INVESTIGATION OF ALKALINE PRETREATMENT PARAMETERS ON A MULTI-PRODUCT BASIS FOR THE CO-PRODUCTION OF GLUCOSE AND HEMICELLULOSE BASED FILMS FROM CORN COBS

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INVESTIGATION OF ALKALINE PRETREATMENT PARAMETERS ON A MULTI-PRODUCT BASIS FOR THE CO-PRODUCTION OF GLUCOSE AND HEMICELLULOSE BASED FILMS FROM CORN COBS

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ABSTRACT

INVESTIGATION OF ALKALINE PRETREATMENT PARAMETERS ON A MULTI-PRODUCT BASIS FOR THE CO-PRODUCTION OF GLUCOSE AND HEMICELLULOSE BASED FILMS FROM CORN COBS

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There is an increasing trend in the world for using renewable sources of fuels and chemicals due to the continuous depletion of fossil fuel reserves besides the environmental issues related with the exploitation of these resources. Lignocellulosic biomass is seen as the most promising candidate to be used instead of fossil sources because of its availability, relatively low price and less competition with food and feed crops. In this study, corn cobs, a lignocellulosic agricultural waste, were subjected to alkaline pretreatment for the co-production of glucose and hemicellulose based films with a multi-product approach in order to diversify the product range and to increase the revenues of the process. The pretreatment applied to lignocellulosic agricultural waste has a significant impact on the quantities and properties of the products that can be produced from the lignocellulosic feedstock upon pretreatment. Within the context of this study, the parameters utilized during the alkaline pretreatment of corn cobs were investigated in terms of their effect on the amount of glucose obtained through the enzymatic hydrolysis of the cellulosic portion and on the mechanical properties of the films obtained through the solvent casting of the hemicellulosic portion of corn cob. The pretreatment parameters including the alkaline type and concentration, addition and type of boron compound as well as the duration of pretreatment, were optimized with respect to the amounts and the properties of the products. Following the pretreatments conducted with 24 % KOH and 1% $NaBH_4$, which were the initial pretreatment parameters in the study, a glucose yield of 22 % and a tensile energy to break of 2.1 MJ/m^3 were obtained. Upon the optimization of the pretreatment procedure, the optimum pretreatment conditions were determined as 5 % NaOH, 1 % $NaBH_4$ and 3 hours and a glucose yield of approximately 31% and a tensile energy to break of around 1.7 MJ/m^3 were obtained.

Keywords: Lignocellulosic, Pretreatment, Hemicellulose, Cellulose, Film, Glucose, Cellulases

ÖZ

MISIR KOÇANINDAN GLİKOZ VE HEMİSELÜLOZ TEMELLİ FİLMLERİN BERABER ÜRETİMİNE YÖNELİK ÇOKLU ÜRÜN TEMELİNE DAYANAN ALKALİ ÖN İŞLEM PARAMETRELERİNİN İNCELENMESİ

