die were thoroughly cleaned before starting a new trial in order to eliminate any possible effect the residual xylan polymers left from previous runs might have on the new extrusion trial. Polylactic acid (NaturePlast PLI005) was also extruded in the same extruder at a screw speed of 50 rpm where extrusion temperature was set to 170°C (Baouz et al., 2013).

2.11. Tensile testing

The tensile properties of the films and strips obtained from hemicelluloses were determined with a universal testing machine (Zwick/Roell Z250, Zwick GmbH & Co., Germany) found at Middle East Technical University (METU) Central Laboratory. If the samples to be tested were films, they were cut into dog-bone shaped specimens with a manual cutting press. If strips were to be tested, rectangular specimens wereobtained by cutting the strip obtained from the extruder to pieces with 8 cm length. For the testing of the dog bone shaped film samples, a 100 N load cell and pneumatic grips were used while for the testing of the strips a 10 kN load cell together

with wedge grips was installed to the testing machine. The tests were performed at a cross-head speed of 5 mm/min. The conditions of the testing room, in terms of relative humidity and temperature, were controlled by means of a climatic room conditioner (Tecnair LV) with special designed air ducts installed to the testing room. During the test, the relative humidity and temperature were between the limits of 50 ± 5% and 23 ± 1°C. The results of the tests were obtained from the software (Testexpert 2) accompanying the universal testing machine. Ultimate tensile strength (UTS), elongation at break (e_b), elastic modulus (E) and toughness were the determined properties during tensile testing measurements. The corresponding locations of these values in the stress-strain diagram are shown in Figure 2.2. At least 5 specimens were tested for each type of material.

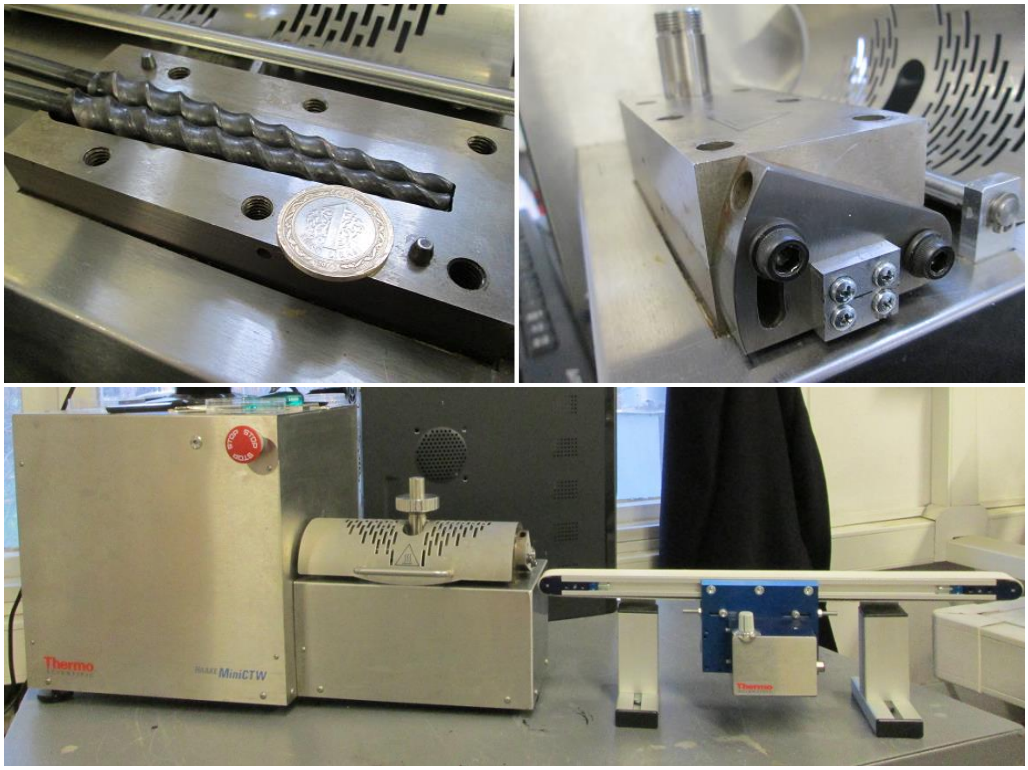

Figure 2.1. Pictures of the extruder used for the extrusion of hemicelluloses into strips. Top left: Upper half of the extruder barrel removed to show the twin screws. Top right: Extruder barrel closed with the die assembly placed at the exit. Bottom: Complete view of the extrusion system including the mini conveyor belt.

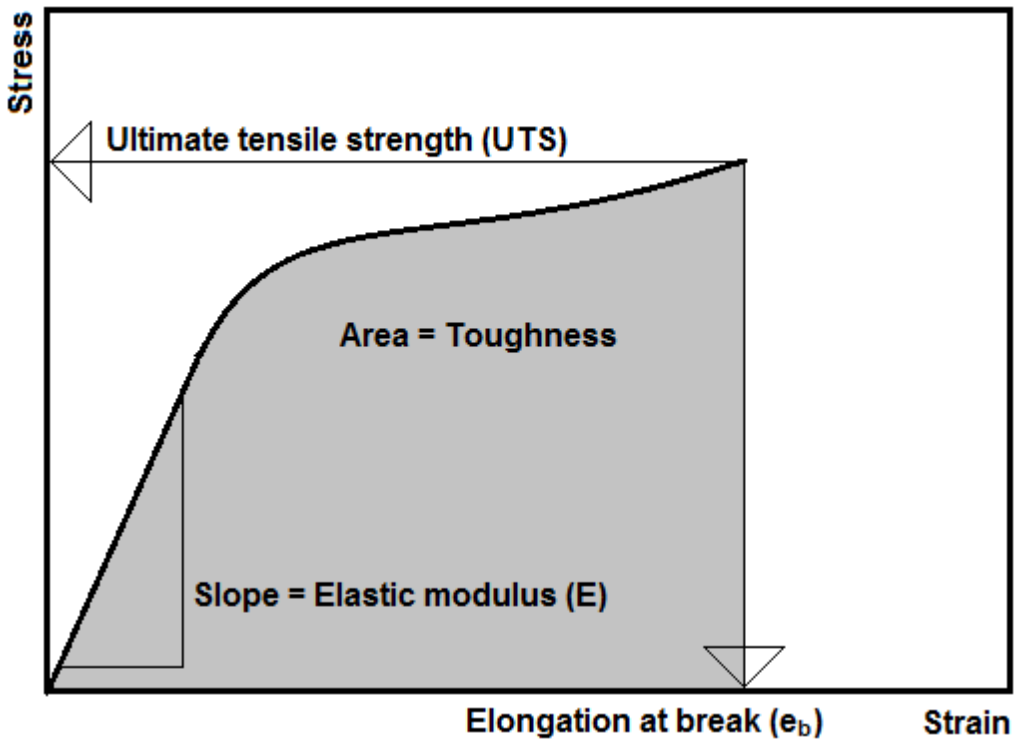

Figure 2.2. Illustration of the different tensile properties on the stress-strain curve.

2.12. Indentation testing

The indentation tests were conducted at METU Central Laboratory with a nanoindentation tester (CSM Instruments, Switzerland). The load was applied to the specimens with a Berkovich tip and the maximum value of the load was fixed at 30 mN. The data were analyzed by the nanoindentation tester's software by using Oliver-Pharr method (Oliver and Pharr, 1992) in order to determine the hardness and elastic modulus values. The water content of the films was determined from the weight loss of the films that took place upon drying at 105°C for 24 hours. The films were conditioned for 2 days at 33% relative humidity at a temperature of 23°C prior to indentation tests and water content measurements.

2.13. Scanning electron microscopy (SEM)

SEM analysis were performed at the METU Central Laboratory. A Quanta 400F scanning electron microscope was used to characterize the cross-sectional morphology of the films and strips at a voltage of 5 kV. The samples were immersed into liquid

nitrogen and fractured in order to obtain the necessary cross-sectional surface. The samples were coated with gold/palladium prior to SEM analysis.

2.14. X-ray diffraction analysis

Rigaku Ultima IV X-ray diffractometer (Japan) was used for the X-ray diffraction analysis of samples, which was conducted between 2θ = 5–40° with a scanning speed of 0.5° min^{-1} at a step size of 0.02°. The analysis were performed at METU Central Laboratory.

2.15. Equations used during the calculations

The following equations were used to calculate the results obtained upon pretreatment and the following enzymatic hydrolysis including solid recovery, digestibility, conversion to glucose and glucose yields. The explanation of each symbol is given below the corresponding equation.

$$\text{Solid recovery } (\%) = \frac{W_{PRT}}{W_{UT}} \times 100$$

W_{PRT} is the weight of biomass recovered after pretreatment and W_{UT} is the weight of untreated biomass subjected to pretreatment.

$$\text{Digestibility } (\%) = \frac{C_{RS} \times V}{W_B} \times 100$$

$$\text{Conversion to glucose } (\%) = \frac{C_G \times V}{W_B} \times 100$$

C_{RS} and C_G are the reducing sugar and glucose concentrations in the enzymatic hydrolysates, respectively. V is the volume of the enzymatic hydrolysis system, W_B is the weight of biomass subjected to enzymatic hydrolysis.

$$\text{Glucose yield } (\%) = \frac{\text{Rec}(\%) \times [\text{Con}(\%)/100]}{\text{Cellulose } (\%) \times 1.11} \times 100$$

Rec (%) is the solid recovery (taken as 100 if no pretreatment was applied), Con (%) is conversion to glucose and Cellulose (%) is the cellulose content of untreated biomass. 1.11 is the correction factor for glucose since 1 g of cellulose liberates 1.11 g glucose upon hydrolysis.

CHAPTER 3

UTILIZATION OF BLOOD GLUCOSE MONITORS AS A RAPID AND PRACTICAL METHOD FOR THE DETERMINATION OF GLUCOSE IN THE ENZMATIC HYDROLYSATES OF PRETREATED LIGNOCELLULOSIC BIOMASS

The following chapter investigates the possibility of using blood glucose monitors (BGMs) for measuring the glucose concentration in the enzymatic hydrolysates of lignocellulosic biomass. BGMs are affordable devices that are available in the drug stores and they can measure the glucose concentration in the blood in around 5 seconds. BGMs are generally used by people who have diabetes so that they can easily monitor their blood glucose levels by themselves at home without going to a hospital or a fully equipped laboratory.

A BGM has 2 main components, the first component is the device itself equipped with a digital display that shows the measured glucose concentration. The second component is a strip (single use, consumable) that gets into contact with the patient's blood. In order to perform a measurement with a BGM, the strip is first inserted to the device and then the other end of the strip is lightly contacted to a very small drop of the patient's blood, which is created by the patient by lightly piercing his/her finger's tip. The blood is drawn into the strip upon contact while in some BGM models the blood has to be dropped on to a specific area on one side of the strip.

The enzymes loaded into the strips of the BGMs are redox enzymes (oxidoreductases), which are categorized into two groups: Glucose oxidases (GOx) and glucose dehydrogenases (GDH) where the GDH has three different types depending on its cofactor (Ferri et al., 2011). These cofactors are flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD) and pyrroloquinoline quinone (PQQ) and the GDH enzymes utilizing these cofactors are designated as GDH-PQQ, GDH-FAD and GDH-NAD (Ferri et al., 2011). In other words, the BGMs of today use one of the GOx, GDH-PQQ, GDH-FAD or GDH-NAD enzymes during the determination of glucose concentration in the blood. All four of these enzymes catalyze the oxidation of the glucose molecule by acting on its reducing end, and depending on the type and measurement mechanism of BGMs the oxidation products are detected amperometrically or colorimetrically (Heller and Feldman, 2008; Wang, 2008; Ferri et al., 2011). An important issue related to the enzymes used in the strips of the BGMs is their selectivity towards glucose and their sensitivity to environmental factors. Measurements made with BGMs utilizing GOx enzyme are sensitive to the oxygen

concentration in the blood (Heller and Feldman, 2008; Ferri et al., 2011) while BGM utilizing GDH-PQQ have a selectivity problem associated with the rather broad range of sugars it is sensitive to, as discussed in the upcoming sections of this chapter. The enzymes GDH-FAD and GDH-NAD can thus be considered as more suitable enzymes for BGMs since they take advantage of both the substrate specificity and the oxygen insensitivity of the GOx and GDH-PQQ enzymes, respectively (Heller and Feldman, 2008).

Measuring the glucose concentration in the enzymatic hydrolysates of lignocellulosic biomass is among the most frequently performed tasks in a research lab working on lignocellulosic biomass pretreatment. Glucose in a solution can be determined via HPLC, bench top biochemistry analyzers and enzymatic glucose determination kits (Choy et al., 2007; Decker et al., 2009). HPLC can be considered as the standard method for measuring the glucose concentration in the enzymatic hydrolysates. Certain types of glucose determination kits that rely on the UV/Vis spectrophotometry are also quite popular in many research labs since HPLC is a rather expensive device and thus for some researchers access to a HPLC system can be hard or not possible at all. Here the word "hard" refers to the case where multiple research groups uses a single HPLC with each group using a different HPLC column. Furthermore, HPLC is a time consuming technique and need a lot of preparation before performing the actual measurements. For instance, before an HPLC analysis, a period of 2 days may be needed for the conditioning of the HPLC column, preparation, filtration and degassing of the mobile phase and filtration of each sample one by one. In other words, it might not be always possible to obtain the results instantly with a HPLC system. Such efforts can especially be tiresome if only a few samples need to be analyzed. Also someone with a certain level of experience is needed to operate the HPLC and also to prepare the samples for analysis. Problematic issues related to time consumption, personnel training and cost (including both the capital and the operational cost) can also arise during the utilization of biochemistry analyzers or glucose determination kits (Choy et al., 2007).

Fitzgerald and Vermerris (2006) investigated the performance of two BGMs that utilize GOx and GDH-PQQ enzymes for the determination of glucose in two different buffers. Although a comparison with a reference method such as HPLC was not made, the authors reported the accuracy of the GDH-PQQ utilizing device to be higher compared to the GOx utilizing device based on the known glucose concentration values. The GDH-PQQ utilizing device was also shown to be less affected from the changes in the pH of the buffer compared to GOx utilizing device. The authors have thus suggested the utilization of the GDH-PQQ using BGM and measured the glucose concentration in the enzymatic hydrolysates of untreated maize stover with this BGM.

Sopade and Gidley (2009) used a GDH-PQQ utilizing BGM and compared its performance to a GOx based colorimetric enzymatic assay for the determination of glucose during the enzymatic hydrolysis of starch. The authors have reported similar results for the glucose concentrations determined via BGM and GOx assay. Choy et al. (2007) used GOx and GDH-PQQ utilizing BGMs for the determination of glucose in the fermentation medium where results obtained via BGMs were reasonably close to those obtained via HPLC and biochemistry analyzer. In addition to these studies where the performance of the BGMs were evaluated in terms of their accuracy, BGMs utilizing GOx or GDH-PQQ enzymes were also used for the determination of glucose concentrations in the enzymatic hydrolysates of dilute acid pretreated bagasse (Alriksson et al., 2009), waste fiber sludge (Cavka et al., 2011) and native or ionic liquid pretreated micro crystalline cellulose (Jones and Vasudevan, 2010).

In a lignocellulosic biomass conversion research lab, there might be numerous occasions where one wants to easily and quickly obtain the results of an enzymatic hydrolysis in terms of glucose produced from the lignocellulosic biomass subjected to enzymatic hydrolysis. BGMs can be good tools to utilize at this stage since they are inexpensive, easy to access and operate. Virtually, no one needs to be trained to use a BGM and results are obtained approximately in 5 seconds.

There are different brands and models of BGMs in the market and as mentioned previously an important difference between these is the enzyme used in the strips of these devices. Considering this issue, this chapter investigates the applicability of four different BGMs for the determination of glucose concentration in the enzymatic hydrolysates of pretreated lignocellulosic biomass where each of the BGMs uses a different enzyme (GOx, GDH-PQQ, GDH-FAD or GDH-NAD) to determine the glucose. Since pretreatment is a key issue at this stage, the lignocellulosic biomass, which is corn cob in this case, was pretreated with three different techniques (alkaline, acid and ionic liquid pretreatments) in order to determine if the type of pretreatment has any effect on the accuracy of the readings made by the BGMs regarding the glucose concentration with respect to the values obtained via HPLC.

3.1. Selectivity and linearity of the response demonstrated by BGMs to glucose and cellobiose

While choosing or developing a method for the determination of a specific compound in a medium, one of the most important issues is the selectivity of the method towards the compound that needs to be determined. In the current case, glucose is the target compound to be determined. However, in the enzymatic hydrolysate of a given lignocellulosic biomass, glucose will not be the only molecule that is liberated from the lignocellulosic structure. Especially, due to the incomplete hydrolysis of cellulose, the glucose dimers called cellobiose will also be present in the hydrolysate together

with other cellooligomers. So it is important for the tested BGMs not to respond to various cellobiose concentrations since if they do so, the glucose concentrations calculated by the BGMs would be misleading as these would be overestimated values.

Among the tested BGMs, only Accu-Chek Active responded to cellobiose while the other three BGMs did not respond to any of the cellobiose concentrations tested, which were between 0.5 – 4 mg/ml. As shown in Figure 3.1, the response of the Accu-Chek Active to cellobiose had a fine linearity.

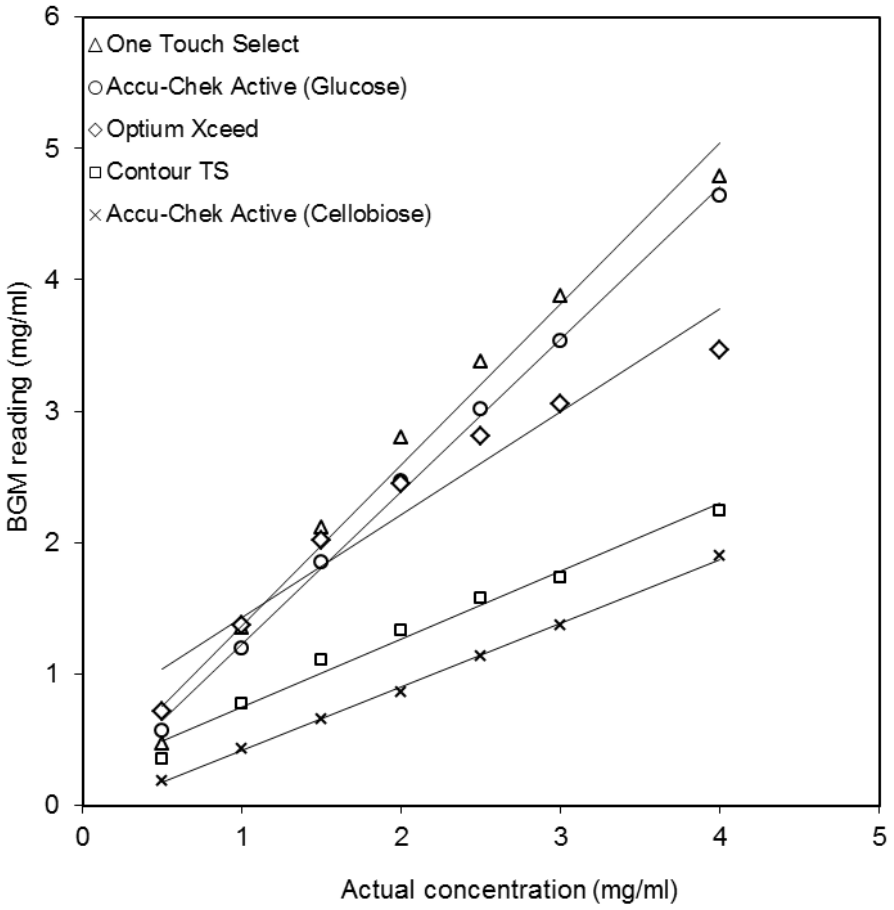

Figure 3.1. Values displayed by the blood glucose monitors vs. the actual glucose and cellobiose concentrations.

This response is likely related to the enzyme used in Accu-Chek Active, which is GDH-PQQ. Tatsumi et al. (2006) have previously shown that this enzyme could be used in a biosensor to detect cellobiose. Other Accu-Chek BGM models, which also use the enzyme GDH-PQQ, were shown to respond to molecules other than glucose including xylose and arabinose (FitzGerald and Vermerris, 2005) and maltose and lactose (Sopade and Gidley, 2009).

The response of the all four BGMs to increasing glucose concentrations from 0.5 – 4 mg/ml is shown in Figure 3.1. A comparison of the BGM readings (The glucose concentration value digitally displayed by the devices) with the actual glucose concentrations shows that the devices either overestimate or underestimate the glucose concentration. Contour Ts was the only device that displayed underestimated values while the other three displayed overestimated values. Taking into account that these devices were produced for the determination of the glucose concentration on the human blood, such a result is not surprising since the calibrations of these devices must have been made according to the human blood. However in the current case, glucose concentration in 0.05 M sodium citrate buffer (pH=4.8) was measured, which resulted in the utilization of these devices in an originally unintended medium. Nevertheless the response of all four BGMs to increasing glucose concentrations were quite linear with certain differences between the BGMs. As shown in Figure 3.1, among the all four BGMs, Accu-Chek Active gave the most linear response in the 0.5 – 4 mg/ml concentration range. The linearity of the responses given by the other BGMs was lost to a certain extent as the glucose concentration increased, which was especially obvious for Optium Xceed. Therefore for the three BGMs other than Accu-Chek Active, the 0.5 – 2 mg/ml region was selected as the concentration range in which the calibration equation was valid. As shown in Table 3.1, The R^2 value is greater than 0.99 for Optium Xceed and One Touch Select in this concentration range while R^2=0.998 for Accu-Chek Active in the 0.5 – 4 mg/ml range indicating a very high linearity for the response of this BGM to glucose in sodium citrate buffer. The lowest R^2 value between 0.5 – 2.0 mg/ml was obtained for Contour Ts as 0.981.