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Dünyada fosil yakıtların kullanılmasının bir sonucu olarak ortaya çıkan çevresel sorunların yanında fosil yakıt rezervlerinin sürekli bir biçimde azalmasından dolayı yenilenebilir yakıt ve kimyasalların kullanımına yönelik giderek artan bir eğilim bulunmaktadır. Lignoselülozik biyokütle bulunabilirliği, maliyetinin nispeten daha düşük olması ve tüketim amacı açısından gıda ve besi tahıllarıyla daha az rekabet içinde olması gibi özelliklerinden dolayı fosil kaynakların yerine kullanılabilecek en umut verici aday olarak görülmektedir. Bu çalışmada, ürün çeşitliliğini ve sürecin karlılığını arttırmak için tarımsal bir lignoselülozik atık olan mısır koçanı çoklu ürün yaklaşımıyla glikoz ve hemiselüloz temelli filmlerin üretiminin gerçekleştirilmesi için alkali temelli ön işleme tabi tutulmuştur. Lignoselülozik tarımsal atıklara uygulanan ön işlemin lignoselülozik hammaddeden üretilebilen ürünlerin miktar ve özellikleri üzerinde büyük bir etkisi bulunmaktadır. Bu çalışma kapsamında, mısır koçanlarına uygulanan alkali ön işleminin gerçekleştirilmesi sırasında kullanılan parametrelerin mısır koçanının hem selülozik kısmına uygulanan enzimatik hidroliz yoluyla elde edilen glikoz miktarına ve hem de hemiselülozik kısmına uygulanan çözelti dökme yöntemiyle elde edilen filmlerin mekanik özelliklerine olan etkisi incelenmiştir. Alkali tipi, alkali konsantrasyonu, bor bileşiğinin eklenmesi, bu eklenen bileşiğin tipi ve aynı zamanda da ön işlemin süresini kapsayan ön işlem parametreleri ürünlerin miktarları ve özelliklerine göre optimize edilmiştir. Bu çalışmada öncül parametreler olan % 24 KOH and % 1 $NaBH_4$ ile yapılan ön işlemlerin sonucunda % 22'lik bir glikoz verimi ve 2.1 MJ/m^3'lük bir kopma enerjisi elde edilmiştir. Ön işlem prosedürünün optimizasyonuna bağlı olarak, optimum ön işlem koşulları % 5 NaOH, % 1 $NaBH_4$ ve 3 saat olarak belirlenmiştir ve yaklaşık % 31'lik bir glikoz verimi ile 1.7 MJ/m^3'lük bir kopma enerjisi elde edilmiştir.

Anahtar Kelimeler: Lignoselülozik, Ön İşlem, Hemiselüloz, Selüloz, Film, Glikoz, Selülazlar

To my beloved family,

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LIST OF ABBREVIATIONS

AIL: Acid insoluble lignin

AIL: Acid soluble lignin

E: Young's modulus

e_B : Elongation at break

g : Grams

L : Liter

ml : Mililiter

TEB: Tensile energy to break

UTS: Ultimate tensile strength

wt % : weight %

w/v : weight/volume

CHAPTER 1

INTRODUCTION

Production processes based on renewable resources has become one of the most important research subjects of today, especially upon the continuous depletion of non-renewable resources such as petroleum. The dependence of products such as fuels and plastics to petroleum causes the need for the production of these products from renewable resources to emerge. The negative environmental impacts of petroleum based products during or after their usage also bring up the development of alternative environmentally friendly products. Biomass is one of the most promising alternatives by providing a renewable source for the production of chemicals, materials and energy (Cherubini et al., 2009).

1.1 Biorefinery

The traditional oil refinery takes crude oil and refines it into products that may be used as transport fuel, electricity, commodity and high value chemicals (approximately 5% by volume). This technology is considered as advantageous because it is highly predictable and is not based on the seasonality of supply. Traditional oil refineries are also highly optimized and are thought to make use of "mature technologies". On the other hand, there are major disadvantages that have become obvious in recent years such as volatility in price, security of supply, competition from developing markets for a limited reserve and the production of atmospheric contamination and greenhouse gases. Therefore, it could be said that the world needs feedstocks that are extensively accessible, relatively cheaper, renewable and can be produced and managed in a sustainable fashion. It is indubitable that biomass can satisfy these necessities (Cherubini et al., 2009).

Several definitions for biorefinery are currently being used based on the type of activity and shareholders involved (Cherubini et al., 2009). Some examples are (Van Ree and Annevelink, 2007):

- “A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass. The biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum” (NREL, 2012).

- “A facility that processes and converts biomass into value-added products. These products can range from biomaterials to fuels such as ethanol or important feedstocks for the production of chemicals and other materials. Biorefineries can be based on a number of processing platforms using mechanical, thermal, chemical, and biochemical processes” (DOE, 2012).