Table 3.1. The linear concentration range and corresponding calibration data for BGMs

BGM Model	Enzyme	Calibration Range (mg/ml glucose)	Calibration equation	R^2
Optium Xceed	GDH-NAD	0.5 - 2.0	y = 1.17x + 17.78	0.991
One Touch Select	GOx	0.5 - 2.0	y = 1.55x - 25.00	0.997
Contour Ts	GDH-FAD	0.5 - 2.0	y = 0.65x + 7.75	0.981
Accu-Chek Active	GDH-PQQ	0.5 - 4.0	y = 1.16x + 6.38	0.998

3.2. Performance of BGMs as compared to HPLC and DNS methods for the measurement of glucose concentration in the enzymatic hydrolysates of pretreated corn cobs

All four BGMs were used to determine the glucose concentration in the enzymatic hydrolysates of corn cobs pretreated with 3 different methods, which were dilute acid, alkaline and ionic liquid pretreatments. The values displayed by the BGMs were substituted into the calibration equations given in Table 3.2 in order to calculate the glucose concentrations in the hydrolysates. These values were then compared with the values obtained via HPLC and dinitrosalicylic acid (DNS) assay (Miller, 1959) in order to assess the accuracy and thus the suitability of each BGM for the determination of glucose in the hydrolysates. The DNS assay is used to measure the reducing sugar concentration in a solution on a colorimetric basis and involves the utilization of a UV/Vis spectrophotometer.

The glucose concentration values measured by the BGMs as well as the values measured by HPLC and DNS methods are shown in Figure 3.2 for both the 1st and the 24th hour of enzymatic hydrolysis. Taking the HPLC values as the actual or reference values, it can be seen that the accuracy of the BGMs depend not only on the type of pretreatment but also on the duration of the enzymatic hydrolysis.

A rather interesting result shown in Figure 3.2 is the close values obtained from the Accu-Chek Active BGM and the DNS method, which is especially true for the 24th hour of enzymatic hydrolysis regardless of the type of the pretreatment applied to corn cobs. For instance, among the pretreated samples, the largest difference between the Accu-Chek Active BGM and the DNS method occurred for the enzymatic hydrolysates of the ionic liquid pretreated sample. While Accu-Chek Active measured a concentration of 19.7 mg/ml, DNS method gave a reducing sugar concentration of 18.6 mg/ml, which results in a difference of approximately 6% between the two methods. Such a difference might be regarded as tolerable, provided that very precise measurements are not necessary. For the dilute acid pretreatment case, this difference was smaller where the measured concentrations were 20.0 mg/ml and 20.8 mg/ml for the BGM and DNS method, respectively, which correspond to a difference less than 4% between the two methods. The two methods gave the closest results for the alkaline pretreatment case (31.9 mg/ml and 31.7 mg/ml, respectively for BGM and DNS method) where the difference was less than 1%.

The DNS method is frequently used for the colorimetric determination of reducing sugars via UV/Vis spectrophotometry. Thus it appears that Accu-Chek Active BGM might be a suitable device that can be used for the determination of reducing sugars especially towards the later stages of enzymatic hydrolysis. Using the Accu-Chek Active BGM instead of the DNS method for the determination of reducing sugars in

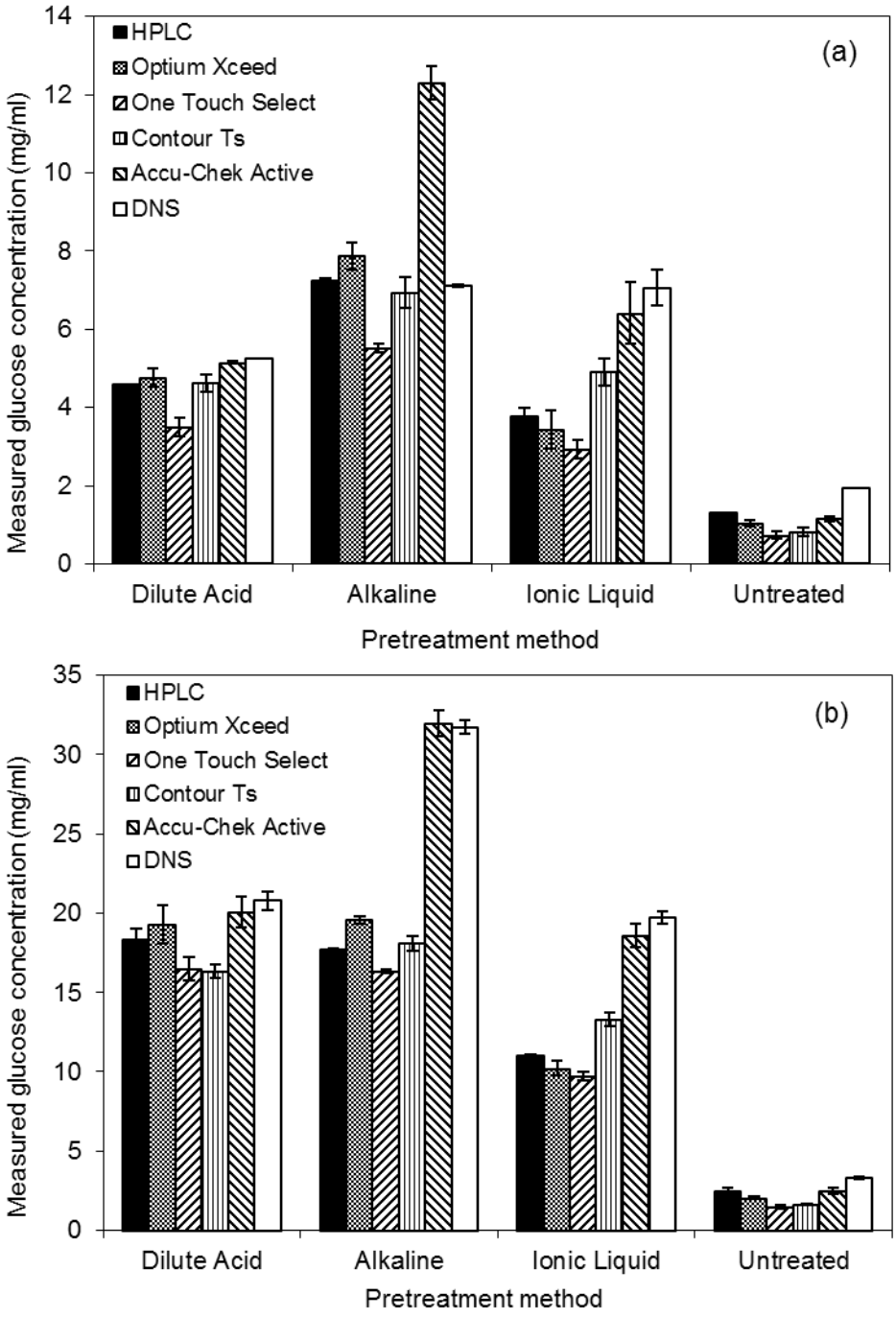

Figure 3.2. Glucose concentrations of the enzymatic hydrolysates measured with the blood glucose monitors, HPLC and DNS method following different pretreatments. (a) Glucose concentrations at the end of 1st hour (a) and 24th hour (b) of enzymatic hydrolysis are shown separately.

the enzymatic hydrolysates might be advantageous since DNS method is much more dependent on the individual that performs it as compared to the BGM measurements. Additionally, DNS method is time consuming as compared to BGM measurements since it includes boiling and cooling stages.

Table 3.2 shows the % errors made by the BGMs for the measurement of glucose concentration as compared to the glucose concentration values obtained via HPLC for both the 1st and the 24th hour of enzymatic hydrolysis. As expected due to its response to cellobiose in addition to its response to xylose, which was previously demonstrated by FitzGerald and Vermerris (2005), Accu-Chek Active, which utilizes the enzyme glucose dehydrogenase with pyrroloquinoline quinone co-factor (GDH-PQQ), made the highest errors overall that were between 70 – 80% for the alkaline and ionic liquid pretreated samples. An important point here is that this BGM made much lower errors for the dilute acid pretreated samples (around 10% to 12% depending on the duration of the enzymatic hydrolysis). The most likely reason for this decrease in the error is that dilute acid pretreatment already causes a depolymerization of cellulose during the pretreatment stage prior to enzymatic hydrolysis, resulting in the absence of cellobiose at the end of enzymatic hydrolysis (Kumar and Wyman, 2009). Indeed, the HPLC analysis of the enzymatic hydrolysates in the present study also showed that cellobiose was absent for the dilute acid pretreatment case unlike the alkaline and ionic liquid pretreatments.

For the 1st hour of enzymatic hydrolysis, the lowest errors considering the analysis of the pretreated samples was made by Contour Ts for the dilute acid and alkaline pretreated samples where the errors were 0.7% and 4.2%, respectively. This BGM made a much higher error of 30% regarding the ionic liquid pretreated samples, which was also repeated at the end of 24th hour of enzymatic hydrolysis. It thus appears that residual ionic liquid present in the enzymatic hydrolysates interfere with the measurement mechanism of this device since the error was at least doubled compared to the dilute acid and alkaline pretreated samples. Such an undesired interference does not appear to take place with the other three BGMs.

Optium Xceed also performed well for the 1st hour of enzymatic hydrolysis where the errors were between 4 – 9% depending on the type of the pretreatment applied. The error range was also similar (between around 5% – 11%) when the enzymatic hydrolysis time was increased to 24 hours. This BGM thus seems to be consistent with the readings it makes regardless of the pretreatment type or the enzymatic hydrolysis duration.

One Touch Select was the BGM that effected most significantly from the duration of the enzymatic hydrolysis in terms of its accuracy. While the error made by this device was around 23% for all three types of pretreatments, it was reduced to a range between

Table 3.2. Errors made by each blood glucose monitor as compared to the values obtained via HPLC. Errors related to both the duration of enzymatic hydrolysis and the pretreatment method are shown.

Pretreatment Method	% Error (1st hour of enzymatic hydrolysis)				% Error (24th hour of enzymatic hydrolysis)			
	Optium Xceed	One Touch Select	Contour Ts	Accu-Chek Active	Optium Xceed	One Touch Select	Contour Ts	Accu-Chek Active
Dilute Acid	3.9	23.8	0.7	12.0	5.4	9.7	10.7	9.6
Alkaline	8.7	23.9	4.2	70.0	10.5	7.8	2.2	80.3
Ionic Liquid	9.0	22.5	29.9	69.9	7.1	11.7	21.0	69.2
Untreated	20.5	44.1	37.1	12.0	17.8	39.4	34.3	0.6

around 8 – 12%, the lowest error resulting from the alkaline pretreated samples. It thus appears that while pretreatment is the critical parameter for the accuracy of some BGMs, the duration of the enzymatic hydrolysis might be critical for the accuracy of others.

The final important point related to the results shown in Table 3.2 is the low accuracy of all three BGMs (except Accu-Chek Active) regarding the measurement of the glucose concentration of the enzymatic hydrolysates of the untreated or native samples. While the Accu-Chek Active made an error of 12% and 0.6% for the hydrolysis durations of 1 hour and 24 hours, other BGMs made an error of at least 18% at the 24th hour of enzymatic hydrolysis. The least error was made by Optium Xceed where the errors were more for all the BGMs when the enzymatic hydrolysis time was reduced to 1 hour (between 21 – 44%). The most likely problem regarding the untreated samples is the low glucose concentration, which results in the presence of other compound at higher proportions as compared to the pretreated samples where the glucose concentration is much higher. Since the calibration equations for the BGMs were obtained by using solely glucose and sodium citrate buffer, these foreign compounds (such as residual pretreatment agents, enzymes or lignin related phenolic compounds that are liberated during the enzymatic hydrolysis due the depolymerization of the lignocellulosic structure) might be interfering with the measurement mechanism of the devices resulting in poor accuracies.

3.3. Comparison of the efficiencies of different pretreatments applied to corn cobs in terms of glucose yields

As mentioned in the previous sections, three different types of pretreatments were applied to corn cobs, which were the dilute acid, alkaline and the relatively recent ionic liquid pretreatment. Sulfuric acid and sodium hydroxide were the agents used for the dilute acid and alkaline pretreatments, respectively where the ionic liquid 1-ethyl-3-methyl imidazolium acetate (EMIMAc), which is in liquid form at room temperature, was used for the ionic liquid pretreatment. The composition of the corn cobs were determined as 35.3 ± 1.1% cellulose, 31.1 ± 0.8% hemicellulose and 18.0 ± 0.3% lignin.

The results of the pretreatments are shown in Table 3.3, including the solid recoveries in addition to the conversion to glucose values and glucose yields. The results in Table 3.3 include those obtained with the HPLC system as well as those obtained via a BGM in order to provide additional comparison when it comes to evaluating the final values of the pretreatment and the following enzymatic hydrolysis process. Based on the consistent results obtained from Optium Xceed regardless of the pretreatment type the 24th hour of enzymatic hydrolysis, this BGM was chosen for this part of the study to accompany the HPLC.

The term “solid recovery” in Table 3.3 refers to the weight of the corn cobs that was collected at the end of pretreatment on a dry basis. As seen here, the highest solid recovery was obtained for the ionic liquid pretreatment where 87% of the corn cobs subjected to pretreatment were recovered at the end of the pretreatment period. Dilute acid and alkaline pretreatments had lower solid recoveries around 46 – 53%, implying that corn cobs subjected to these pretreatments lost more of their material during pretreatment as compared to ionic liquid pretreatment.

“Conversion to glucose” is defined in the present study as the fraction of the pretreated corn cobs that was converted to glucose. For instance the conversion to glucose value for the dilute acid pretreatment case is 61% as determined via HPLC. This value means that 61% of the fraction that was recovered at the end of dilute acid pretreatment was converted to glucose upon enzymatic hydrolysis. In terms of the conversion to glucose values, dilute acid and alkaline pretreatments had almost 2 times higher conversion values (~60%) compared to ionic liquid pretreatment. This result is not surprising since as implied by the low solid recovery values, the fraction of cellulose in the dilute acid and alkaline pretreated samples should be higher than the fraction of cellulose in the ionic liquid pretreated samples.

The glucose yield in Table 3.3 is defined on the basis of the cellulose content of the corn cobs prior to being subjected to pretreatment. In other words, the glucose yields given in Table 3.3 are the percentiles of the theoretical maximum amount of glucose that could be obtained from native corncobs. All three kinds of pretreatments gave similar glucose yields, which were approximately around 73 – 84%, being around 3.5 times higher compared to the case where no pretreatment was applied to the corn cobs.

Table 3.3 can also be used to compare the above mentioned values with those obtained via a BGM instead of the HPLC. As it can be predicted from the results given in the previous section, the largest difference between the HPLC and BGM results occurred for the untreated samples. On the other hand, the differences between the two methods for all three kinds of pretreatments were reasonably small, indicating the suitability of using the Optium Xceed BGM as an alternative to HPLC if a high level of accuracy is not a necessity. However, it must also be noted that if the only type of pretreatment applied to corn cobs would be alkaline pretreatment, then One Touch Select or Contour Ts would be better options than Optium Xceed since these two BGMs had lower error values compared to Optium Xceed for the alkaline pretreatment case.

Table 3.3. Solid recovery, conversion to glucose and glucose yield values obtained from corncobs upon three different types of pretreatments and the following enzymatic hydrolysis.

Pretreatment method	Solid recovery (%)	Glucose determination method			
		HPLC		Blood glucose monitor	
		Conversion to glucose (%)	Glucose yield (%)	Conversion to glucose (%)	Glucose yield (%)
Dilute acid	45.6 ± 1.0	61.0 ± 2.4	73.0 ± 1.3	64.3 ± 4.0	76.9 ± 3.1
Alkaline	52.5 ± 0.6	59.1 ± 0.2	81.4 ± 1.0	65.3 ± 0.8	90.0 ± 1.8
Ionic liquid	87.0 ± 1.7	36.7 ± 0.4	83.7 ± 0.8	34.1 ± 1.6	78.0 ± 2.6
Untreated	100 ± 0.0	8.3 ± 0.8	21.8 ± 2.1	6.8 ± 0.3	17.8 ± 0.8

CHAPTER 4

EFFECT OF LIGNOCELLULOSIC BIOMASS PARTICLE SIZE ON THE IONIC LIQUID PRETREATMENT EFFICIENCY

As shown in the previous chapter, applying pretreatment to a lignocellulosic biomass significantly enhances the following enzymatic hydrolysis step, dramatically increasing the amount of glucose produced compared to the enzymatic hydrolysis of untreated biomass. A given type of pretreatment has several parameters depending on its kind, such as the pretreatment temperature, time, pressure as well as the concentration of the pretreatment agents and the solid to liquid ratio, meaning the ratio of the lignocellulosic biomass to the pretreatment agent. In addition to these, one of the most important parameters of the pretreatment is the particle size of the lignocellulosic biomass prior to pretreatment. The importance of this parameter is mainly due to the fact that the particle size reduction (grinding) is a very energy consuming and thus economically costly step. This issue makes the pretreatment systems capable of working at larger biomass particle sizes more advantageous and feasible compared to the pretreatments that demand smaller particle sizes to work efficiently (Yang and Wyman, 2008; Zhu and Pan, 2010; Vidal Jr. et al., 2011).

The present chapter focuses on the effect of lignocellulosic biomass particle size on the pretreatment efficiency of two ionic liquids, namely 1-ethyl-3-methyl imidazolium acetate (EMIMAc) and 1-ethyl-3-methyl imidazolium chloride (EMIMCl). Cotton stalks were subjected to grinding and the collected particles were separated into four different particle size ranges via sieving. The cotton stalk particles were subjected to ionic liquid pretreatment followed by enzymatic hydrolysis. The changes that took place in the appearances, lignin content and crystallinity of the particles were investigated. Following the pretreatment, pretreated cotton stalk particles were subjected to enzymatic hydrolysis in order to determine the differences in their enzymatic digestibility and the glucose yields they give.

4.1. Effect of different ionic liquids on the structural properties of pretreated cotton stalks with respect to different biomass particle sizes

After the pretreatment, the recovered cotton stalk particles were dried and they were pictured together with the untreated cotton stalk particles in order to inspect their visual appearances in a comparative manner as shown in Figure 4.1. Each row in Figure 4.1 represents a different pretreatment category where for the top row the particles were not subjected to pretreatment. Each column represents a different

particle size where as you go from left to right the particle size of cotton stalks prior to pretreatment increases from less than 0.15 mm up to the 1.0 – 2.0 mm range. All the photographs depicted in Figure 4.1 were taken from the exact same vertical distance in order to enable a realistic comparison between the images.

Inspection of the middle row reveals that the particles having the two smallest particles sizes underwent obvious changes as a result of the EMIMCl pretreatment as compared to their untreated counterparts. However, increasing the particle size to 0.5 – 1 mm range and further to 1 – 2 mm range resulted in particles having similar appearances with those of the untreated particles. There was only a slight darkening in the color of the particles where the appearances looked almost identical implying that a lower degree of modification might have taken place with these particle sizes as compared to the smaller ones.

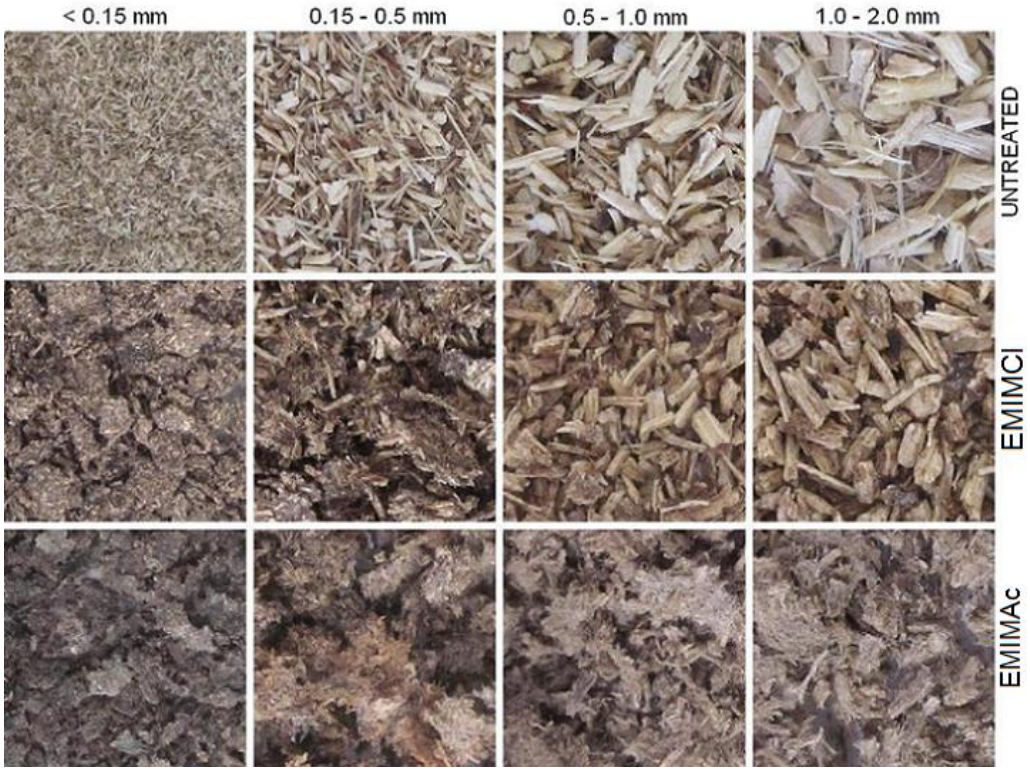

Figure 4.1. Appearance of cotton stalks at different particle sizes prior to and after ionic liquid pretreatment with two different ionic liquids.

On the other hand EMIMAc pretreatment appears to have caused more significant changes during pretreatment independent of the particle size. Especially for the 0.5 – 1 mm and 1 – 2 mm particles sizes, the large and intact body of the particles prior to pretreatment seems to be lost and these were replaced with rather fibrous like structures found together in the form of lumps. Therefore, it can be predicted that EMIMAc was more successful in terms of modifying or deconstructing the lignocellulosic structure of cotton stalks compared to EMIMCl especially at larger particle sizes when the appearance of the native cotton stalks are considered.
A key and a beneficial aspect of the ionic liquid pretreatment from the enzymatic hydrolysis perspective is its effect on the crystalline structure of cellulose as well as lignocellulosic biomass as a whole. Ionic liquid pretreatment renders the cellulose and the lignocellulosic biomass more amorphous compared to its native form, which in turn enhances the enzymatic hydrolysis that follows the pretreatment step (Kilpelainen et al., 2007; Samayam et al., 2011; Wu et al., 2011). Determining the changes in the crystallinity of the lignocellulosic biomass samples after the pretreatment step is therefore important.

The X-ray diffractograms of the cotton stalks before and after ionic liquid pretreatment are given in Figures 4.2 (a) and (b). As it can be seen in the diffractograms of untreated cotton stalks, there are two peaks appearing in the native sample at $2\theta \approx 22°$ and at $2\theta \approx 16°$, which are indicative of the samples' crystallinity. For the EMIMAc pretreatment (Figure 4.2 a) the peak at $2\theta \approx 16°$ completely disappears except for the largest particle size (1 – 2 mm) where for this sample this peak is decreased in its intensity and takes the form of a shoulder. The peak at $2\theta \approx 22°$ shifts to lower angles for all the EMIMAc pretreated samples while it also broadens and loses its intensity where these changes are most evident for the three smallest particle sizes. These changes that took place following the EMIMAc pretreatment show that the crystalline structure of cotton stalks were disrupted as a result of the pretreatment leading to a more amorphous form, especially for the samples with particle sizes of <0.15 mm, 0.15 – 0.5 mm and 0.5 – 1.0 mm. In other words EMIMAc was successful at altering the native crystalline structure of cotton stalks regardless of the change in their particle sizes prior to the ionic liquid pretreatment where this alteration was most obvious for the three smallest particle sizes.

Inspection of Figure 4.2 (b) reveals that EMIMCl pretreatment resulted in different outcomes compared to EMIMAc. The two largest particle sizes (0.5 – 1.0 mm and 1.0 – 2.0 mm) gave almost identical diffractograms with both of the peaks in place. For the particle size of 0.15 – 0.5 mm, intensity decrease can be observed for both of the peaks indicating that for EMIMCl to alter the crystalline structure of cotton stalks, this particle size might be regarded as a minimum threshold. With that said the change that took place for this particle size is not as dramatic as its EMIMAc counterpart.

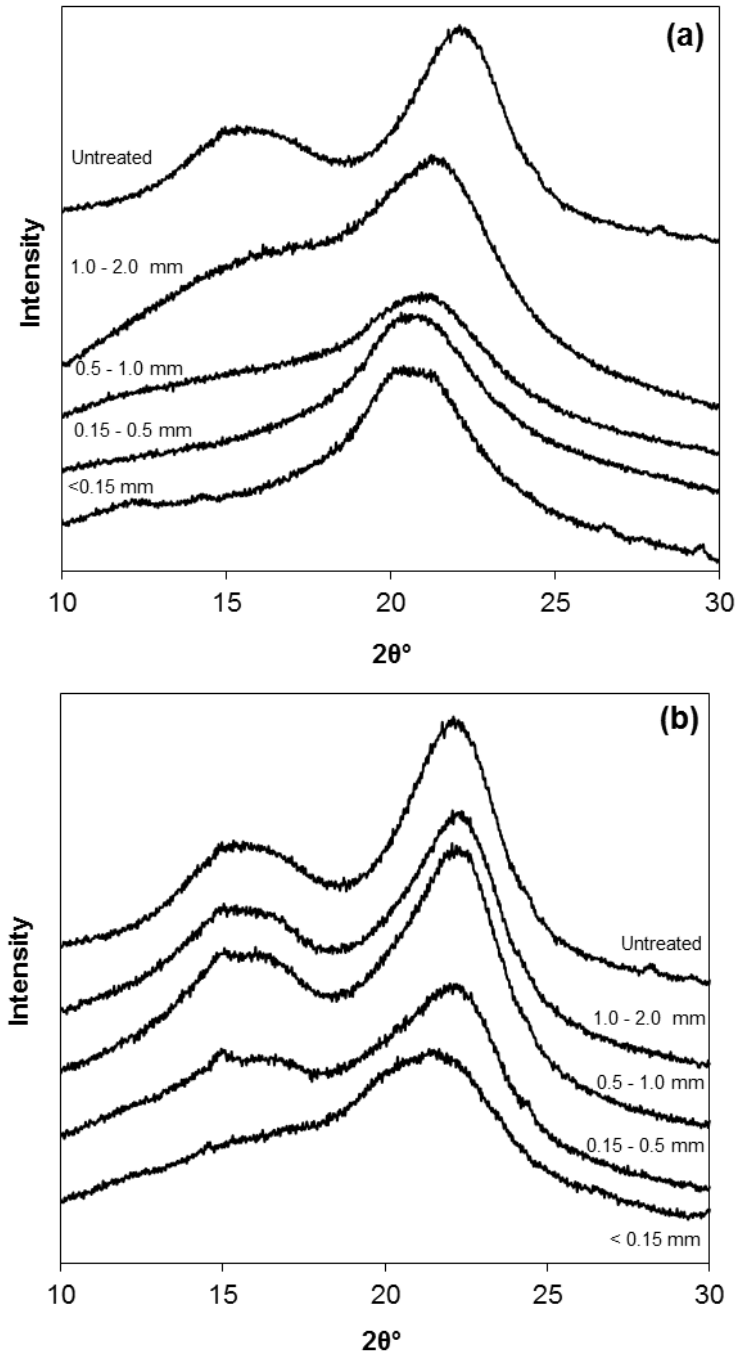

Figure 4.2. X-ray diffractograms of cotton stalks prior to and after ionic liquid pretreatment. (a) EMIMAc pretreated and (b) EMIMCl pretreated cotton stalks.

The most significant change regarding the peak shapes and positions for the EMIMCl the peak at 2θ ≈ 16° mostly disappeared while the peak at 2θ ≈ 22° broadened and shifted to lower angles together with a decrease in its intensity. These results regarding the EMIMCl pretreatment show that this ionic liquid needs smaller particle sizes to be effective in disrupting the native crystallinity of cotton stalks and that overall it is not as good as EMIMAc in transforming the original structure of cotton stalks into a more amorphous form.

Other than the crystallinity, another important property of a lignocellulosic biomass that will be subjected to enzymatic hydrolysis is its lignin content. The presence of lignin in lignocellulosic biomass is known to hinder the enzymatic hydrolysis by assuming different roles such as acting as a physical barrier for the enzymes or binding to them irreversibly (Chang and Holtzapple, 2000; Eriksson et al., 2002; Studer et al., 2011). Therefore the presence of lignin in the substrates during the enzymatic hydrolysis is generally considered as a negative aspect and lignin removal as a result of a given type of pretreatment is desired, which makes monitoring the lignin content of a lignocellulosic biomass prior to enzymatic hydrolysis an important issue. The compositional analysis of cotton stalks shows that it contains a significant amount of lignin (25.6 ± 0.6%) while the cellulose and hemicellulose contents were determined as 40.7 ± 0.6 and 16.0 ± 0.2, respectively.

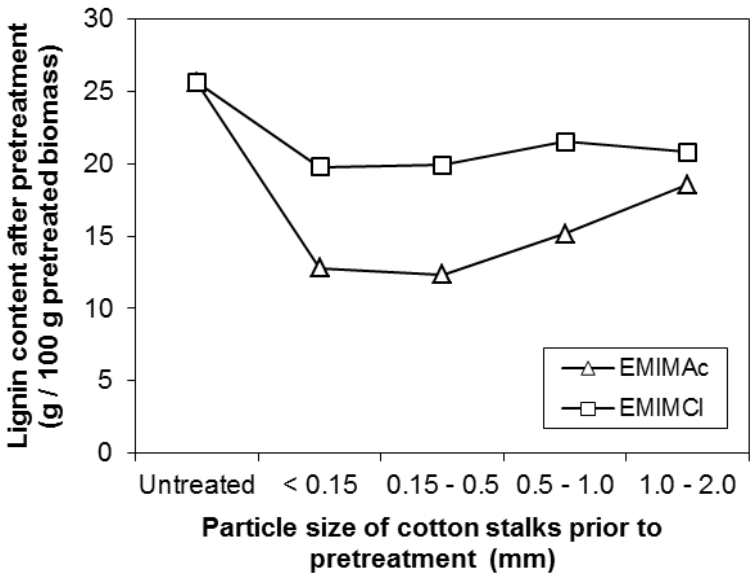

Figure 4.3. Lignin content of cotton stalks prior to and after ionic liquid pretreatment.

The lignin content of the EMIMAc and EMIMCl pretreated cotton stalks with respect to different particle sizes is shown in Figure 4.3 where untreated stands for the case in which no pretreatment was applied to the cotton stalks. Overall, EMIMAc appears to be better agent compared to EMIMCl for the removal of lignin. For the EMIMCl pretreatment, the reduction of the lignin content was rather stable with respect to increasing particle size where lignin content was reduced from approximately 26% to around 20 – 22% with a slight increase taking place for the two larger particle sizes. It thus appears that particle size did not have a dramatic effect on the lignin extraction capability of EMIMCl. On the other hand, the reduction of lignin content was more dramatic for the EMIMAc pretreatment where the initial lignin content of 26% was reduced to the 12 – 19% range. As opposed to the EMIMCl case, EMIMAc was much more sensible to the increase in the cotton stalk particle size during pretreatment in terms of its lignin extraction capability. While the two smallest particle sizes had similar lignin contents between 12 – 13%, increasing the particle size beyond the 0.5 – 1.0 mm range caused an increase in the lignin content of the samples where for the largest particle size the lignin content was quite close to that obtained with the EMIMCl pretreatment.

4.3. Effect of different ionic liquids on the digestibility of cotton stalks with respect to different biomass particle sizes

A quite practical as well as a realistic way to assess the efficiency of a pretreatment process applied to a lignocellulosic biomass is to measure the digestibility of the pretreated biomass during a given enzymatic hydrolysis period. The digestibility of pretreated and untreated cotton stalks during the course of enzymatic hydrolysis at different time periods (1^{st}, 5^{th} and 24^{th} hours of enzymatic hydrolysis) are shown in Figure 4.4. The digestibility values given here are calculated on the basis of the reducing sugars released from cotton stalks as determined via the DNS method (Miller, 1959). In other words, a higher reducing sugar yield means a higher enzymatic digestibility, which is taken as a measure of the efficiency of the ionic liquid pretreatments conducted via EMIMCl (Figure 4.4 b) and EMIMAc (Figure 4.4 c) as compared to the digestibility of untreated samples (Figure 4.4 a) at four different particle sizes.

As shown in Figure 4.4 (a), there is a consistent relation between the particle size of untreated cotton stalks prior to pretreatment and their digestibility. Increasing the particle size successively from < 0.15 mm to 1.0 – 2.0 mm range caused a decrease in the digestibility values for all the three time intervals. This result is quite expected since smaller particle size means a larger surface area, enabling a better interaction of the enzymes with the biomass. The highest digestibility in this case was approximately 17%, which was obtained for native cotton stalks smaller than 0.15 mm while a

digestibility of only 8% could be obtained for the cotton stalks with the largest particle size.

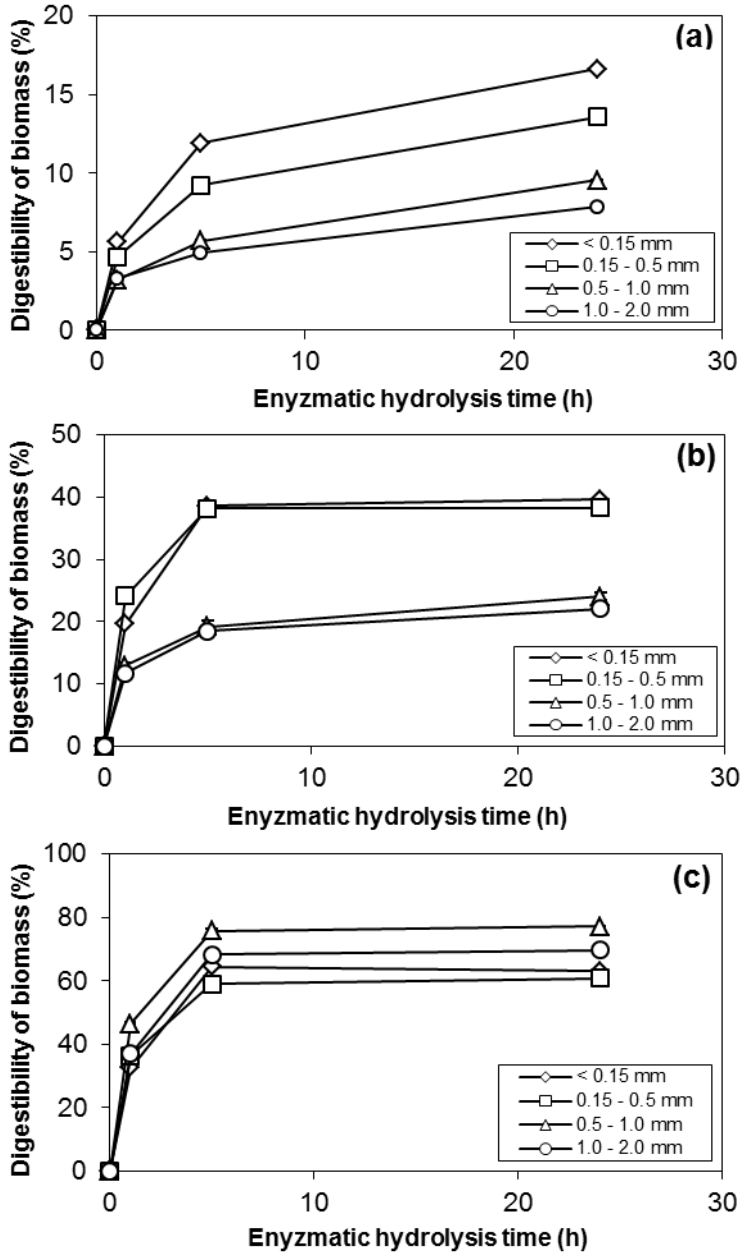

Figure 4.4. Digestibility of cotton stalks prior to (a) and after EMIMCl (b) and EMIMAc (c) pretreatments with respect to different particle sizes.

For EMIMCl pretreatment (Figure 4.4 b), there appears to be 2 different outcomes depending on the particle size prior to pretreatment. The digestibility values for the two smallest particle sizes were significantly higher compared to the digestibility values of the other larger particle sizes (0.5 – 1.0 mm and 1.0 – 2.0 mm). The two smaller particle sizes (< 0.15 mm and 0.15 – 0.5 mm) gave around 40% digestibility at the end of 24 hours of hydrolysis where this value was decreased to 22% for the larger particle sizes, indicating a 2 fold increase in the digestibility depending on the particle size prior to pretreatment. Another important point to mention at this point regarding the course of the enzymatic hydrolysis is that the process was almost completed at the end of 5 hours enzymatic hydrolysis since no dramatic change has taken place after this period regarding the digestibility unlike the untreated samples where the hydrolysis continued after the 5th hour (Figure 4.4 a). This is indicative of the fact that the EMIMCl pretreatment applied to cotton stalks enabled a faster enzymatic hydrolysis, which is beneficial from the economical point of view.

The results obtained for the EMIMAc pretreatment case are rather surprising since unlike the untreated or EMIMCl pretreated cotton stalks, smaller particle sizes had lower digestibility values compared to the two larger particle sizes (Figure 4.4 c). The digestibility values for both of the smaller particle sizes (< 0.15 mm and 0.15 – 0.5 mm) were around 61%, while it was 70% for the 1.0 – 2.0 mm particles and 77% for the 0.5 – 1.0 mm ones. Similar to EMIMCl pretreatment, cotton stalks pretreated with EMIMAc were also hydrolyzed rapidly with the enzymatic hydrolysis reaction being almost completed at the end of 5th hour. The enzymatic hydrolysis speed of the EMIMAc pretreated samples can be better understood when it is considered that the digestibility of the particles with 0.5 – 1.0 mm size reached 46% just at the end of the 1st hour of hydrolysis, being higher than the digestibility of any of the EMIMCl pretreated samples at the end of 24 hours of hydrolysis.

Considering the digestibility results overall, EMIMAc pretreatment was much more effective than the EMIMCl pretreatment where the difference between the digestibility values reached up to around 3 fold for the two larger cotton stalk particle sizes in favor of the EMIMAc pretreatment.

It is worth mentioning that the digestibility values shown in Figure 4.4 are in very good agreement with the previous results on the visual appearances and the crystallinity of the cotton stalks before and after the pretreatments. It was mentioned previously that the two larger particles sizes pretreated with EMIMCl had very similar appearances to the untreated cotton stalks at the same particle size where these also had very similar diffractograms to the untreated cotton stalks. Indeed these two particle sizes (0.5 – 1.0 mm and 1.0 – 2.0 mm) had the lowest digestibility values following the EMIMCl pretreatment and these values very close to each other. On the other hand all the EMIMAc pretreated samples, regardless of their particle sizes prior

to pretreatment, had deconstructed visual appearances and obviously different diffractograms compared to the untreated samples. In accordance with these results, EMIMAc pretreated samples had the highest digestibility values overall where the digestibility was enhanced at least more than 3 fold (< 0.15 mm particle size) and up to approximately 9 fold (1.0 – 2.0 mm particle size) compared to the untreated samples.

On the basis of the results obtained so far, EMIMAc pretreatment appears to be more effective compared to EMIMCl pretreatment applied to cotton stalks. The primary difference between these two ionic liquids is their anion where EMIMAc bears an acetate anion with chloride being the anion of EMIMCl. Previous studies report the key importance of the ionic liquid anion regarding the lignocellulosic biomass pretreatment ((Brandt et al., 2010; Brandt et al., 2011; Doherty et al., 2010; Nguyen et al., 2010; Wu et al., 2011). The important issue at this point is the basicity of the anion since it was shown that higher anion basicity positively influences the ionic liquid pretreatment efficiency, resulting in higher glucose yields during the enzymatic hydrolysis (Doherty et al., 2010). Therefore, a possible explanation for the better performance of EMIMAc as a pretreatment agent for cotton stalks compared to EMIMCl could be the higher anion basicity of EMIMAc.

4.4. Effect of different ionic liquids on the glucose yields obtained from cotton stalks at different biomass particle sizes via enzymatic hydrolysis

In addition to digestibility, the enzymatic hydrolysis process was also evaluated from the glucose yield perspective as shown in Figure 4.5. Here the glucose yield is defined on the basis of the theoretical maximum amount of glucose that can be obtained from native cotton stalks with respect to its cellulose content, which was determined as 40.7 ± 0.6% (Haykir 2013).

In a good agreement with the digestibility results given in the previous section, EMIMAc pretreated cotton stalks gave higher glucose yields for all the four particle sizes compared to EMIMCl pretreated samples. The glucose yield obtained from untreated cotton stalks showed a quite linear decrease with increasing biomass particle size where the glucose yield ranged between 9 – 20%. The increase in the biomass particle size had a different effect on the glucose yield obtained from EMIMCl pretreated samples. The glucose yield in this case was approximately 50% for the two smallest particle sizes. However increasing the biomass particle size further caused a dramatic decrease in the glucose yield down to 33%, indicating that EMIMCl favors smaller particle sizes to function more efficiently in terms of the glucose yields. This situation was different for the EMIMAc pretreatment since the smallest particle size (< 0.15 mm) gave the lowest glucose yield, which was 57%. As opposed to the EMIMCl pretreatment, increasing the biomass particle size further caused an increase in the

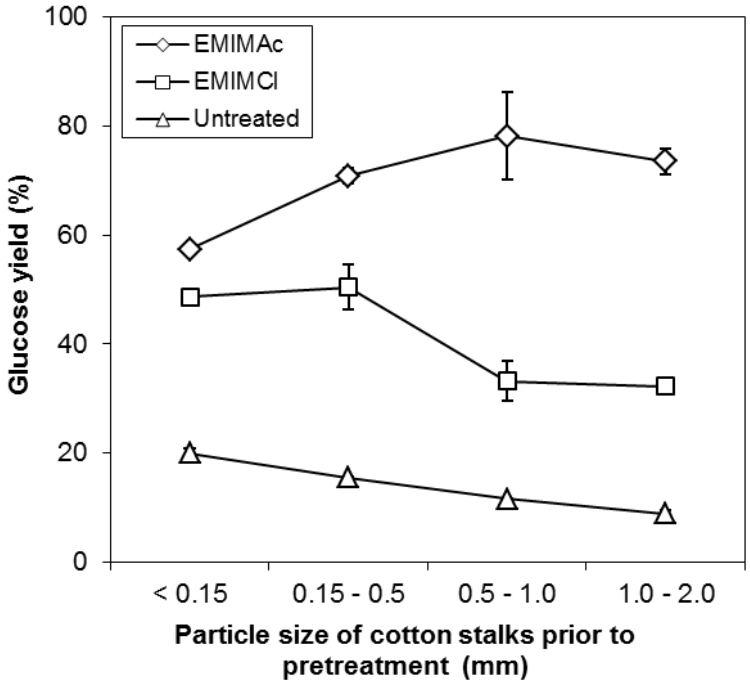

Figure 4.5. Glucose yields obtained at the end of 24 hours of enzymatic hydrolysis applied to cotton stalks at different particle sizes prior to ionic liquid pretreatment.

glucose yields, which were similar and were between 71 – 78%. Considering this different response of the EMIMAc and EMIMCl pretreatments to increasing particle sizes, it appears that the correct particle size of a lignocellulosic biomass prior to pretreatment can be different for each type of ionic liquid. The glucose yields presented in Figure 4.5 also show that using small lignocellulosic biomass particle sizes during pretreatments does not always gives the best results.

The critical question to ask at this stage would be: Why did the smallest particle size give the lowest glucose yield for the EMIMAc pretreatment? Biomass particles with the smallest size had the largest surface area, which should result in a better interaction with the ionic liquid compared to the larger particle sizes having smaller surface areas. A better interaction with EMIMAc should have resulted in a more effective pretreatment, finally resulting in a higher glucose yield at the end of enzymatic hydrolysis. However, this was not the case for the EMIMAc pretreatment applied to cotton stalks with the smallest particles size of < 0.15 mm. The results given in Table 4.1 can be useful at this stage.

Table 4.1. Cotton stalk recovery data following the ionic liquid pretreatments and the conversion of pretreated cotton stalks to glucose at the end of 24 hours of enzymatic hydrolysis.

Particle size of cotton stalks subjected to pretreatment (mm)	Biomass recovery (%)		Conversion to glucose (%)		
	EMIMAc	EMIMCl	EMIMAc	EMIMCl	Untreated
< 0.15	54.1 ± 1.9	76.1 ± 0.8	47.9 ± 0.9	28.9 ± 0.3	9.0 ± 0.4
0.15 - 0.5	75.9 ± 9.2	81.7 ± 0.9	42.7 ± 4.4	27.9 ± 2.0	7.0 ± 0.1
0.5 - 1.0	71.1 ± 0.2	88.7 ± 0.7	49.6 ± 5.2	16.9 ± 2.0	5.2 ± 0.0
1.0 - 2.0	77.7 ± 0.2	91.9 ± 2.5	42.7 ± 1.3	15.8 ± 0.03	4.0 ± 0.3

As shown in Table 4.1, while the biomass recovery was between 71 – 78% for the three larger particle sizes, it was reduced to 54% for the smallest particle size. This means that around half of the biomass was lost during the pretreatment cotton stalks with a particle size of < 0.15 mm. However, the conversion of the remaining fraction to glucose (48%) was similar to that of the others, which were between 43 – 50%, indicating that the decrease in the glucose yield was due to the lower amount of material to be hydrolyzed. In other words, there must have been less amount of substrate during the enzymatic hydrolysis where the substrate for the production of glucose is cellulose. It thus appears that during the EMIMAc pretreatment of cotton stalks with a particle size of < 0.15 mm, some of the cellulose in the lignocellulosic structure was lost. The most likely way for this to happen is that cellulose was degraded during pretreatment down to the water soluble cellooligomer range or to even smaller molecular weights. As a result of this degradation, during the precipitation step, which was conducted by adding water to the pretreatment medium, the water soluble cellooligomers were retained together with the water and thus they could not be recovered together with the pretreated biomass. In other words, the degraded cellulose fractions were retained in the filtrate during the separation of the pretreated biomass from the water-ionic liquid-biomass suspension formed at the end of the precipitation step.