- “Biorefining is the transfer of the efficiency and logic of fossil-based chemistry and substantial converting industry as well as the production of energy onto the biomass industry” (Kamm et al., 2006).

- “Biorefineries are integrated bio-based industries, using a variety of technologies to produce chemicals, biofuels, food and feed ingredients, biomaterials (including fibres) and power from biomass raw materials” (EU Biorefinery Euroview, 2007).

- “Biorefinery is efficient use of the entire potential of raw materials and by-streams of the forest-based sector towards a broad range of high added-value products (by co-operation in between chains)” (Biorefinery Taskforce FTP, 2007).

- “Biorefinery is the sustainable processing of biomass into a spectrum of marketable products and energy” (IEA Bioenergy task 42 on Biorefineries, 2007).

Seven different biorefinery concepts are recognized based on the definition of IEA Bioenergy task 42 on Biorefineries (2007) which are:

- Conventional Biorefineries (CBR)
- Green Biorefineries (GBR)
- Whole Crop Biorefineries (WCBR)
- Lignocellulosic Feedstock Biorefineries (LCFBR)
- Two Platform Concept Biorefineries (TPCBR)
- Thermochemical Biorefineries (TCBR)
- Marine Biorefineries (MBR)

The main characteristics of the biorefinery concepts in terms of type of feedstock and predominant technology can be seen below in Table 1.1.

Table 1.1 Summary of the characteristics of biorefinery concepts (Van Ree and Annevelink, 2007).

Type of biorefinery	Type of feedstock	Leading technology
Green Biorefineries	Green grasses and green crops such as clover and lucerne	Pretreatment, pressing, fractionation, separation, digestion
Whole Crop Biorefineries	Whole crop cereals such as wheat, rye and maize	Dry or wet milling, biochemical conversion
Lignocellulosic Feedstock Biorefineries	Lignocellulosic biomass such as straw, wood, corn stover, corn cob, cotton stalk	Pretreatment, enzymatic hydrolysis, fermentation, separation
Two Platform Concept Biorefineries	All kinds of biomass	Combination of sugar platform (biochemical conversion) and syngas platform (thermochemical conversion)
Thermochemical Biorefineries	All kinds of biomass	Thermochemical conversion
Marine Biorefineries	Aquatic biomass such as microalgae and macroalgea	Cell disruption, product extraction and separation

1.1.1 Lignocellulosic Feedstock Biorefineries (LCFBR)

The LCFBR depends on the fractionation of lignocellulosic biomass sources into the intermediate output streams which are cellulose, hemicellulose and lignin which can be further processed into a broad spectrum of bio-based end-products, fuels, chemicals, and power and/or heat. It is expected that these bio-based products will have a good place on both the petrochemical and the expected future bio-based markets. Lignocellulosic biomass is estimated to become the most important biomass source of the future because of its availability, relatively low price, and less competence with food and feed crops.

1.2 Lignocellulosic Biomass

Lignocellulose is the major building block of plant cell walls. Plant biomass is mostly made up of cellulose, hemicellulose, and lignin (Figure 1.1), together with relatively small amounts of protein, pectin, extractives (soluble nonstructural materials such as nonstructural sugars, chlorophyll, waxes and nitrogenous material), and ash (Jorgensen et al., 2007; Kumar et al., 2009). The composition of lignocellulosic biomass depends on its source and so it can vary from one plant species to another (Harmsen et al., 2010). The composition of lignocellulose in several sources can be seen in Table 1.2.

1.2.1 Corn Cob

The average corn production in the world is approximately 800 million tons and corn ranks second after wheat in terms of world grain production. The production of corn in Turkey is about 4.2 million tons in 2011 giving rise to approximately 0.63-0.84 million tons of corn cobs (Sokhansanj et al., 2002; www.tuik.gov.tr). In the United States which is the largest producer of corn in the world, the largest acreage crop is corn grain with an approximate annual harvest value of 84 million acres in 2011 (USDA-NASS). In Turkey, it ranks third after wheat and barley in terms of the grain production (Ministry of Food, Agriculture and Animal Husbandry-Turkey). Therefore, as an abundant agricultural by-product with low commercial value, corn cob seems as an ideal raw material to establish a cost-effective technology for conversion of lignocellulosic structures to value-added products.