The degradation of cellulose during ionic liquid pretreatment was reported previously in various studies. A reduction in the cellulose recovery following the EMIMAc pretreatment of rice straws was reported for a rice straw particle size of < 2mm compared to larger particle sizes (Nguyen et al., 2010). Furthermore, the formation of water soluble compounds emerging from the polysaccharides dissolved in ionic liquids was reported in a series of previous studies (Miyafuji et al., 2009; Nakamura et al., 2010a; Nakamura et al., 2010b). It was shown that the composition of the atmosphere had an essential influence on the formation of these water soluble compounds in which the air, as compared to other atmospheres deficient in air, was found to strongly promote the degradation of cellulose dissolved in the ionic liquid EMIMCl to smaller molecular weight compounds including not only cellooligomers but also cellobiose and glucose (Nakamura et al., 2010b). On the basis of this finding reported by Nakamura et al. (2010b), it would be rational to propose that a fraction of the cellulose in the lignocellulosic structure of cotton stalks was lost due to degradation since the experiments were performed under air atmosphere. Of course the high temperature of the ionic liquid pretreatment process (140°C) should also have a major role in the degradation process.

Finally, it would be wise to question why the proposed degradation of cellulose took place for the smallest particle size studied but not occurred (or not occurred as dramatically as in the case of the smallest particle size) with the larger particle sizes. As reported previously, lignocellulosic biomass with smaller particle sizes dissolve

faster in the ionic liquids compared to larger particle sizes (Viell and Marquardt, 2011; Kilpelainen et al., 2007) where this faster dissolution is related to the larger surface area of the biomass with the smaller particle size (Sun et al., 2009). Therefore cotton stalks with a particle size of < 0.15 mm must have dissolved in the EMIMAc before the cotton stalks with larger particle sizes. As mentioned shortly, dissolution is closely related to degradation and thus cotton stalks with the smallest particle size must have more time to degrade into smaller molecular weight water soluble compounds compared to the particles with larger sizes. Eventually, this extra time likely resulted in the loss of cellulose during the pretreatment and finally to a lower glucose yield due to the partial absence of the substrate to the enzymes during the hydrolysis period.

CHAPTER 5

EFFECT OF ALKALINE PRETREATMENT TEMPERATURE ON THE GLUCOSE YIELD AND ON THE MECHANICAL PROPERTIES OF HEMICELLULOSE BASED FILMS OBTAINED FROM COTTON STALKS

In the previous two chapters, the emphasis was on the production of glucose from the lignocellulosic biomass by conducting a pretreatment step followed by the enzymatic hydrolysis of the pretreated biomass. As mentioned in the introduction chapter, production of multiple products is a very important aspect of a feasible lignocellulosic biorefinery. Therefore the production of the principal product, which is generally considered to be a fuel such as ethanol that is produced from glucose, should be coupled with additional products (Zhang, 2008; Bozell and Petersen, 2010).

The present chapter considers the co-production of glucose and environmentally friendly biodegradable polymeric materials. Within this framework, all three components of the lignocellulosic biomass were utilized where the cellulose fraction was utilized for the glucose production while hemicellulose and lignin fractions were used for the production of polymeric films. Thus, more of the lignocellulosic biomass, which is cotton stalks in this case, was converted to value added products since not only cellulose but also hemicellulose and lignin were utilized as base biopolymers.

For co-production of glucose and hemicellulose based films cotton stalks were subjected to alkaline pretreatment at three different temperatures. The pretreatment also functioned as a means of extracting hemicelluloses from cotton stalks at different temperatures, which were used for the production of polymeric films by using the solvent casting technique. The effect of the pretreatment temperature on the properties of hemicellulose based films in addition to its effect on the enzymatic hydrolysis process was investigated and the optimum pretreatment temperature for this multi-product approach was determined.

Another important issue about this chapter is that it functions as a bridge between the previous and the upcoming chapters since it includes both the lignocellulosic biomass pretreatment (and the glucose production related to the pretreatment) and the hemicellulose based film production, which is the main topic of the next two chapters. Finally, the present chapter uses the blood glucose monitor (BGM) method described in Chapter 3 for the determination of glucose obtained from the alkaline pretreated cotton stalks via enzymatic hydrolysis.

5.1. Effect of alkaline pretreatment temperature on the yields of cellulosic and hemicellulosic fractions and on the digestibility of the cellulosic fraction obtained from cotton stalks

Two important notions regarding this chapter of the thesis study are the phrases "cellulosic fraction" and "hemicellulosic fraction". Cellulosic fraction is defined as the alkaline insoluble fraction obtained at the end of the alkaline pretreatment. Since this fraction is insoluble in the alkaline medium, it was recovered from the medium via filtration. This fraction is termed "cellulosic" because it is the fraction rich in cellulose since hemicellulose and lignin found in the lignocellulosic structure together with cellulose are dissolved in the alkaline medium during the alkaline pretreatment and thus they are removed from the lignocellulosic structure. On the other hand, hemicellulosic fraction is the name given to the portion that dissolved in the alkaline medium together with lignin where this fraction was obtained by the precipitation of the dissolved polymers followed by the recovery of the precipitates from the suspension via filtration.

The recovery data regarding these two fractions is given in Table 5.1 where the recovery is defined on the basis of the initial weight of cotton stalks subjected to pretreatment. As shown in Table 5.1, the recovery of the cellulosic fraction decreased from approximately 58% to 42% with increasing pretreatment temperature most likely because more hemicellulose and lignin were dissolved in the alkaline medium due to the increasing pretreatment temperature (Lawther et al., 1996). The increase in the recovery of the hemicellulosic portion confirms this assumption where an almost two fold increase in the hemicellulosic fraction recovery was observed. Here, it must be noted that the serious increase in the hemicellulosic fraction recovery was obtained as a result of the increase in the pretreatment temperature from 25°C to 60°C where only a minor increase of around 10% was observed when the pretreatment temperature was increased further to 90°C.

Table 5.1. Recovery of the cellulosic and hemicellulosic fractions obtained from cotton stalks upon alkaline pretreatment conducted at various temperatures.

Pretreatment temperature (ºC)	Recovery (%)	
	Cellulosic Portion	Hemicellulosic Portion
25	57.4 ± 0.1	9.8 ± 0.1
60	49.9 ± 1.0	16.2 ± 0.5
90	41.9 ± 0.3	18.2 ± 0.7

The recovered cellulosic fractions were subjected to enzymatic hydrolysis and their digestibility was assessed by the reducing sugars released from the samples in a similar manner to that described in the previous chapter. As shown in Figure 5.1, increasing the pretreatment temperature caused an increase in the digestibility of the cellulosic fractions while the native (untreated) cotton stalks had the lowest digestibility as expected. The response of the systems in terms of their digestibility to increasing pretreatment temperatures were rather consistent as each increase in the pretreatment temperature resulted in an increase of 1.2 fold considering the digestibility at the 24th hour of enzymatic hydrolysis. For all the pretreatment temperatures, the digestibility of the samples increased dramatically compared to the untreated cotton stalks with 4.8, 5.8 and 7 folds for the pretreatments conducted at 25°C, 60°C and 90°C, respectively.

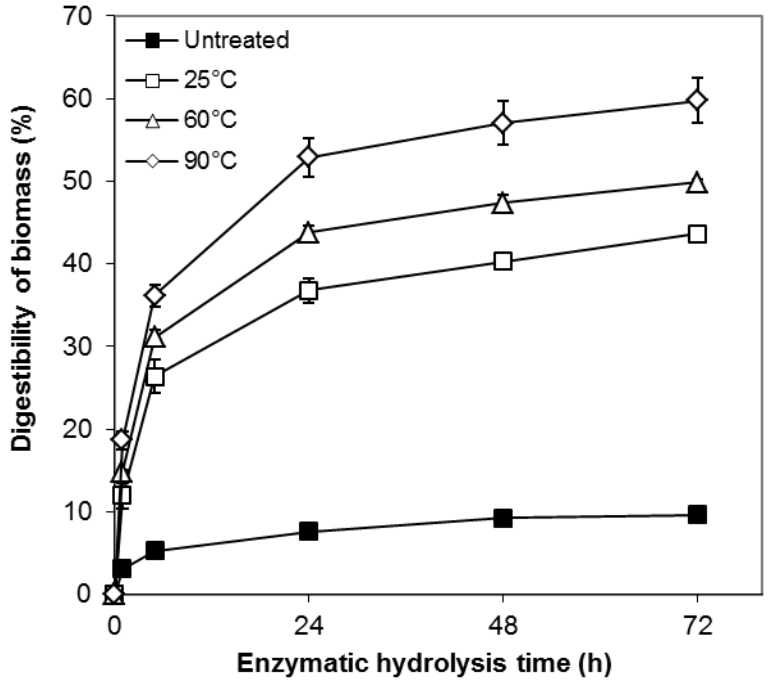

Figure 5.1. Digestibility of alkaline pretreated (at three different temperatures) and untreated cotton stalks during the course of enzymatic hydrolysis.

Another result that can be drawn from Figure 5.1 is that when compared with the digestibility results regarding the pretreated cotton stalks given in Section 4.3 of Chapter 4, it appears that EMIMAc pretreatment enables a much faster hydrolysis

compared to the alkaline pretreatment. While the enzymatic hydrolysis reaction was almost over at the end of 5th hour for the EMIMAc pretreatment (Figure 4.4), the hydrolysis kept on going even after the 48th hour for the alkaline pretreated cotton stalks. Although the pretreatment conditions differ considerably for the two cases, such comparison still gives an idea about how fast ionic liquid pretreated cotton stalks, particularly those pretreated with EMIMAc, can be hydrolyzed.

5.2. Effect of alkaline pretreatment temperature on the properties of hemicellulose based polymeric films

The hemicellulosic fraction, which was extracted from cotton stalks during the alkaline pretreatment, was converted into polymeric films via the solvent casting technique. Film forming solutions prepared by the dissolution of the hemicellulosic fraction in water, were poured into petri dishes and following the evaporation of the solvent via drying, the films were formed on the surface of the petri dishes. The films were removed from the petri dishes and they were then subjected to various analysis. It is worth mentioning that the films did not contain any additives that could impair their biodegradability.

The visual appearance of the films following their removal from the petri dishes is shown in Figure 5.2. While the films produced from the hemicellulosic fraction obtained at 25°C had a yellowish color, hemicellulosic fractions obtained at higher temperatures had darker colors where the color of the film obtained from the hemicellulosic fractions isolated at 90°C was dark brown. Polymers or polymeric films with inherent colors, although these are of natural origin, particularly dark ones, might be undesired during certain packaging applications. From this point of view, the pretreatment temperature of 25°C seems to be the best choice while the pretreatment temperature of 90°C would be the least preferable temperature.

Another important point regarding the appearance of the film in Figure 5.2 is the film forming property of the isolated hemicellulosic fraction. It is seen in Figure 5.2 that the film produced from the biopolymers isolated at 90°C is not intact or totally continuous as it has large cracks that almost entirely crosses the diameter of the film. Such a situation is not observed for the films produced from the polymers obtained atlower temperatures since these films does not have any crack related defects on them and they are totally intact. Therefore it can be commented that the alkaline pretreatment temperature of 90°C hinders the film forming property of the hemicellulosic fraction isolated from cotton stalks and is not a suitable pretreatment temperature if a multi-product perspective considering the co-production of glucose and hemicellulose based polymeric films is to be embraced.

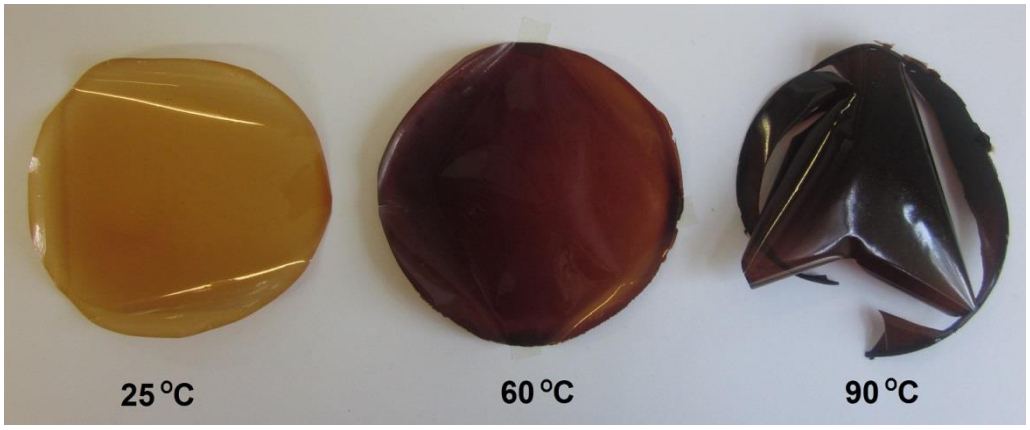

Figure 5.2. Appearance of the polymeric films produced from the hemicellulosic fractions isolated from cotton stalks at different pretreatment temperatures.

The mechanical properties of the films, including ultimate tensile strength (UTS), elongation at break (e_b) and elastic modulus (E) values are given Table 5.2 together with the water contents and lignin contents of the films. The water content of the films was measured by drying the films for 24 h at 105°C. The water contents of the films were determined following the conditioning of the films at 50% RH for 24 h. The lignin content of the films increases consecutively by more than 2 fold and 3 fold as the pretreatment temperature is increased from 25°C to 60°C and to 90°C. Solely, lignin itself has a quite dark brown color. Therefore the reason for the darkening of the film colors shown in Figure 5.2 must be due to the increased lignin content of the films with increasing pretreatment temperature.

Overall the e_b values of the films shown in Table 5.2 are generally low since they were between 1.6 – 3.1%, indicating that the films were rather brittle. Increasing the pretreatment temperature from 25°C to 60°C did not cause a notable change in any of the mechanical property values. However, further increase of the pretreatment temperature to 90°C decreased the UTS from approximately 52 MPa to 46 MPa while also decreasing the e_b by a half. Westbye et al. (2007) has shown that lignin induced the agglomeration of the hemicellulose-xylan complexes. Such an agglomeration eventually affects the mechanical properties of the material in a negative way. Recalling that the highest amount of lignin was present in the film produced from the polymers isolated at a pretreatment temperature of 90°C, the lower mechanical properties of this type of film in terms of the UTS and e_b values can be attributed to its high lignin content. Water content of the films is also another important parameter capable of affecting the mechanical properties since water is a plasticizer for hemicellulose based films (Gröndahl et al., 2004; Sternemalm et al., 2008; Ying et al.,

Table 5.2. Ultimate tensile strength (UTS), elongation at break (e_b) and elastic modulus (E) values of the hemicellulose based films together with their water and lignin contents.

Pretreatment	Mechanical property			Water Content (%)	Lignin Content (%)
Temperature (°C)	UTS (MPa)	e_b (%)	E (MPa)		
25	51.7 ± 3.1	3.1 ± 0.5	3086.0 ± 244.5	13.3 ± 0.2	5.2 ± 0.3
60	51.2 ± 2.8	2.5 ± 0.6	3257.5 ± 128.9	13.2 ± 0.3	11.7 ± 0.5
90	45.6 ± 3.0	1.6 ± 0.3	3265.0 ± 283.4	12.9 ± 0.1	18.2 ± 0.5

2011). However, the water content of all three types of films are very similar to each other (between 12.9% and 13.3%), which further strengthens the claim that high lignin content was responsible for the decrease in the mechanical properties of the films.

In spite of the negative aspects discussed above, presence of lignin in the hemicellulose based films has a positive effect as well. Zhu Ryberg et al. (2011) has shown that presence of lignin in the hemicellulose based films enhances the oxygen barrier performance of the films by more than 5 fold as compared to the films obtained from hemicelluloses deficient in lignin. Taking into account this finding, it might be thought that the pretreatment temperature of 60°C is more advantageous to 25°C as the former enables the production of films with more than twice the lignin content of the latter without negatively affecting the mechanical properties of the films. This could enable these films to act as better oxygen barriers compared to the films produced following the pretreatment conducted at 25°C.

5.3. Effect of alkaline pretreatment temperature on the co-production of glucose and hemicellulose based films with respect to glucose yield and film toughness

In order to evaluate the influence of the alkaline pretreatment temperature on the proposed multi-product approach, glucose yields obtained from the cellulosic fraction was considered together with the toughness values of the films produced from the hemicellulosic fraction. Toughness, also known as tensile energy to break, was the chosen mechanical property to consider at this stage, since its value is dependent on both the UTS and e_b providing an overall measure of the mechanical properties of the hemicellulose based films rather than just focusing on one of these properties. The toughness value is obtained by calculating the area under the stress-strain curve, therefore higher UTS and e_b values lead to higher toughness values.

The response of the glucose yield and film toughness to the pretreatment temperature is given in Figure 5.3. The untreated cotton stalks gave the lowest glucose yield as expected, and after alkaline pretreatment glucose yields obtained were between 37 – 39%, which were around 4 times higher than the glucose yield obtained from untreated cotton stalks. The response of the films to increasing pretreatment temperature in terms of their toughness was different where a large range of toughness values from 1.1 MJ/m^3 down to 0.4 MJ/m^3 were observed with increasing pretreatment temperature from 25°C to 90°C. It appears that while the cellulosic portion of the process was not sensible to the changes in the pretreatment temperature, increasing the pretreatment temperature adversely affected the mechanical properties of the films to a certain extent. When Figure 5.3 is observed, a decrease in the toughness value

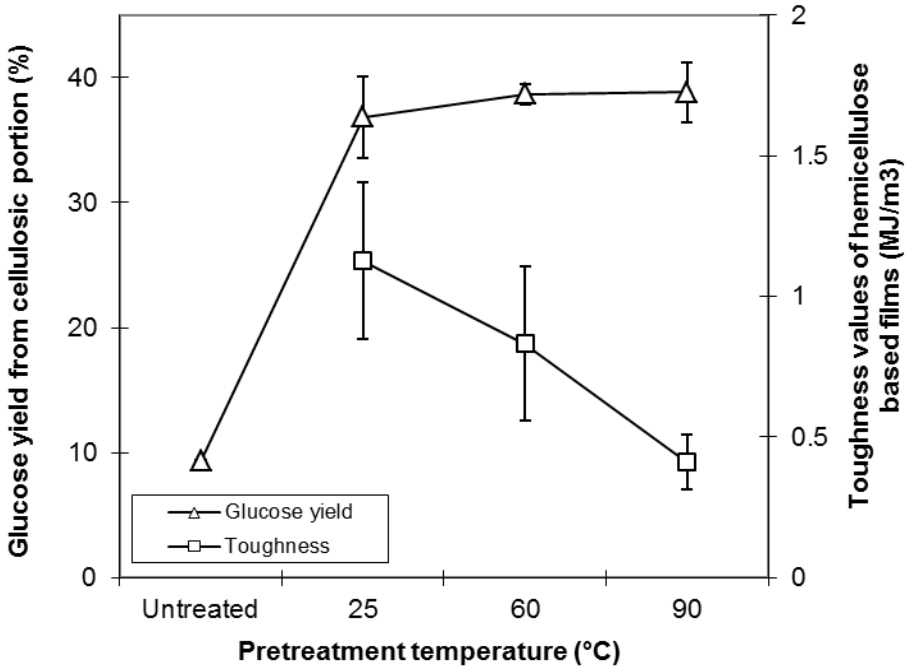

Figure 5.3. Effect of alkaline pretreatment temperature on the glucose yields and on the toughness values of hemicellulose based films.

from approximately 1.1 MJ/m^3 to 0.8 MJ/m^3 might be noted as the pretreatment temperature is increased from 25°C to 60°C. However this decrease in the toughness value is not statistically significant on a 95% confidence level (p-value = 0.16) while the statistically significant decrease occurs for the case in which the pretreatment was conducted at 90°C as compared to 60°C (p-value = 0.027). Thus the pretreatment temperature of 90°C is detrimental for the toughness of the films and it does not cause any notable increase on the glucose yield. In other words, it is apparent that increasing the pretreatment temperature up to 90°C would be irrational within the context of the proposed multi-product framework. Additionally, considering that the hemicellulosic part of the proposed multi-product strategy was much more sensitive to the changes in the pretreatment temperature compared to the cellulosic part of the process that involves glucose production, it appears that the pretreatment temperature should be determined by considering the hemicellulosic part of the process.

5.4. Effect of alkaline pretreatment temperature on the total product yield produced from cotton stalks

The final issue to take into account is the total product yield obtained from cotton stalks for each of the pretreatment temperatures, which is shown in Figure 5.4. Here total product yield is defined as the sum of the glucose and hemicellulosic fraction produced from cotton stalks. Since the entire hemicellulosic fraction is converted into films, hemicellulosic fraction itself is considered as a product together with glucose. Since no separation can occur without any pretreatment, the only product obtained in the case of no pretreatment is glucose, which is produced via the enzymatic hydrolysis of native cotton stalks where the product yield in this case is only around 4%. Applying room temperature pretreatment to cotton stalks dramatically improves the product yield to 26% where the major contributor to this yield is the cellulosic portion of the process since glucose produced accounts for around 16% of cotton stalks initially subjected to pretreatment. Increasing the pretreatment temperature to 60°C improves the total product yield by approximately 30% compared to the room temperature pretreatment case. Here the contribution of the hemicellulosic part of the process to total product yield is almost equal to that of the cellulosic part and the 30% increase in the total product yield is almost entirely due to the increase in the amount of hemicellulosic fraction extracted from cotton stalks compared to the room temperature pretreatment case. Further increasing the pretreatment temperature to 90°C causes only a 5% increase in the total product yield.

Considering the results given in Figure 5.4 together with the previously reported results in this chapter, the optimum alkaline pretreatment temperature for the co-production of glucose and hemicellulose based appears to be 60°C. Compared to the case in which the pretreatment was conducted at 25°C, the higher temperature of 60°C resulted in obtaining considerably more products from cotton stalks without any significant decrease in the material properties of the films where the higher lignin content of these films might also be beneficial in terms of their oxygen barrier properties (Zhu Ryberg et al., 2011). The pretreatment temperature to 90°C is the worst choice among all three temperatures since it causes only a minor increase in the total product yield while adversely affecting the material properties of the films including the formation of continuous and intact films.

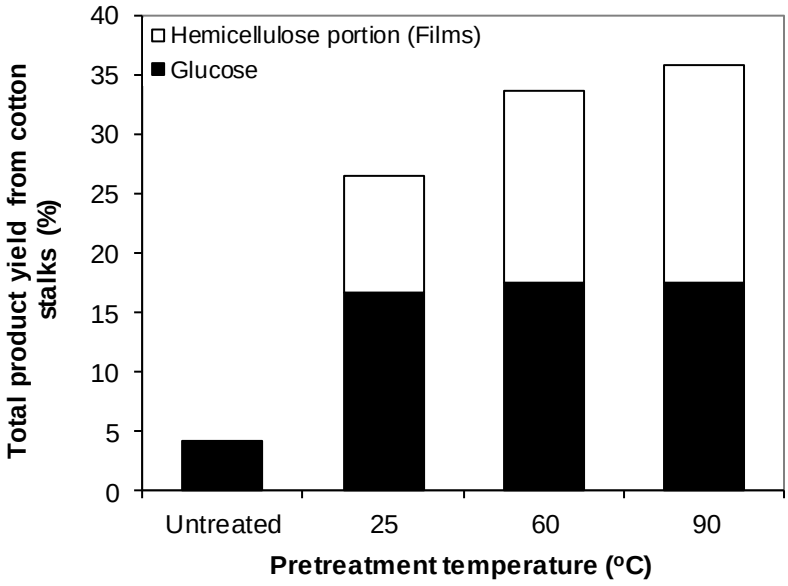

Figure 5.4. Effect of alkaline pretreatment temperature on the total product yield obtained from cotton stalks.

CHAPTER 6

EFFECT OF THE SALTS FORMED DURING THE ISOLATION OF HEMICELLULOSES VIA ALKALINE EXTRACTION ON THE PROPERTIES OF HEMICELLULOSE BASED FILMS

As also demonstrated in the previous chapter, alkaline extraction is one of the most popular techniques for the isolation of hemicelluloses from various lignocellulosic bioresources. This isolation process typically involves a neutralization step conducted by the addition of an acid into the medium, resulting in the formation of salts due to the reaction between the base and the acid, which are typically removed from the medium via techniques such as dialysis or ultrafiltration. It was previously shown that the presence of these salts, including sodium chloride and sodium acetate, together with the hemicelluloses has an adverse effect on the mechanical properties of the hemicellulose based films and therefore it was suggested that purity of the hemicelluloses should be carefully monitored prior to their conversion into hemicellulose based films (Mikkonen et al., 2009). This view can be considered as typical across a wide range of studies that deal with the hemicellulose based films.

The present chapter investigates whether or not all the salts are undesired impurities that adversely affect the properties of the hemicellulose based films. This is an important point since the purification step conducted in order to remove the salts increases the costs associated with the production of hemicellulose based films by adding an extra step to the process. Thus the demonstration of the beneficial effect of a salt on the film properties would be a significant improvement towards the feasible production of hemicellulose based films.

Two different kinds of salts, sodium chloride (NaCl) and potassium acetate (KAc) were investigated for their effects on the properties of hemicellulose based films. These salts were chosen on the basis that they are frequently encountered during alkaline extractions and the subsequent neutralizations conducted with rather popular bases and acids. KAc is formed as a result of the alkaline extraction conducted by using KOH if acetic acid is used during the neutralization stage. NaCl is also formed in a similar manner if NaOH and HCl are used during the extraction and neutralization steps.

6.1. Film forming capability of isolated hemicelluloses

Following the extraction of hemicellulose from corn cobs into the alkaline solution, the polymers dissolved in the potassium hydroxide solution were precipitated by the addition of acetic acid and ethanol. In order to remove the KAc formed during the precipitation step as a result of the reaction between potassium hydroxide and acetic acid, the recovered hemicelluloses were partially dissolved in water and re-precipitated by the addition of ethanol. This process was repeated three times in order to remove the salts present together with the precipitated hemicelluloses. The polymers obtained this way are referred as "desalted hemicelluloses" throughout the present text. Alternatively, KAc was retained together with hemicelluloses simply by not conducting the solubilization and re-precipitation steps where these polymers are referred to as "salted hemicelluloses". The ICP-OES analysis revealed that the KAc content of the salted hemicelluloses was 14% where it was reduced to 1.8% for the desalted hemicelluloses. Once the recovered polymers were dried, film forming solutions were prepared by the dissolution of the desalted and salted hemicelluloses in water. Film forming solutions of desalted hemicellulose also contained sorbitol and KAc separately.

The film forming solutions were cast into petri plates and upon the evaporation of water, films or film fragments were formed as shown in Figure 6.1. The desalted hemicellulose was not capable of forming an intact film where large cracks were observed between the film fragments (Figure 6.1 a). Addition of sorbitol (10% w/w on dry basis) improved the film formation, however cracks ranging approximately from 0.5 to 2 cm in length could still be observed in the sorbitol containing desalted hemicellulose based films (Figure 6.1 d). Unlike desalted hemicellulose, retaining the salt together with hemicellulose at the end of the extraction resulted with the formation of intact films without any visible cracks (Figure 6.1 c). Addition of 10% KAc (w/w on dry basis) into the film forming solution composed of desalted hemicellulose also enabled the formation of a whole film (Figure 6.1b). Thus, it appears that KAc is more effective than sorbitol in terms of enabling the formation of a crack free intact hemicellulose based film and retaining KAc at the end of the extraction process is useful in terms of film formation.

6.2. Mechanical properties of hemicellulose based films

The mechanical properties of polymeric films are of prime importance for various applications including packaging. The mechanical properties of the strips obtained from the films or film fragments were characterized by means of a universal testing machine. The UTS, e_b and E values for each type of film were determined in addition to toughness values. As in the previous chapter, toughness was taken as a measure of a film's overall mechanical property since both the UTS and e_b values contribute to the

toughness values. The mechanical property data for different type of films are given in Figure 6.2. As mentioned in the previous section, the desalted hemicellulose did not form films but instead film fragments upon solution casting. However, the mechanical properties of these fragments were still determined so that the effect of the presence of additives KAc and sorbitol in the films could be better evaluated.

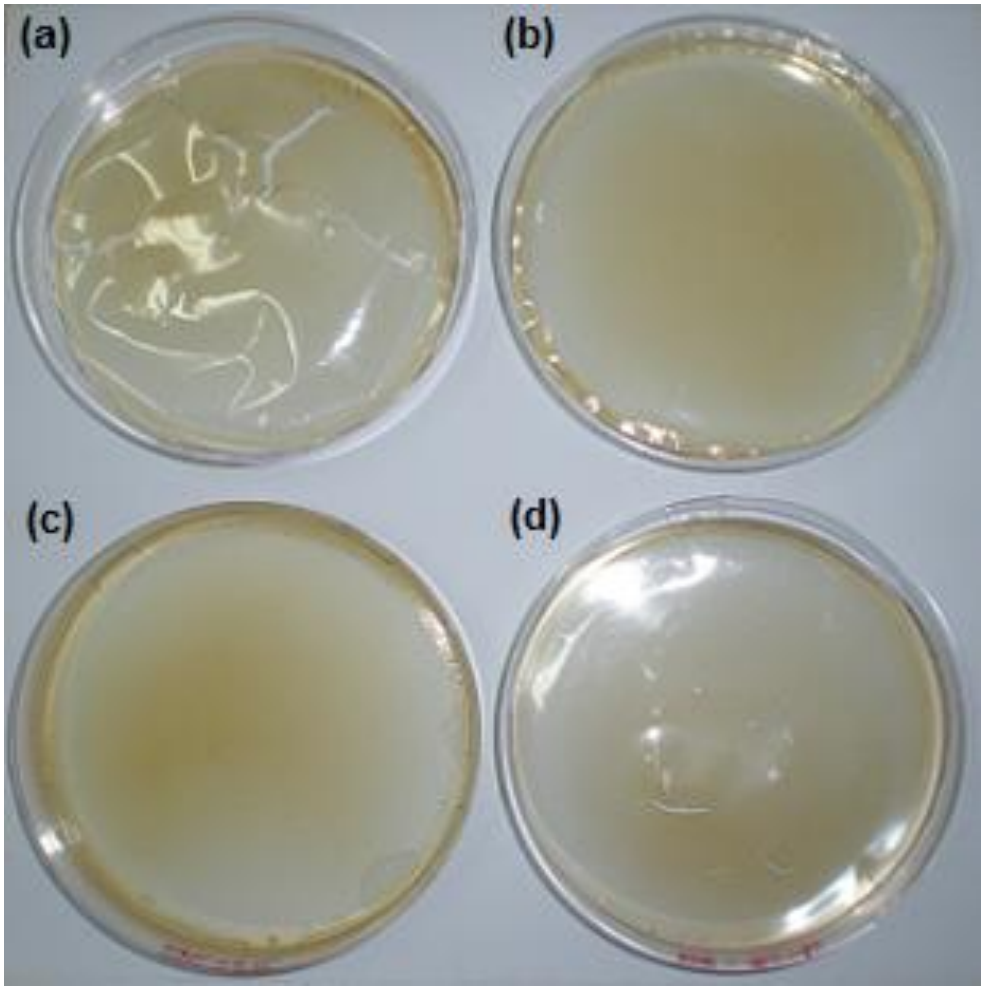

Figure 6.1. Appearance of hemicellulose based films with different compositions. (a) Film fragments obtained from desalted hemicellulose. (b) Desalted hemicellulose based film containing 10% KAc as an additive (c) Film obtained from hemicellulose retaining KAc formed during hemicellulose isolation (d) Desalted hemicellulose based film containing 10% sorbitol as an additive.

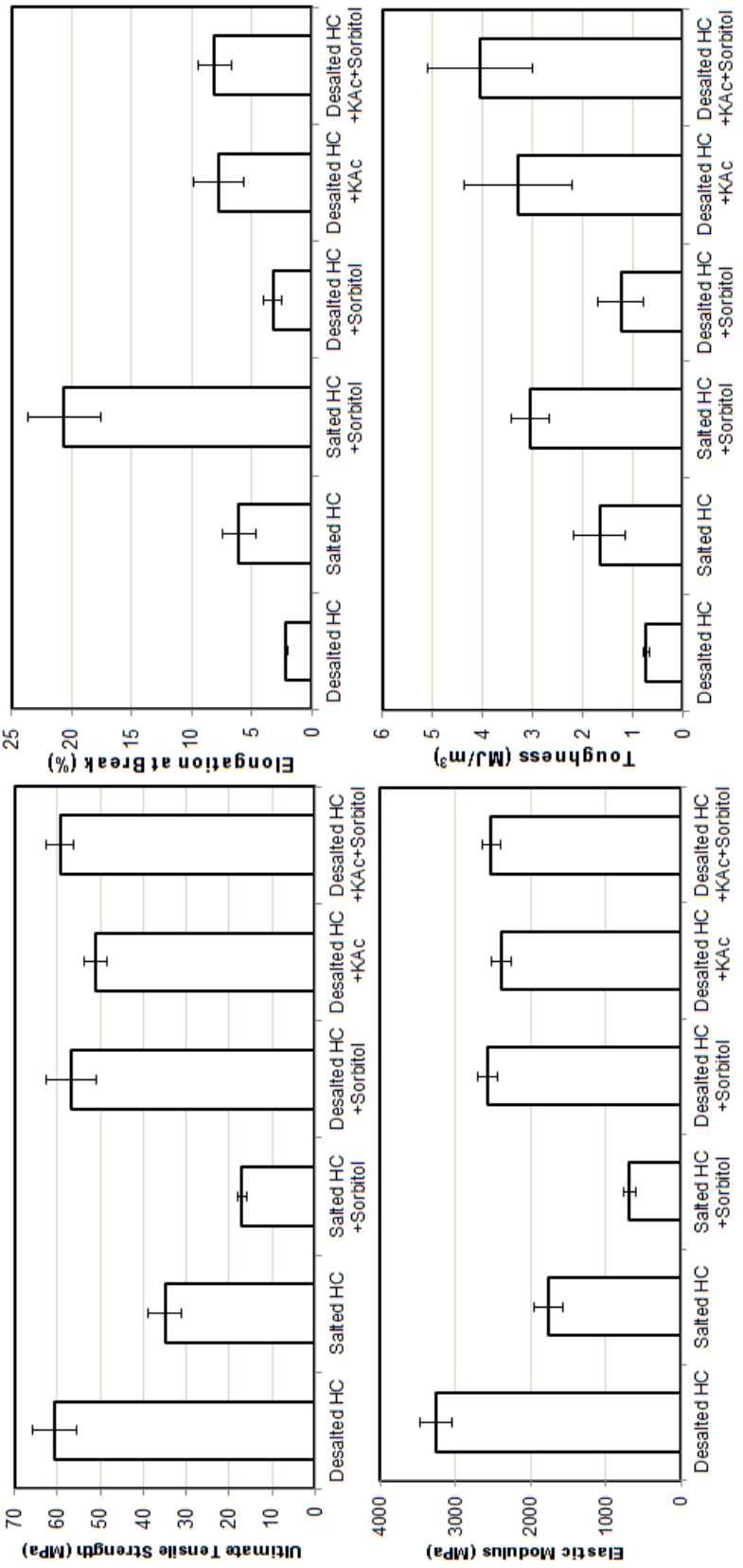

Figure 6.2. Mechanical properties of hemicellulose based films in the presence and absence of KAc and sorbitol. HC stands for hemicellulose.

hemicellulose based films. This result is quite surprising since it implies that the presence of KAc in the films results in the increased plasticization of the films compared to sorbitol, which is one of the most frequently used plasticizers in the hemicellulose based film literature. Furthermore sorbitol appears to work more efficiently as a plasticizer when it is present together with KAc in the films. Films containing 10% sorbitol together with salts had the highest e_b value among all the films (20.6%). Presence of 5% (w/w) sorbitol together with 5% (w/w) KAc in the desalted hemicellulose based films also results in a higher e_b value of 8.1% as compared to the films containing 10% sorbitol alone, which has an e_b value of 3.3%.

It was previously shown that the presence of 5% and 10% sodium chloride (NaCl) or sodium acetate (NaAc) in the hemicellulose based films containing 40% sorbitol lowered the UTS of the films by approximately 1.5 to 2 fold, while no significant change in the e_b values of the films were determined (Mikkonen et al., 2009). On the contrary, it was found in the present study that in addition to the improved film formation induced by KAc, presence of 10% KAc in the films only slightly reduced the UTS of the films from 60.7 MPa to 51.2 MPa while increasing the e_b from 2.2% to 7.8% compared to the additive free film made up of desalted hemicellulose. Furthermore, presence of 5% KAc together with 5% sorbitol in the films also increased the e_b up to 8.1% while resulting in similar UTS values with the additive free film.

Based on their observations regarding the undesired effect of NaCl and NaAc salts on the mechanical properties of hemicellulose based films, Mikkonen et al. (2009) was right to suggest that the purity of the isolated hemicelluloses, in terms of being free of residual salts, is crucial in order to obtain films with good mechanical properties. However, from the comparison of the two studies, it appears that not all the salts are the same in terms of their effect on the mechanical properties of hemicellulose based films. Purity of a product is a critical issue but from an industrial point of view, more purity almost always means more cost as this will raise the need for additional purification steps, which is obviously undesirable for the large scale production of the intended commodity.

Regarding the hemicellulose based coatings and films, this issue was also emphasized from a different perspective in two recent studies where it was shown that instead of pure hemicelluloses, using rather crude hemicellulose fractions obtained from the wood hydrolysate could be advantageous when it comes to achieving lower oxygen permeability (Ryberg et al., 2011; Ibn Yaich et al., 2012) Based on the mechanical properties of the KAc containing films in the present study, it appears that it is not always necessary to remove the salts formed at the end of the alkaline extraction of hemicelluloses from lignocellulosic biomass in order to obtain hemicellulose based films with satisfactory mechanical properties.

6.3. Effect of KAc and NaCl concentration on the indentation hardness and modulus of the films

In order to understand the effect of KAc on the film properties more thoroughly, KAc at two different concentrations (10% and 25%) was added to the desalted hemicellulose based films and these films were subjected to indentation testing in order to characterize their indentation hardness and modulus. This procedure was also repeated for the films containing NaCl at the same concentrations with that of KAc, with the thought that the comparative data obtained in this manner could provide more insight about the positive effect of KAc on the film properties.

Figure 6.3 shows the indentation hardness test results for the films containing different salts at different concentrations. Compared to the film that was composed solely of desalted hemicelluloses, increasing the KAc concentration in the films resulted in an increase in the penetration depth while an inverse trend was observed for the NaCl containing films. This implies that the presence of KAc made the films softer while the presence of NaCl resulted in harder films compared to the film made up of desalted hemicellulose, which is also supported with the indentation hardness data presented in Table 6.1. At a salt concentration of 10%, NaCl containing films are around 1.6 times harder than the KAc containing films, while the gap is increased to 4 fold in the favor of NaCl when the concentration of the salts in the films is increased to 25%, indicating that the presence of NaCl results with significantly harder films as compared to KAc. Similar to their effect on the film hardness, the presence of KAc reduced the elastic modulus of the films while increasing the concentration NaCl in the films resulted in increased modulus values as shown in Table 6.1. Based on these findings, it is apparent that the hemicellulose based films might give totally different responses to the presence of different salts in their structure.

The softening of the films with increasing KAc concentration implies that the presence of KAc results in the plasticization of the films. Taking into account that KAc is a highly hygroscopic salt, which is capable of absorbing significant amounts of moisture from the surroundings, the conventional explanation to the observed plasticization of the films would be related to their water contents. Since water acts as a plasticizer for hemicellulose based films (Gröndahl et al., 2004; Sternemalm et al., 2008; Ying et al., 2011) higher KAc content in a film could have resulted in a higher water content, which would decrease the hardness of the films by acting as a plasticizer. However, as shown in Table 6.1 the water contents of the films containing KAc are not significantly different from the salt-free film under the conditions in which the indentation tests took place. Therefore, it appears that it is not the water that is responsible for the plasticization of the films, which takes place with increasing KAc content. It was recently shown that the salt choline chloride itself acts as a plasticizer when it is included into the hydrophilic polymer starch, thereby increasing the

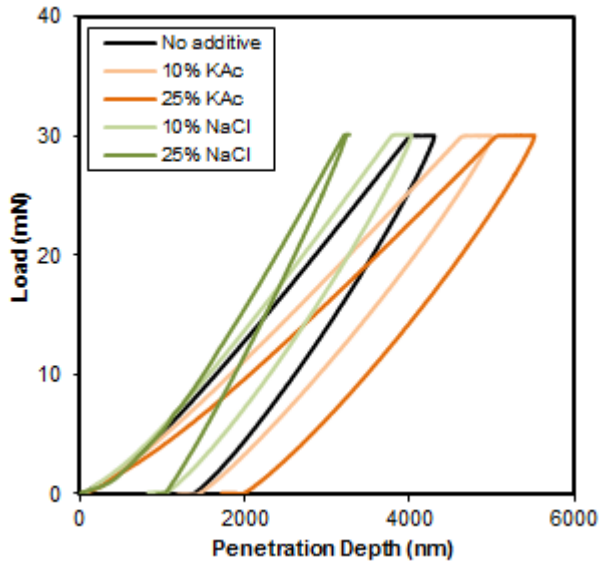

Figure 6.3. Load-Displacement curves obtained from indentation testing of the hemicellulose based films containing different salts at two different concentrations.

flexibility of the starch based films (Abbott et al., 2012). In the light of this finding and taking into account the similar water contents of the films with and without KAc, a possible explanation for the softening of the films in the presence of KAc could be that KAc acts as a plasticizer in the hemicellulose based films, which was not the case for NaCl. The reason for this could be the different compatibilities of these two salts with the polymer matrix in addition to their different solubility in water since the films contain around 8 – 9% water.

6.4 Cross-sectional and surface morphologies of the films containing different types of salts

In order to understand the reason behind the different response of the hemicellulose based films to KAc and NaCl, the cross-sectional and surface morphologies of the films, which contained only desalted hemicellulose or either one of the salts as additives, were characterized by means of scanning electron microscopy (SEM) and optical transmission light microscopy, respectively.

Table 6.1. Hardness, elastic modulus and water content data for hemicellulose based films containing different additives

Additive	Hardness (MPa)	Elastic Modulus (MPa)	Water Content (%)
No salt added	172.6 ± 15.0	755 ± 16	8.9 ± 0.2
KAc 10%	139.5 ± 12.6	543 ± 9	9.0 ± 0.1
KAc 25%	93.4 ± 5.7	469 ± 6	9.2 ± 0.1
NaCl 10%	222.3 ± 26.4	841 ± 26	8.4 ± 0.3
NaCl 25%	366.6 ± 12.4	1285 ± 11	7.7 ± 0.4

Figures 6.4, 6.5 and 6.6 show the SEM images obtained from the cross-sections (fracture surfaces) of the desalted hemicellulose based film and the films containing 10% KAc or NaCl as additives. As it can be easily noted from the SEM images, the three types of films displayed totally different cross-sectional morphologies. Compared to the film made up of desalted hemicellulose (Figure 6.4), addition of KAc resulted in a much more continuous and homogeneous appearance (Figure 6.5). This smoother appearance as compared to the rather coarse and irregular appearance of the desalted hemicellulose based film might be attributed to the plasticization induced by the presence of KAc. A quite similar transition from an irregular structure to a smoother one was also observed by Abbott et al., when choline chloride salt was used in addition to urea in order to plasticize starch (Abbott et al., 2012). On the other hand, dendrite like shapes were observed in the cross-sections of NaCl containing film (Figure 6.6) and its bottom fraction was swarming with cubic structures (Figure 6.7), which were identified as NaCl particles via EDX, indicating a serious compatibility problem of NaCl with the polymer matrix unlike KAc. The dramatic differences in the cross-sectional morphologies of the KAc and NaCl containing films make it evident that different salts may behave differently in a hemicellulose based polymer matrix.