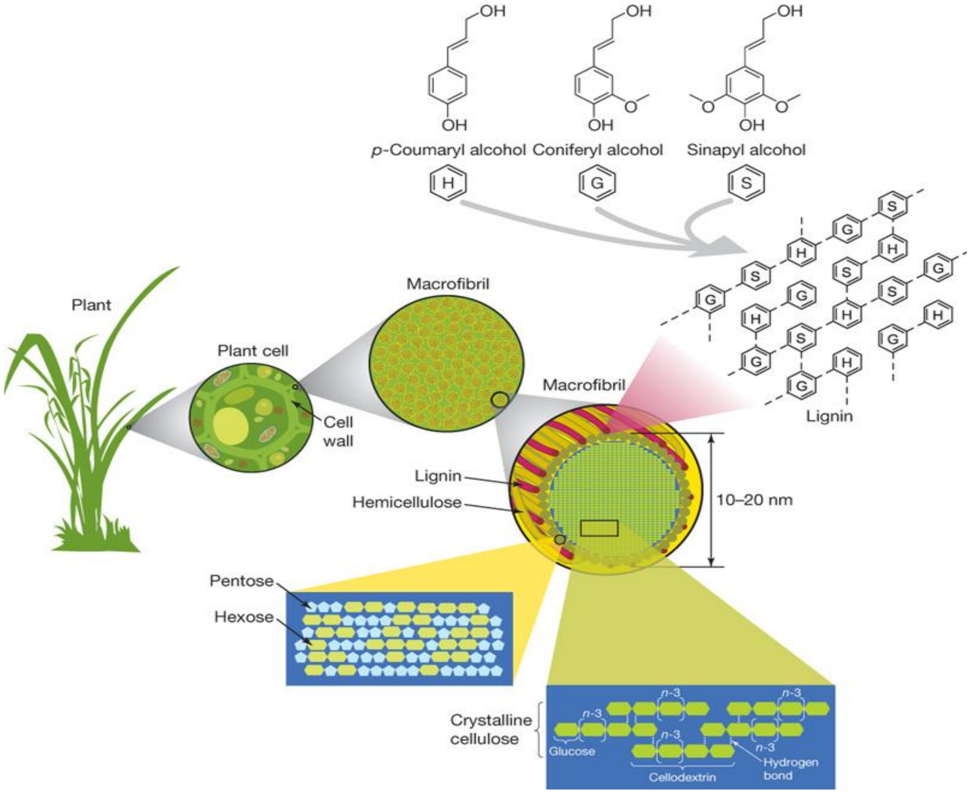

Figure 1.1 The structure of lignocellulose (Rubin, 2008)

Table 1.2 The composition of lignocellulosic material in common agricultural residues and wastes (Jorgensen et al., 2007; Kumar et al., 2009).

Lignocellulosic Material	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Wheat straw	30	50	15
Coastal bermudagrass	25	35.7	6.4
Switchgrass	45	31.4	12

1.2.2 Cellulose

Cellulose is the major structural constituent in plant cell walls. It is produced biosynthetically by photosynthetic higher plants, algae and nonphotosynthetic organisms such as certain bacteria, fungi, marine invertebrates, slime molds and amoebae (Jarvis, 2003; Lynd et al., 2002; Tomme et al., 1995; Zhang and Lynd, 2004).

The chemical formula of cellulose is $(C_6H_{10}O_5)_n$ and the structure is shown in Figure 1.2.