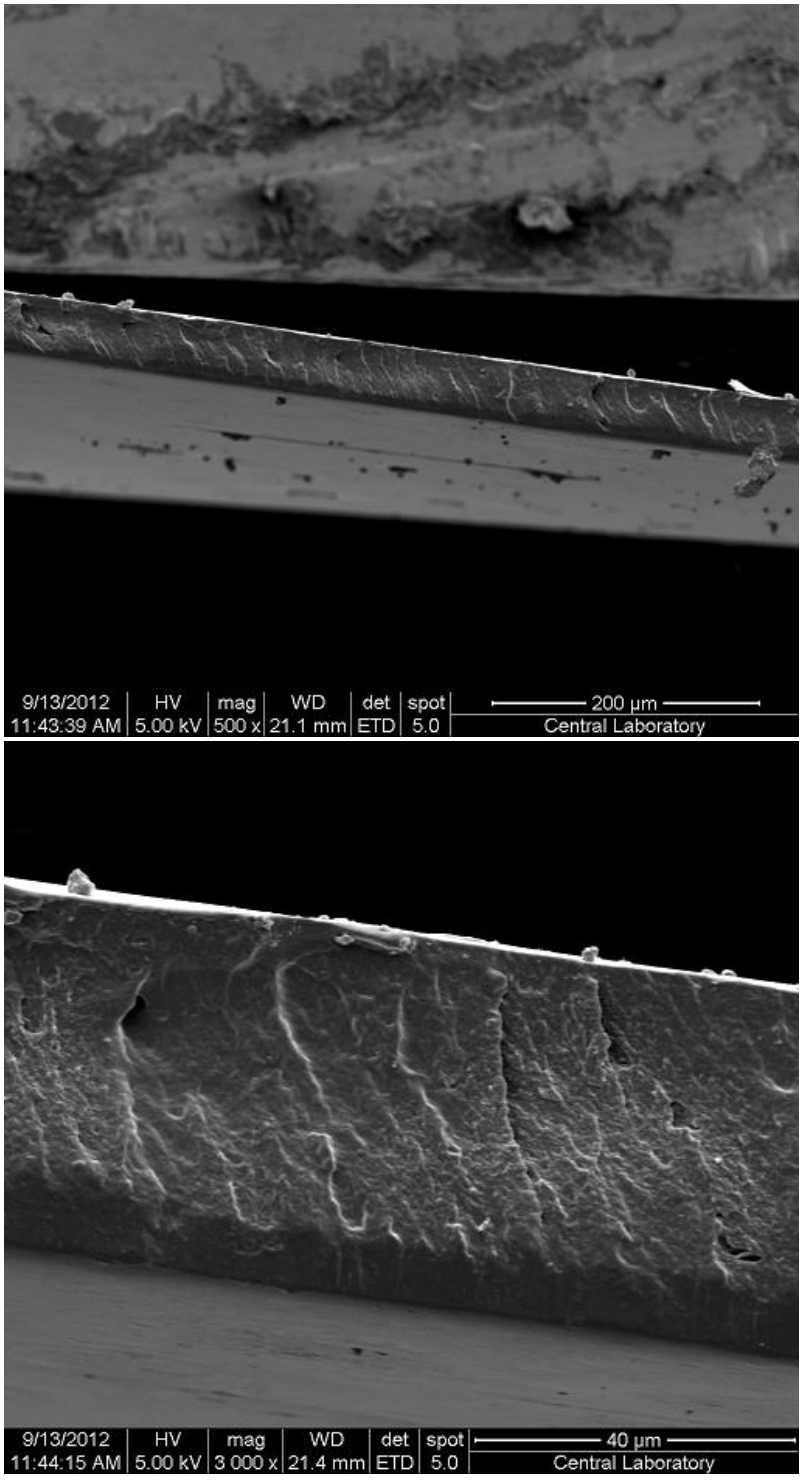

Figure 6.4. Scanning electron microscopy images of the hemicellulose based film prepared without any additives at 500× (top) and 3000× (bottom) magnification.

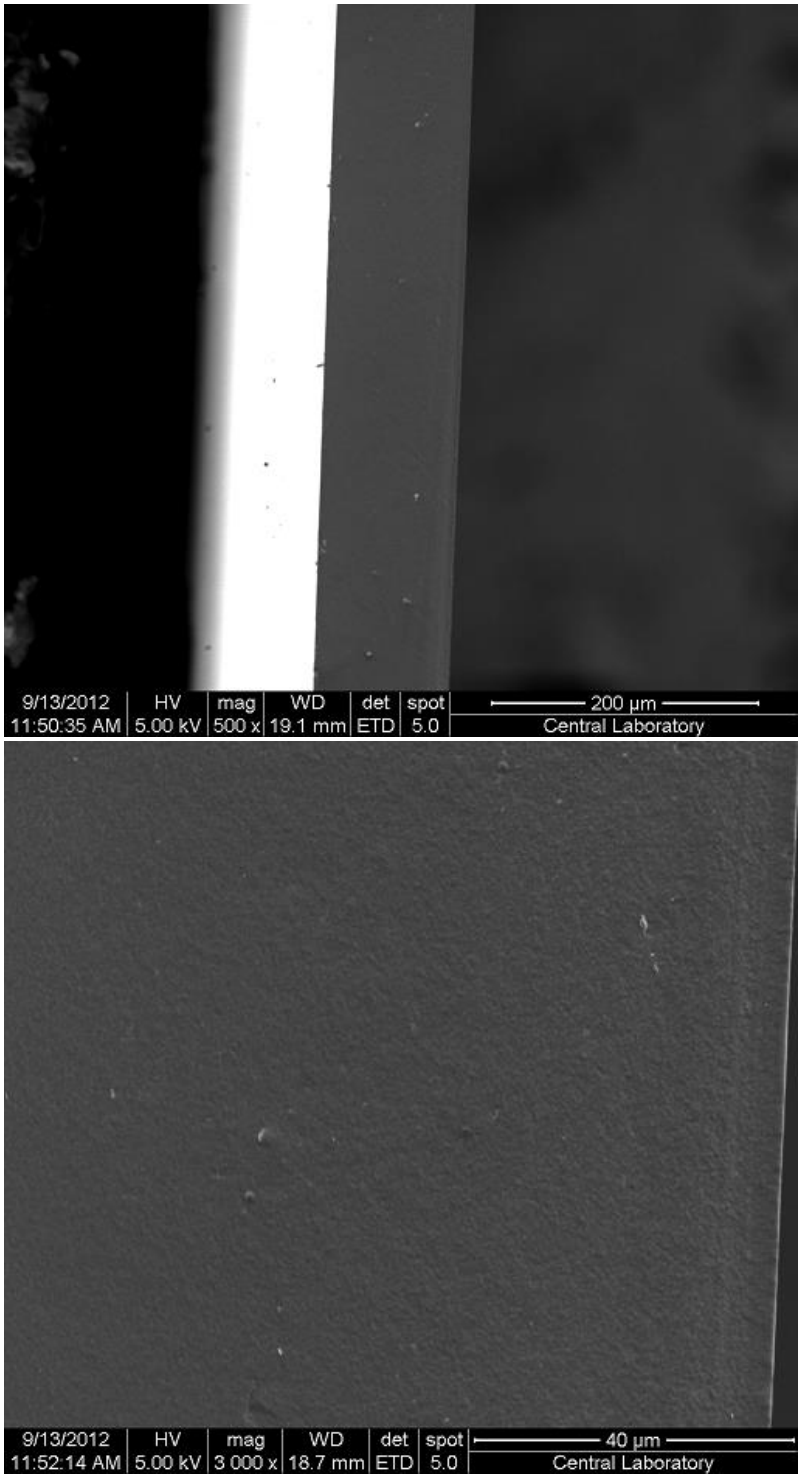

Figure 6.5. Scanning electron microscopy images of the hemicellulose based film containing 10% potassium acetate at 500× (top) and 3000× (bottom) magnification.

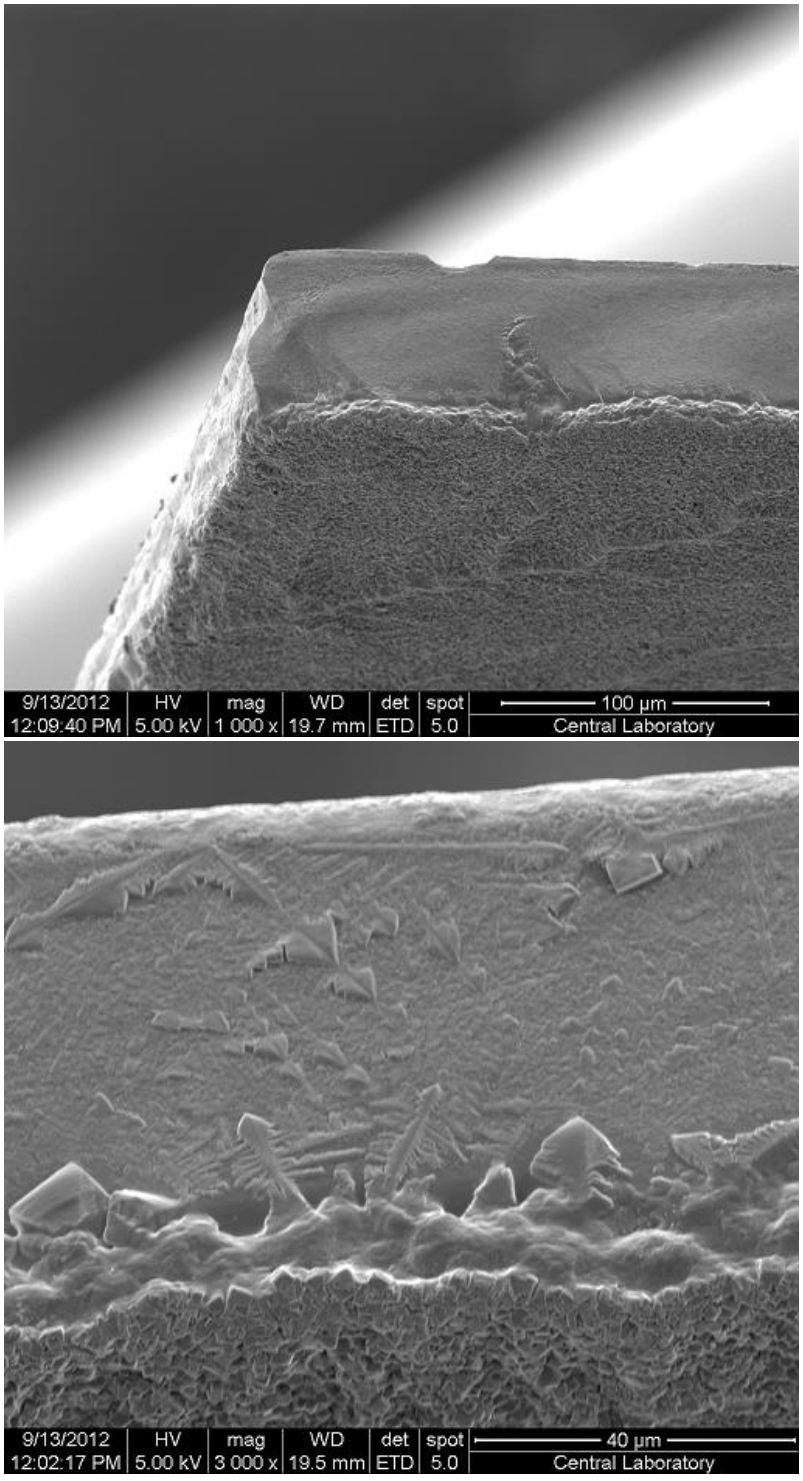

Figure 6.6. Scanning electron microscopy images of the hemicellulose based film containing 10% sodium chloride at 1000× (top) and 3000× (bottom) magnification.

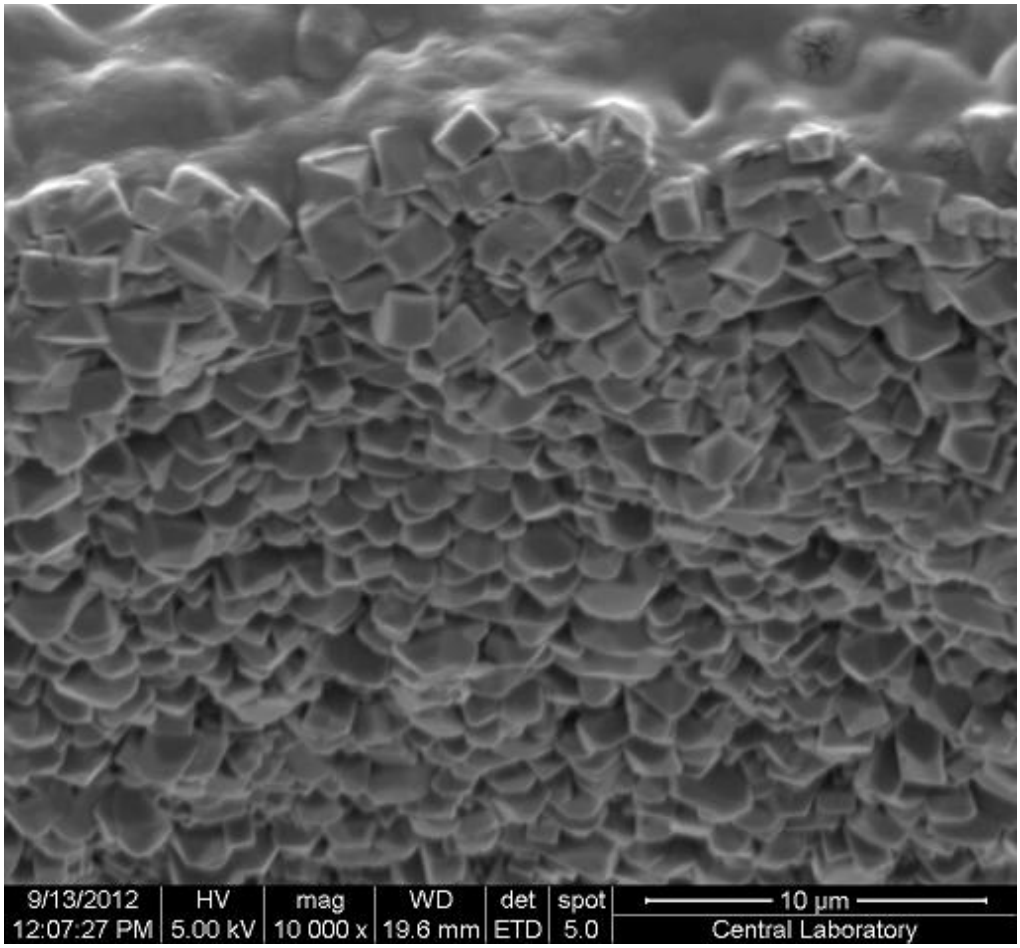

Figure 6.7. Scanning electron microscopy image (at 10000× magnification) of the sodium chloride cubes located at the bottom surface of the hemicellulose based film containing 10% sodium chloride.

The surface morphologies of the films were characterized by means of optical transmission light microscopy and they were consistent with the observations made on the cross-sectional morphologies of the films. As shown in Figure 6.8, the KAc containing films (Figure 6.8 b and c) and the additive free film (Figure 6.8 a) had smooth and homogeneous surface morphologies while imperfections were present on the rough surface of the NaCl containing films (Figures 6.8 d and e). The surface of the NaCl containing films were crowded with tiny particles, which were most likely made up of NaCl due to its segregation from the polymer matrix, where pathways could be observed between them. Thus, the surface images of the films comply with and further support the observation that different salts may dramatically vary in their compatibility to the hemicellulose matrix.

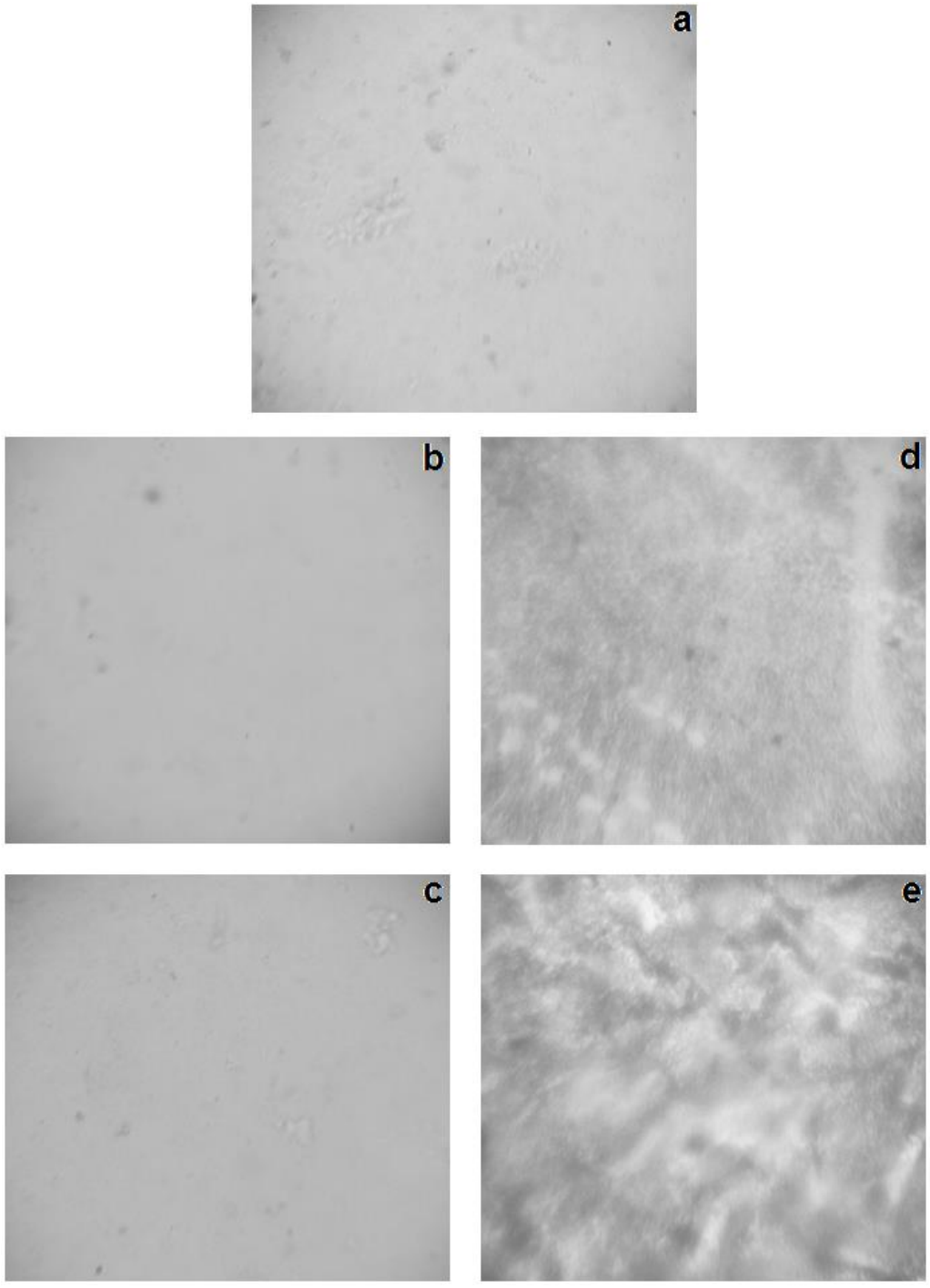

Figure 6.8. Optical transmission light microscopy images of the film surfaces containing; No additive (a), 10% KAc (b), 25% KAc (c), 10% NaCl (d) and 25% NaCl (e).

CHAPTER 7

PRODUCTION OF HEMICELLULOSE BASED MATERIALS VIA EXTRUSION

In Chapters 5 and 6, hemicelluloses based films were produced via the solvent casting technique, which is based on the dissolution of the polymer in a suitable solvent followed by the evaporation of the solvent from the solution, which was cast on a flat surface. Solvent casting is a favorable method on the lab scale, since it is relatively easy to apply. However this is not the case from an industrial point of view since solvent casting is a costly procedure as it requires solvents for the solubilization of the polymers and the subsequent evaporation of the solvents from the solution in order to obtain the desired material. Furthermore, most of the polymer processing plants today do not have solvent casting equipment since a great majority of commercial polymers products of today are not produced via solvent casting causing problems related to the infrastructure for the production of hemicellulose based materials via solvent casting.

Unlike solvent casting, extrusion is mostly a single step production method where the polymer is fed into an extruder and flows through the extruder barrel and extruder screws where it is forced out of the extruder die, which enables the shaping of the polymers to the desired profile at this stage. Dissolution of the polymers and evaporation of the solvent are not necessary steps during extrusion making it a faster, easier and a more feasible way of producing polymeric materials compared to solvent casting. Since the production process of a very large majority of polymer products on the market already includes the utilization of extruders, the infrastructures needed for the processing of a polymer via extrusion is readily available in the polymer processing plants.

Despite the above mentioned superiority of extrusion compared to solvent casting, solvent casting is the dominant method in the literature for the production of hemicellulose based materials where extrusion is not utilized for the production of materials from hemicelluloses.

The present chapter investigates the applicability of the extrusion technique for this purpose. Hemicelluloses were extracted from corncobs via alkaline extraction as in the previous two chapters. The polymers were then loaded with different water contents and extrusion trials were made at different extrusion temperatures. The extruded materials, which were in the form of strips, were characterized for their cross-sectional morphology and mechanical properties.

7.1. Effect of water content and extrusion temperature on the extrudability of hemicelluloses

Following the alkaline extraction and drying, the hemicelluloses extracted from corn cobs were loaded with different water contents by keeping them in desiccators at different relative humidities. At the end of 24 hours, polymers with three different water contents were obtained and these polymers were fed to the twin-screw micro extruder at three different temperatures to see if any extrudate could be obtained from the extruder. As shown in Table 7.1, only a single combination of water content and extrusion temperature resulted in the formation of the hemicellulose extrudate, which was 90°C extrusion temperature and 27% polymer water content. In other words, none of the other two extrusion temperatures (60°C and 120°C) gave hemicellulose extrudates while the two lower water contents, which were approximately 7% and 12%, also gave no extrudates at any of the three temperatures studied. Therefore it appears that both the water content of the hemicelluloses prior to extrusion and the extrusion temperature are essential factors to render hemicelluloses extrudable.

Table 7.1. Extrusion temperatures, water content of hemicelluloses prior to extrusion and the resulting extrudability of the hemicelluloses.

$T_{Extrusion}$ (ºC)	Water Content (%)	Extrudability
60	7.2	**X**
	11.4	**X**
	28.5	**X**
90	6.7	**X**
	12.9	**X**
	26.9	✓
120	6.6	**X**
	12.1	**X**
	28.3	**X**

Sugar beet pulp, which is composed of cellulose and pectin in addition to hemicellulose, was previously converted into polymeric materials via extrusion (Rouilly et al., 2006a; Rouilly et al., 2006b; Rouilly et al., 2009). Water content was shown to be a crucial parameter for the extrusion of sugar beet pulp too, since under the correct temperature, sufficient water content enabled the desired polymer flow within the extruder because of the plasticization induced by water to the system, which

subsequently resulted in sugar beet pulp extrudates (Rouilly et al., 2006b). Considering the results given in Table 7.1 together with this finding, it might be thought that within the set of the water contents studied, a water content of 27% was needed together with an extrusion temperature of 90°C to create the necessary polymer flow within the extruder so that the extrudates could be obtained through the extruder die. When the extrusion temperature was increased to 120°C, the water content of the hemicelluloses within the extruder was probably lost to a certain extent due to evaporation, which subsequently disabled their flow properties due to the reduced plasticization of the polymers within the extruder barrel. On the contrary, insufficient temperature must be responsible for the unsuccessful extrusion trials made at 60°C, since compared to the two higher temperatures studied, the loss of water content must have been much lower in this case.

The extrusion of hemicelluloses with a water content of 27% at 90°C resulted in the formation of an extrudate, which was obtained in the shape of a strip with a thickness and width of roughly 0.5 mm and 5 mm, respectively, as shown in Figure 7.1. It should be noted that the strips contained neither plasticizers nor any other types of additives that could be detrimental for their biodegradation properties. This strip was collected on to a mini conveyor belt with adjustable speed that was placed right after the extruder die. The length of the extruded strip eventually depends on the amount of material fed to the extruder. In the present case, 5 g of hemicellulose was fed to the extruder and this resulted in a strip with approximately 1.5 m in length.

Closer inspection of the hemicellulose strip by transmission optical light microscopy reveals an important aspect, which further supports the claim that the water content is of key importance for the extrusion of the hemicelluloses. The beginning and the end of the hemicellulose strip or in other words the portion of the strip that initially comes out of the die and the portion that comes at the end of the extrusion when the entire polymer is depleted, was subjected to inspection. Although both portions were intact and continuous to the naked eye, the initial portion of the strip was more homogeneous (Figure 7.2 a) compared to the final part of the strip (Figure 7.2 b), which contained segments comprised of small cracks separated with quite clear borders from the rest of the strip. The speed at which the strip comes out of the die slows down towards the final stages of extrusion. Due to this reason, the residence time of the polymers towards the final stages of extrusion is more than the ones that are extruded at the initial stages of the extrusion. Therefore the polymers with higher residence times must have lost more of their water content compared to those with lower residence times, which likely caused the inhomogeneous structure shown in Figure 7.2 (b) due to insufficient water content.

Figure 7.1. Appearance of hemicelluloses extracted from corn cobs prior to being fed to the extruder (left) together with a hemicellulose strip produced from these polymers via extrusion (right).

The SEM images of the cross-sections of the hemicellulose strip is shown in Figure 7.3, where it can be seen that the internal structure of the strip is quite homogeneous indicating that extrusion technique indeed appears to be suitable for the production of hemicellulose based materials.

7.2 Mechanical properties of hemicellulose based strips obtained via extrusion

The strips obtained from the extruder were cut into samples 8 cm long and they were subjected to tensile testing. In addition to the hemicellulose strips, strips obtained from polylactic acid (PLA), which were also produced with the same extruder used for the production of hemicellulose strips, were also tested for their mechanical properties. The reason for this is to compare the mechanical performance of the hemicellulose strips with one of the most popular biodegradable polymers, which is already available in the market. The PLA polymers, which were in the form of pellets, were extruded into a PLA strip at an extrusion temperature of 170°C as suggested previously (Baouz et al., 2013).

The mechanical properties of the strips together with the literature values given for the materials produced from sugar beet pulp (SBP) via extrusion are given in Table 7.2.

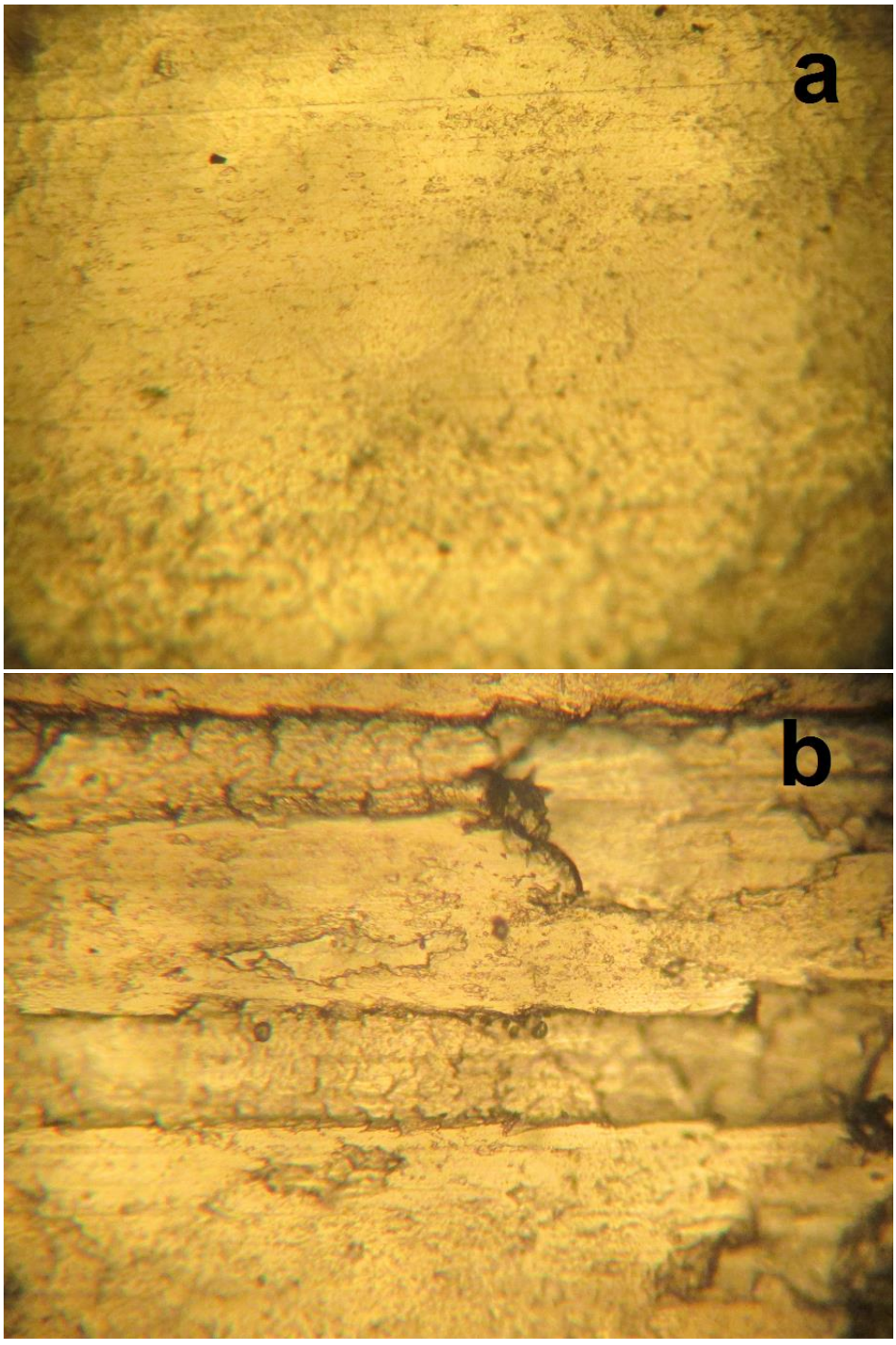

Figure 7.2. Transmission optical microscopy images of the initial (a) and final (b) portions of the hemicellulose strip's surface.

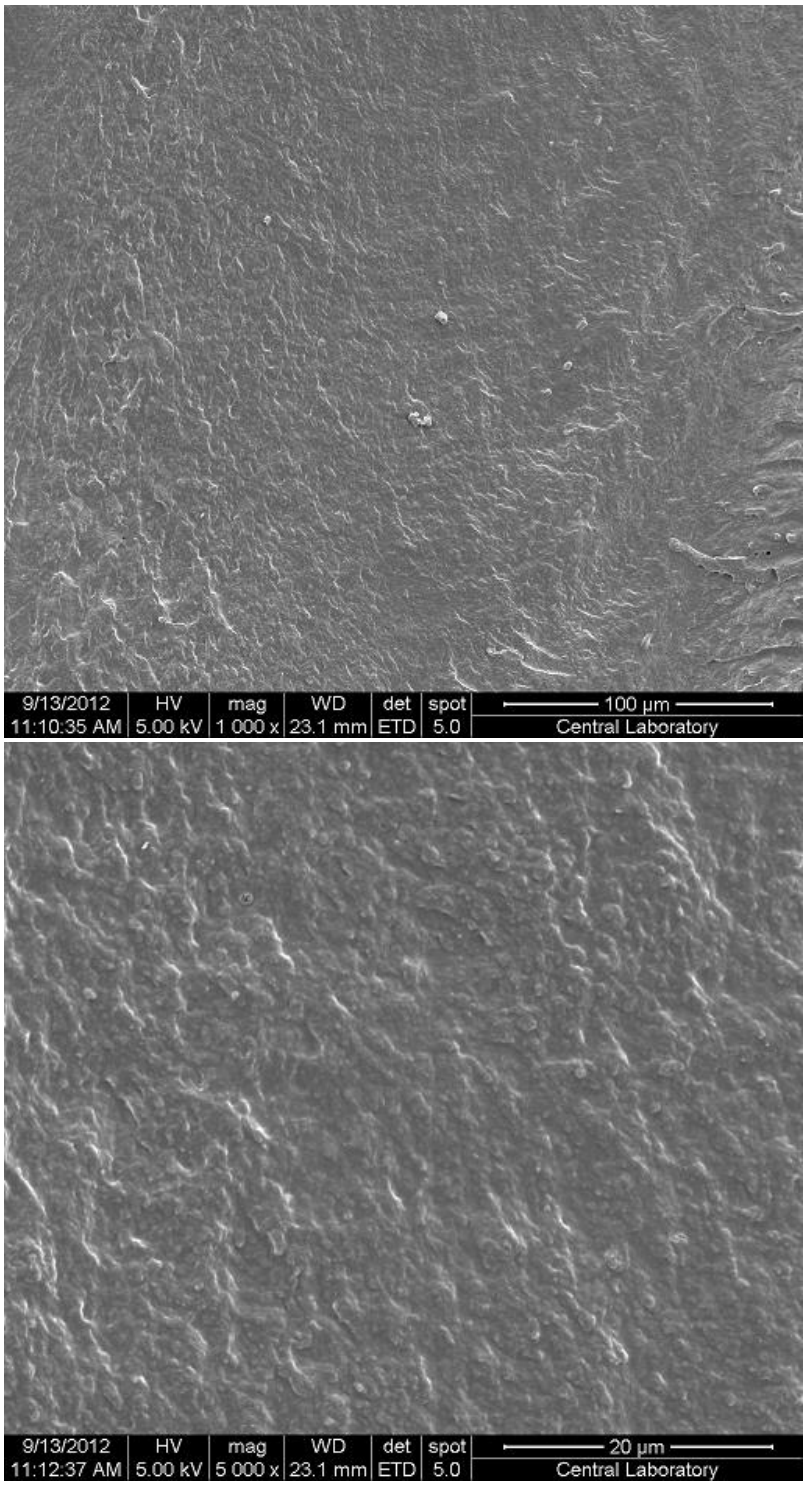

Figure 7.3. Cross-sectional appearances of a hemicellulose strip as obtained via scanning electron microscopy at 1000× (top) and 5000× (bottom) magnification.

The hemicellulose strips had good mechanical properties, which were superior to those of the PLA strips where the UTS and e_b values of hemicellulose strips were around 1.5 and 3 times higher, respectively, compared to the PLA strips. Additionally the hemicellulose strips also had much better mechanical properties compared to the materials produced from SBP via extrusion as shown in Table 7.2. These results indicate that polymeric materials produced from hemicelluloses can be promising candidates, at least in terms of their mechanical properties, for being popular biodegradable polymeric materials of the near future.

Table 7.2. Ultimate tensile strength (UTS), elongation at break (e_b) and elastic modulus (E) values for polymeric materials obtained from three different types biodegradable polymers including polylactic acid (PLA) and sugar beet pulp (SBP).

Polymer	UTS (MPa)	e_b (%)	E (MPa)	Reference
Hemicellulose	76 ± 6	35 ± 8	1073 ± 86	Present study
PLA	52 ± 2	12 ± 2	708 ± 88	Present study
SBP	~3-19	~1-7	~100-2000	Rouilly et al., 2009
SBP	6.5-9.2	4.1-11.5	317-744	Liu et al., 2011

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

Lignocellulosic agricultural wastes are renewable, cheap, widely available and excessively abundant sources of biopolymers, which can be converted into a large variety of products capable of substituting those produced from non-renewable fossil resources. In this context, second generation biorefineries that utilize lignocellulosic biomass as raw materials for the production of these products are considered to be of primary importance. A feasible as well as flexible second generation biorefinery should be capable of producing more than just a single product while utilizing all the three biopolymers (cellulose, hemicellulose and lignin) found in the lignocellulosic biomass as much as possible. On the basis of these points, the present study has focused on the production of glucose and biodegradable polymeric materials from different components of lignocellulosic agricultural wastes. Various process parameters employed during the conversion of the lignocellulosic agricultural wastes to glucose and biodegradable polymeric materials were evaluated with respect to their effects on the quantity and properties of the products.

Three different types of pretreatments, namely dilute acid (via H_2SO_4), alkaline (via NaOH) and ionic liquid (via 1-ethyl-3-methylimidazolium acetate) pretreatments, were applied to corn cobs. The glucose yields obtained via the enzymatic hydrolysis of the pretreated biomass samples were between 73 – 84%, where the highest glucose yield was obtained from the ionic liquid pretreated samples. The effect of ionic liquid pretreatment on the lignin content, crystallinity and digestibility of the biomass was investigated in addition to the glucose yields with respect to increasing lignocellulosic biomass particle size prior to ionic liquid pretreatment conducted with 1-ethyl-3-methylimidazolium acetate (EMIMAc) and 1-ethyl-3-methylimidazolium chloride (EMIMCl). The particle size of the biomass samples prior to pretreatment was determined to have different effects on the performances of the two ionic liquids. Among the four biomass particle size studied (<0.15 mm, 0.15 – 0.5 mm, 0.5 – 1.0 mm, 1.0 – 2.0 mm), EMIMCl pretreatment gave the highest glucose yields for the two smallest particle sizes (49%) while for the EMIMAc pretreatment the smallest particle size gave the lowest glucose yield (57%), compared to the glucose yields obtained upon the pretreatment of larger particle sizes with EMIMAc (≥71%) most likely due to the degradation of cellulose during pretreatment. It was therefore shown that the ideal particle size of lignocellulosic biomass depends on the selection of the ionic liquid that is to be used in the pretreatment step. The pretreatment process was also investigated in terms of its effect on the co-production of glucose and hemicellulose

based films where alkaline pretreatment was used for the extraction of hemicelluloses from lignocellulosic biomass in order to separate the biomass into cellulosic and hemicellulosic fractions, which were used for glucose and polymeric film production, respectively. Alkaline pretreatment was conducted at three different temperatures (25, 60 and 90°C) and it was determined that while the increase in the pretreatment temperature did not impose any significant change in the glucose yield obtained from pretreated cotton stalks, the toughness values of the films produced by the solvent casting technique were decreased with increasing pretreatment temperature from 1.1 MJ/m^3 down to 0.4 MJ/m^3. The highest pretreatment temperature studied (90°C) was also shown to impair the film forming property of the isolated hemicelluloses. The pretreatment temperatures were also evaluated in terms of the total amounts of the final products (glucose + hemicellulose based films) where increasing the pretreatment temperature form 25°C to 60°C was shown to be useful since an increase in the total product yield was obtained upon this increase to a certain extent. Upon the evaluation of the overall results, 60°C was determined as the ideal alkaline pretreatment-extraction temperature.

The alkaline extraction also results in the formation of salts together with hemicelluloses during the hemicellulose recovery step, which are regarded as undesired impurities during the preparation of hemicellulose based films. This issue was investigated by either retaining or excluding the potassium acetate salts formed during the alkaline extraction of hemicelluloses from corn cobs. Polymers and films containing the salt potassium acetate was determined to show better film forming and mechanical properties compared to the desalted samples where the toughness values were improved for 2 – 5 folds. Furthermore comparison of potassium acetate with sodium chloride revealed that different salts induce dramatically different effects on the properties and structure of the solvent cast films due to their level of compatibility with the polymer matrix. Increasing potassium acetate concentration in the films resulted in softening, which was indicative of the plasticization of the films where opposite was true for the sodium chloride. While the presence of potassium acetate made the cross-sections of the films smoother and more homogeneous compared to the desalted films, sodium chloride resulted in the formation of dendrites and different sections in the films. The results related to the presence of potassium acetate in the films imply that the salt removal step, which is conventionally conducted prior to the preparation of the films via solvent casting, is not always necessary and it can be avoided. This finding has important implications from the economic point of view since it reduced the number of steps and separation needs associated with the production of hemicellulose based films. Another economically essential issue that was studied regarding the production of hemicellulose based materials was the utilization of the extrusion technique instead of solvent casting, which involves solvents, long processing times as well as multiple steps including dissolution of the polymer and evaporation of the solvent. It was found that the trick that made

hemicelluloses extrudable was their water content where hemicellulose polymers with a water content of 27% could be extruded at 90°C extrusion temperature into hemicellulose strips. The extruded strips had a homogeneous cross-sectional appearance and showed good tensile properties with an ultimate tensile strength of 76 MPa and elongation at break of 35% that were superior to the tensile properties of the strips extruded from polylactic acid, which is one of the most popular biodegradable polymers on the market, demonstrating the potential of hemicelluloses and polymeric materials produced from them.

Further studies related to the topics covered in the present thesis can focus on a number of points. Ionic liquid pretreatment can be conducted with an extruder in order to perform a continuous pretreatment instead of the conventional batch style pretreatment. A mixture of ionic liquid and lignocellulosic biomass can be fed to the extruder, which is operated at various screw speeds and temperatures. A synergistic effect could arise from the deconstructive power of the ionic liquid and the shear force of the extruder. Such an approach could also decrease the pretreatment times necessary in order to achieve high glucose yields down to a few minutes.

Another future study could be the modification of the entire lignocellulosic structure with ionic liquids in order to make the entire biomass (not just the hemicellulosic fraction) extrudable. Based on the findings of the present study, it would be a good approach to load the modified biomass samples with sufficient water content where the extrusion temperature would probably be different than 90°C due to the involvement of modified cellulose and lignin. This approach could lead to the formation of a natural composite in a different arrangement compared to its native form in nature and could yield materials with better mechanical properties.

Nanocomposite materials based on hemicelluloses could also be produced with the extrusion technique described in the present study. Addition of plasticizers, which are frequently used during the solvent casting based studies, to the hemicelluloses during or prior to extrusion can also be studied to further enhance the ductility of the hemicellulose based materials.

REFERENCES

Abbott, A.P., Ballantyne, A.D., Conde, J.P., Ryder, K.S., Wise, W.R., 2012. Salt modified starch: sustainable, recyclable plastics. Green Chem. 14, 1302-1307.

Akpınar, O., Levent, O., Bostancı, Ş., Bakır U., Yılmaz, L., 2011. The optimization of dilute acid hydrolysis of cotton stalk in xylose production. Appl. Biochem. Biotechnol. 163, 313–325.

Alriksson, B., Rose, S.H., van Zyl, W.H., Sjöde, A., Nilvebrant, N.-O., Jönsson, L.J., 2009. Cellulase production from spent lignocellulose hydrolysates by recombinant *Aspergillus niger*. Appl. Environ. Microbiol. 75, 2366–2374.

Arora, R., Manisseri, C., Li, C., Ong, M.D., Scheller, H.V., Vogel, K., Simmons, B.A., Singh, S., 2010. Monitoring and analyzing process streams towards understanding ionic liquid pretreatment of switchgrass (*Panicum virgatum* L.). Bioenerg. Res. 3, 134–145.

Bahçegül, E., 2009. Preparation of biodegradable films from agricultural biomass, METU, M.Sc. Thesis.

Baouz, T., Rezgui, F., Yilmazer, U., 2013. Ethylene-methyl acrylate-glycidyl methacrylate toughened poly (lactic acid) nanocomposites. J. Appl. Polym. Sci. 128, 3193–3204.

Bozell, J.J., Petersen, G.R., 2010. Technology development for the production of biobased products from biorefinery carbohydrates—the US Department of Energy's "Top 10" revisited. Green Chem. 12, 539–554.

Brandt, A., Hallett, J.P., Leak, D.J., Murphy, R.J., Welton, T., 2010. The effect of the ionic liquid anion in the pretreatment of pine wood chips. Green Chem. 12, 672–679.

Brandt, A., Ray, M.J., To, T.Q., Leak, D.J., Murphy, R.J., Welton, T., 2011. Ionic liquid pretreatment of lignocellulosic biomass with ionic liquid–water mixtures. Green Chem. 13, 2489–2499.

Brandt, A., Grasvik, J., Hallett, J.P., Welton, T., 2013. Deconstruction of lignocellulosic biomass with ionic liquids. Green chem. 15, 550–583.

Bubner, P., Plank, H., Nidetzky, B., 2013. Visualizing cellulase activity. Biotechnol. Bioeng. 110, 1529–1549.

Cavka, A., Alriksson, B., Rose, S.H., van Zyl, W.H., Jönsson, L.J., 2011. Biorefining of wood: combined production of ethanol and xylanase from waste fiber sludge. J. Ind. Microbiol. Biotechnol. 38, 891–899.

Celebioglu, H.Y., Cekmecelioglu, D., Dervisoglu, M., Kahyaoglu, T., 2012. Effect of extraction conditions on hemicellulose yields and optimisation for industrial processes. Int. J. Food Sci. Technol., 47, 2597–2605.

Chang, V.S., Holtzapple, M.T., 2000. Fundamental factors affecting biomass enzymatic reactivity. Appl. Biochem. Biotechnol. 84–86, 5–37.

Chiellini, E., Solaro, R., 1996. Biodegradable polymeric materials. Adv. Mater. 8, 305-313.

Choy, V., Patel, N., Thibault, J., 2007. Blood glucose monitor: an alternative off-line method to measure glucose concentration during fermentations with *Trichoderma reesei*. Biotechnol. Lett. 29, 1075–1080.

Dadi, A.P., Varanasi, S., Schall, C.A., 2006. Enhancement of cellulose saccharification kinetics using an ionic liquid pretreatment step. Biotechnol. Bioeng. 95, 904–910.

Dale, B.E., Ong, R.G., 2012. Energy, wealth, and human development: why and how biomass pretreatment research must improve. Biotechnol. Prog. 28, 893–898.

Decker, S.R., Brunecky, R., Tucker, M.P., Himmel, M.E., Selig, M.J., 2009. High-Throughput screening techniques for biomass conversion. Bioenerg. Res. 2, 179–192.
Doherty, T.V., Mora-Pale, M., Foley, S.E., Linhardt, R.J., Dordick, J.S., 2010. Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy. Green Chem. 12, 1967–1975.

Ebringerova, A., Heinze, T., 2000. Xylan and xylan derivatives–biopolymers with valuable properties, 1. Naturally occurring xylans structures, isolation procedures and properties. Macromolecular rapid comm. 21, 542–556.

Ebringerova, A., Hromadkova, Z., Alföldi, J., Berth, G., 1992. Structural and solution properties of corn cob heteroxylans. Carbohydr. Polym. 19, 99– 105.

Ebringerova, A., Hromadkova, Z., Heinze, T., 2005. Hemicellulose. Adv. Polym. Sci. 186, 1–67.

Edlund, U., Zhu Ryberg, Y.Z., Albertsson, A.C., 2010. Barrier films from renewable forestry waste. Biomacromolecules 11, 2532-2538.

Eriksson, T., Börjesson, J., Tjerneld, F., 2002. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. Enzyme Microb. Technol. 31, 353–364.

Escalante, A., Gonçalves, A., Bodin, A., Stepan, A., Sandström, C., Toriz, G., Gatenholm, P. 2012. Flexible oxygen barrier films from spruce xylan. Carbohydr. Polym. 87, 2381–2387.

Fernando, S., Adhikari, S., Chandrapal, C., Murali, N., 2006. Biorefineries: Current status, challenges and future direction. Energy Fuels 20, 1727–1737.

FitzGerald, J., Vermerris, W., 2005. The utility of blood glucose meters in biotechnological applications. Biotechnol. Appl. Biochem. 41, 233–239.

Ferri, S., Kojima, K., Sode, K., 2011. Glucose electrochemistry: Review of glucose oxidases and glucose dehydrogenases: A bird's eye view of glucose sensing enzymes. J. Diabetes Sci. Technol. 5, 1068–1076.

FitzPatrick, M., Champagne, P., Cunningham, M.F., Whitney, R.A., 2010. A biorefinery processing perspective: Treatment of lignocellulosic materials for the production of value-added products. Bioresour. Technol. 101, 8915–8922.

Fort, D.A., Remsing, R.C., Swatloski, R.P., Moyna, P., Moyna, G., Rogers, R.D., 2007. Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-*n*-butyl-3-methylimidazolium chloride. Green Chem. 9, 63–69.

Fredon, E., Granet, R., Zerrouki, R., Krausz, P., Saulnier, L., Thibault, J.F., Rosier, J., Petit, C., 2002. Hydrophobic films from maize bran hemicelluloses. Carbohydr. Polym. 49, 1–12.

Fu, D., Mazza, G., Tamaki Y., 2010. Lignin extraction from straw by ionic liquids and enzymatic hydrolysis of the cellulosic residues. J. Agric. Food Chem. 58, 2915–2922.

Gabrielii, I., Gatenholm, P., 1998. Preparation and properties of hydrogels based on hemicellulose. J. Appl. Polym. Sci. 69, 1661-1667.

Gabrielii, I., Gatenholm, P., Glasser, W.G., Jain, R.K., Kenne, L., 2000. Separation, characterization and hydrogel-formation of hemicellulose from aspen wood. Carbohydr. Polym. 43, 367–374.

Gandini, A., 2011. The irruption of polymers from renewable resources on the scene of macromolecular science and technology. Green Chem. 13, 1061-1083.

Glasser, W.G., Kaar, W.E., Jain, R.K., Sealey, J.E., 2000. Isolation options for non-cellulosic heteropolysaccharides (HetPS). Cellulose 7, 299–317.

Goksu, E.I., Karamanlioglu, M., Bakir, U., Yilmaz, L., Yilmazer, U., 2007. Production and characterization of films from cotton stalk xylan. J. Agric. Food Chem. 55, 10685–10691.

Gröndahl, M., Eriksson, L., Gatenholm, P., 2004. Material properties of plasticized hardwood xylans for potential application as oxygen barrier films. Biomacromolecules 5, 1528–1535.

Hansen, N. M. L., Plackett, D., 2008. Sustainable films and coatings from hemicelluloses: a review. Biomacromolecules 9, 1493–1505.

Hartman, J., Albertsson, A.C., Lindblad, M.S., Sjoberg, J., 2006b. Oxygen barrier materials from renewable sources: Material properties of softwood hemicellulose-based films J. Appl. Polym. Sci. 100, 2985–2991.

Hartman, J., Albertsson, A.C., Sjoberg, J., 2006a. Surface- and bulk-modified galactoglucomannan hemicellulose films and film laminates for versatile oxygen barriers. Biomacromolecules 7, 1983–1989.

Haykır, N.I., 2013. Pretreatment of cotton stalks with ionic liquids for enhanced enzymatic hydrolysis of cellulose and ethanol production, METU, Ph.D. Thesis.

Heller, A., Feldman, B., 2008. Electrochemical glucose sensors and their applications in diabetes management. Chem. Rev. 108, 2482–2505.

Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol. 100, 10–18.

Henrissat, B., 1994. Cellulases and their interaction with cellulose. Cellulose, 1, 169–196.

Hoije, A., Grondahl, M., Tommeraas, K., Gatenholm, P., 2005. Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks. Carbohydr. Polym. 61, 266–275.

Hoije, A., Sternemalm, E., Heikkinen, S., Tenkanen, M., Gatenholm, P., 2008. Material properties of films from enzymatically tailored arabinoxylans. Biomacromolecules 9, 2042–2047.

Ibn Yaich, A., Edlund, U., Albertsson, A.C., 2012. Wood hydrolysate barriers: Performance controlled via selective recovery. Biomacromolecules 13, 466-473.

Johansson, C., Bras, J., Mondragon, I., Nechita, P., Plackett, D., Simon, P., Svetec, D.G., Virtanen, S., Baschetti, M.G., Breen, C., Clegg, F., Aucejo, S., 2012. Renewable fibers and bio-based materials for packaging applications–a review of recent developments. BioResources 7, 2506-2552.

Jones, P.O., Vasudevan, P.T., 2010. Cellulose hydrolysis by immobilized *Trichoderma reesei* cellulase. Biotechnol. Lett. 32, 103–106.

Kayserilioğlu, B.Ş., Bakir, U., Yilmaz, L., Akkaş, N., 2003. Use of xylan, an agricultural by-product, in wheat gluten based biodegradable films: mechanical, solubility and water vapor transfer rate properties. Bioresour. Technol. 87, 239-246.

Kilpelainen, I., Xie, H., King, A., Granstrom, M., Heikkinen, S., Argyropoulos, D.S., 2007. Dissolution of wood in ionic liquids. J. Agric. Food Chem. 55, 9142–9148.

Klemm, D., Heublein, B., Fink, H.P., Bohn, A., 2005. Cellulose: fascinating biopolymer and sustainable raw material. Angew. Chem. Int. Ed. 44, 3358–3393.

Kumar, R., Wyman, C.E., 2009. Effect of enzyme supplementation at moderate cellulase loadings on initial glucose and xylose release from corn stover solids pretreated by leading technologies. Biotechnol. Bioeng. 102, 457–467.

Kuo, C.-H., Lee, C.-K., 2009. Enhancement of enzymatic saccharification of cellulose by cellulose dissolution pretreatments. Carbohydr. Polym. 77, 41–46.

Lawther, J.M., Sun, R.C., Banks, W.B., 1996. Effects of extraction conditions and alkali type on yield and composition of wheat straw hemicellulose. J. Appl. Polym. Sci. 60, 1827–1837.

Lee, S.H., Doherty, T.V., Linhardt, R.J., Dordick, J.S., 2009. Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. Biotechnol. Bioeng. 102, 1368–1376.

Li, B., Asikkala, J., Filpponen, I., Argyropoulos, D.S., 2010. Factors affecting wood dissolution and regeneration of ionic liquids. Ind. Eng. Chem. 49, 2477–2484.

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H.V., Auer, M., Vogel, K.P., Simmons, B.A., Singh, S., 2010. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification. Bioresour. Technol. 101, 4900–4906.

Li, Q., He, Y.-C., Xian, M., Jun, G., Xu, X., Yang, J.-M., Li, L.-Z., 2009. Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment. Bioresour. Technol. 100, 3570–3575.

Li, X.M., Pan, X.J., 2010. Hydrogels based on hemicellulose and lignin from lignocellulose biorefinery: A mini-review. J. Biobased Mater. Bioenergy 4, 289–297.
Liu, B., Zhang, J., Liu, L., Hotchkiss, A.T., 2011. Preparation and properties of water and glycerol-plasticized sugar beet pulp plastics. J. Polym. Environ. 19, 559–567.

Liu, B., Zhang, J., Liu, L., Hotchkiss, A.T., 2011. Preparation and properties of water and glycerol-plasticized sugar beet pulp plastics. J. Polym. Environ. 19, 559–567.

Lopes, A.M.C, Joao, K.G., Morais, A.R.C., Bogel-Lukasik, E., Bogel-Lukasik, R., 2013. Ionic liquids as a tool for lignocellulosic biomass fractionation. Sustainable Chem. Processes 1, 1–31.

Lynd, L.R., Cushman, J.H., Nichols, R.J., Wyman, C.E., 1991. Fuel ethanol from cellulosic biomass. Science 251, 1318–1323.

Mikkonen, K. S., Tenkanen, M., 2012. Sustainable food-packaging materials based on future biorefinery products: Xylans and mannans. Trends Food Sci. Technol. 28, 90–102.

Mikkonen, K.S., Heikkinen, S., Soovre, A., Peura, M., Serimaa, R., Talja, R.A., Helen, H., Hyvonen, L., Tenkanen, M., 2009. Films from oat spelt arabinoxylan plasticized with glycerol and sorbitol. J. Appl. Polym. Sci. 114, 457–466.

Mikkonen, K.S., Yadav, M.P., Cooke, P., Willför, S., Hicks, K.B., Tenkanen, M., 2008. Films from spruce galactoglucomannan blended with poly (vinyl alcohol), corn arabinoxylan, and konjac glucomannan. BioResources 3, 178-191.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.

Mora-Pale, M., Meli, L., Doherty, T.V., Linhardt, R.J., Dordick, J.S., 2011. Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass. Biotechnol. Bioeng. 108, 1229–1245.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96, 673–686.

Nguyen, T.-A.D., Kim, K.-R., Han, S.J., Cho, H.Y., Kim, J.W., Park, S.M., Park, J.C., Sim, S.J., 2010. Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. Bioresour. Technol. 101, 7432–7438.

Ninomiya, K., Kamide, K., Takahashi, K., Shimizu, N., 2012. Enhanced enzymatic saccharification of kenaf powder after ultrasonic pretreatment in ionic liquids at room temperature. Bioresour. Technol. 103, 259–265.

Oliver, W.C., Pharr, G.M., 1992. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. J. Mater. Res. 7, 1564–1583.

Peroval, C., Debeaufort, F., Despre, D., Voilley, A., 2002. Edible arabinoxylan-based films. 1. Effects of lipid type on water vapor permeability, film structure, and other physical characteristics. J. Agric. Food Chem. 50, 3977–3983.

Pezoa, R., Cortinez, V., Hyvarinen, S., Reunanen, M., Hemming, J., Lienqueo, M.E., Salazar, O., Carmona, R., Garcia, A., Murzin, D.YU., Mikkola, J.P., 2010. The use of ionic liquids in the pretreatment of forest and agricultural residues for the production of bioethanol. Cellulose Chem. Technol. 44, 165–172.

Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert, C.A., Frederick Jr., W.J., Hallett, J.P., Leak, D.J., Liotta, C.L., Mielenz, J.R., Murphy, R., Templer, R., Tschaplinski, T., 2006. The path forward for biofuels and biomaterials. Science 311, 484–489.

Remsing, R.C., Swatloski, R.P., Rogers, R.D., Moyna, G., 2006. Mechanism of cellulose dissolution in the ionic liquid 1-n-butyl-3- methylimidazolium chloride: a ^{13}C and $^{35/37}Cl$ NMR relaxation study on model systems. Chem. Commun. 2006, 1271–1273.

Rouilly, A., Geneau-Sbartaï, C., Rigal, L, 2009. Thermo-mechanical processing of sugar beet pulp. III. Study of extruded films improvement with various plasticizers and cross-linkers. Bioresour. Technol. 100, 3076–3081.

Rouilly, A., Jorda, J., Rigal, L., 2006a. Thermo-mechanical processing of sugar beet pulp. I. Twin-screw extrusion process. Carbohydr. Polym. 66, 81–87.

Rouilly, A., Jorda, J., Rigal, L., 2006b. Thermo-mechanical processing of sugar beet pulp. II. Thermal and rheological properties of thermoplastic SBP. Carbohydr. Polym. 66, 117–125.

Ryberg, Y.Z.Z, Edlund, U., Albertsson, A.C., 2011. Conceptual approach to renewable barrier film design based on wood hydrolysate. Biomacromolecules 12, 1355–1362.

Samayam, I.P., Hanson, B.L., Langan, P., Schall, C.A., 2011. Ionic-Liquid induced changes in cellulose structure associated with enhanced biomass hydrolysis. Biomacromolecules 12, 3091–3098.

Sannigrahi, P., Pu, Y., Ragauskas, A., 2010. Cellulosic biorefineries-unleashing lignin opportunities. Curr. Opin. Environ. Sustainability, 2, 383–393.

Selig, M., Weiss, N., Ji, Y., 2008. Enzymatic saccharification of lignocellulosic biomass. Laboratory analytical procedure (LAP). Technical report, NREL/TP-510-42629. National Renewable Energy Laboratory, Golden, CO.

Silverstein, R.A., Chen, Y., Sharma-Shivappa, R.R., Boyette, M.D., Osborne, J., 2007. A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresour. Technol. 98, 3000–3011.

Singh, S., Simmons, B.A., Vogel, K.P., 2009. Visualization of biomass solubilization and cellulose regeneration during ionic liquid pretreatment of switchgrass. Biotechnol. Bioeng. 104, 68 – 75.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2011. Laboratory analytical procedure (LAP). Technical report, NREL/TP-510-42618. National Renewable Energy Laboratory, Golden, CO.

Somerville, C., Youngs, H., Taylor, C., Davis, S.C., Long, S.P., 2010. Feedstocks for lignocellulosic biofuels. Science 329, 790-792.

Sopade, P.A., Gidley, M.J., 2009. A rapid *in-vitro* digestibility assay based on glucometry for investigating kinetics of starch digestion. Starch/Stärke 61, 245–255.

Sternemalm, E., Hoije A., Gatenholm, P., 2008. Effect of arabinose substitution on the material properties of arabinoxylan films. Carbohydr. Res. 343, 753–757.

Studer, M.H., DeMartini, J.D., Davis, M.F., Sykes, R.W., Davison, B., Keller, M., Tuskan, G.A., Wyman, C.E., 2011. Lignin content in natural populus variants affects sugar release. Proc. Natl. Acad. Sci. U. S. A. 108, 6300–6305.

Sun, N., Rahman, M., Qin, Y., Maxim, M.L., Rodriguez, H., Rogers, R.D., 2009. Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. Green Chem. 11, 646–655.

Sun, N., Rodríguez, H., Rahman, M., Rogers, R.D., 2011. Where are ionic liquid strategies most suited in the pursuit of chemicals and energy from lignocellulosic biomass? Chem. Comm. 47, 1405–1421.

Swatloski, R.P., Spear, S.K., Holbrey, J.D., Rogers, R.D., 2002. Dissolution of cellulose with ionic liquids. J. Am. Chem. Soc. 124, 4974–4975.

Tadesse, H., Luque, R. 2011. Advances on biomass pretreatment using ionic liquids: An overview. Energy Environ. Sci. 4, 3913–3929.

Tatlı, E., 2013. Pretreatment of peanut shells for co-production of glucose and concrete admixture, METU, M.Sc. Thesis.

Tatsumi, H., Katano, H., Ikeda, T., 2006. Kinetic analysis of enzymatic hydrolysis of crystalline cellulose by cellobiohydrolase using an amperometric biosensor. Anal. Biochem. 357, 257–261.

Toraman, H.E., 2012. Investigation of alkaline pretreatment parameters on a multi-product basis for the co-production of glucose and hemicellulose based films from corn cobs, METU, M.Sc. Thesis.

Turner, M.B., Spear, S.K., Huddleston, J.G., Holbrey, J.D., Rogers, R.D., 2003. Ionic liquid salt-induced inactivation and unfolding of cellulase from *Trichoderma reesei*. Green Chem. 5, 443–447.

U.S. Department of Energy Genomic Science Program. http://genomicscience.energy.gov. Last accessed date: 2 September 2013.

Vidal Jr, B.C., Dien, B.S., Ting, K.C., Singh, V., 2011. Influence of feedstock particle size on lignocellulose conversion – A review. App. Biochem. Biotechnol. 164, 1405–1421.

Vroman, I., Tighzert, L., 2009. Biodegradable polymers. Materials 2, 307-344.

Waltz, E., 2008. Cellulosic ethanol booms despite unproven business models. Nat. Biotechnol. 26, 8–9.

Wang, J., 2008. Electrochemical glucose biosensors. Chem. Rev. 108, 814–825.

Westbye, P., Kohnke, T., Glasser, W., Gatenholm, P., 2007. The influence of lignin on the self-assembly behaviour of xylan rich fractions from birch (*Betula pendula*). Cellulose 14, 603–613.

Wu, H., Mora-Pale, M., Miao, J., Doherty, T.V., Linhardt, R.J., Dordick, J.S., 2011. Facile pretreatment of lignocellulosic biomass at high loadings in room temperature ionic liquids. Biotechnol. Bioeng. 108, 2865–2875.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R., Lee, Y.Y., 2005. Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover. Bioresour. Technol. 96, 2026–2032.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R., Lee, Y.Y, Mitchinson, C., Saddler, J.N., 2009. Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies. Biotechnol. Prog. 25, 333–339.

Xylophane AB. http://www.xylophane.com. Last accessed date: 2 September 2013

Yang, B., Wyman, C.E., 2006. BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. Biotechnol. Bioeng. 94, 611–617.

Yang, B., Wyman, C.E., 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels Bioprod. Bioref. 2, 26–40.

Ying, R., Saulnier, L., Rondeau-Mouro, C., 2011. Films of arabinoxylans and β-glucans extracted from cereal grains: Molecular motions by TD-NMR. Carbohydr. Polym. 86, 812-822.

Yuan, T.Q., Xu, F., Sun, R.C., 2013. Role of lignin in a biorefinery: separation characterization and valorization. J. Chem. Technol. Biotechnol. 88, 346–352.

Zavrel, M., Bross, D., Funke, M., Büchs, J., Spiess, A.C., 2009. High-throughput screening for ionic liquids dissolving (ligno-)cellulose. Bioresour. Technol. 100, 2580–2587.

Zhang, P.Y., Whistler, R.L., 2004. Mechanical properties and water vapor permeability of thin film from corn hull arabinoxylan. J. Appl. Polym. Sci. 93, 2896–2902.

Zhang, Y.H.P., 2008. Reviving the carbohydrate economy via multi-product lignocellulose biorefineries. J. Ind. Microbiol. Biotechnol. 35, 367-375.

Zhang, Y.H.P., Lynd, L.R., 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Biotechnol. Bioeng. 88, 797–824.

Zhao, H., Jones, C.L., Baker, G.A., Xia S., Olubajo, O., Person, V.N., 2009. Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis. J. Biotechnol. 139, 47–54.

Zhu, J.Y., Pan, X.J., 2010. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation. Bioresour. Technol. 101, 4992–5002.

Zilliox, C., Debeire, P., 1998. Hydrolysis of wheat straw by a thermostable endoxylanase: Adsorption and kinetic studies. Enzyme Microb. Technol. 22, 58–63.

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PUBLICATIONS

Peer-reviewed Publications Indexed in SCI

Bahçegül, E., Akınalan, B., Toraman, H.E., Erdemir, D., Özkan, N., Bakır, U., 2013. Extrusion of xylans extracted from corn cobs into biodegradable polymeric materials. Bioresource Technology, In Press.

Haykır, N. I., Bahçegül, E., Bıçak, N., Bakır, U., 2013. Pretreatment of cotton stalk with ionic liquids including 2-hydroxy ethyl ammonium formate to enhance biomass digestibility. Industrial Crops and Products, 41, 430-436.

Bahçegül, E., Apaydın, S., Haykır, N. I., Tatlı, E., Bakır, U., 2012. Different ionic liquids favor different lignocellulosic biomass particle sizes during pretreatment to function efficiently. Green Chemistry, 14, 1896-1903.

Bahçegül, E., Toraman, H. E., Özkan, N., Bakır, U., 2012. Evaluation of alkaline pretreatment temperature on a multi-product basis for the co-production of glucose

and hemicellulose based films from lignocellulosic biomass. Bioresource Technology, 103, 440-445.

Bahçegül, E., Tatlı, E., Haykır, N. I., Apaydın, S., Bakır, U., 2011. Selecting the right blood glucose monitor for the determination of glucose during the enzymatic hydrolysis of corncob pretreated with different methods. Bioresource Technology, 102, 9646-9652.

Other Publications

Bahçegül, E., 2011. Tarımsal atıkların çevre dostu plastiklere dönüşümü. Bilim ve Teknik, 521, 68 – 73.

AWARDS

Best poster award (Biorefineries and industrial materials category). 18th European Biomass Conference and Exhibition, 3 – 7 May 2010, Lyon/France. "Preparation of biodegradable hemicellulose-based films from agricultural biomass" (with N. Özkan and U. Bakır).

REFERENCES

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