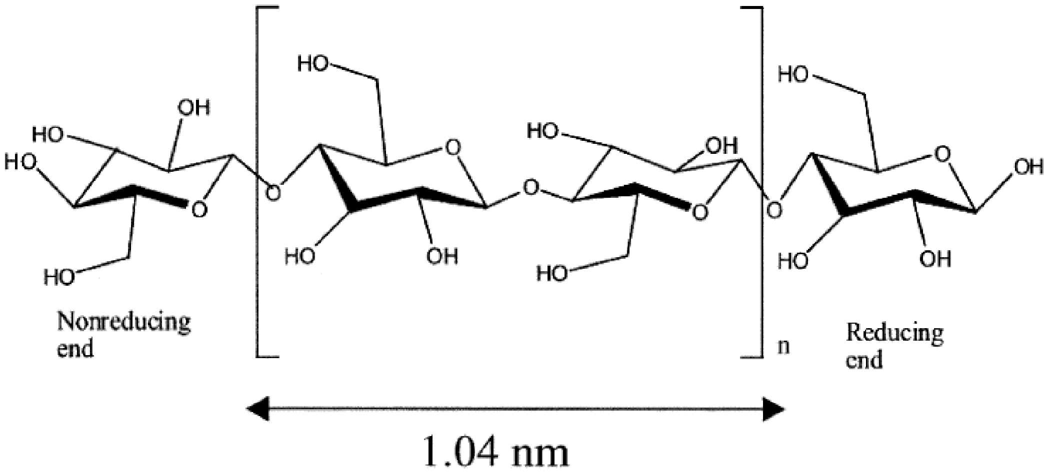

Figure 1.2 Structure of cellulose chain (Zhang and Lynd, 2004).

Cellulose is a linear condensation polymer in which there are β-1,4-glycosidic bonds linking D-anhydroglucopyranose molecules together. The repeating unit of cellulose is anhydrocellobiose since adjacent anhydroglucose molecules are rotated 180° with respect to their neighbors. As a result of this rotation, cellulose becomes symmetrical, since each side of the chain possesses an equal number of hydroxyl groups. Coupling interaction of adjacent cellulose molecules through van der Waal's forces and hydrogen bonds result in a crystalline structure and a parallel alignment. The hydrogen bonds of intrachain (2-3 per anhydroglucopyranose) and interchain (2 per anhydroglucopyranose) cause stable, straight supramolecular fibers with good tensile strength. Native cellulose is coined as cellulose I and there are two different crystalline forms of native cellulose. The first type of these crystalline native celluloses is I_{α}, and this native cellulose can be found in algal and bacterial cellulose. The second type is denoted as I_{β}, and this native cellulose can generally be found in higher plants (Zhang and Lynd, 2004). Conversion of cellulose I can take place towards other crystalline forms (II–IV) with the aid of various treatment methods (O'Sullivan, 1997; Zhang and Lynd, 2004).

Cellulose is made up of sheets of glucopyranose rings which lies in a plane with consecutive sheets fixed on top of each other to create a three-dimensional structure.

Many properties of cellulose are based on its degree of polymerization (DP), which is defined as the number of glucose units that constitute one polymer molecule. Degree of polymerization of cellulose can be as high as 17000, although generally the number of units present is 800-10000. For example, the wood pulp cellulose has a DP value of between 300 and 1700 (Harmsen et al., 2010).

Fermentable D-glucose can be obtained from cellulose by the action of either acid or enzymes breaking the β-(1,4)- glycosidic bonds. Cellulose exists in both crystalline and amorphous forms. It is mainly composed of crystalline cellulose together with small fraction of amorphous cellulose and it is more subject to enzymatic hydrolysis in its amorphous form (Beguin et al., 1994; Kumar et al., 2009).

Under normal conditions (20°C, 60% relative humidity), cellulose behaves as a moderately hygroscopic material absorbing 8-14% water. However, it is not soluble in water, in which it swells. Also, cellulose is not soluble in dilute acid solutions at low temperature. It becomes soluble at higher temperatures since sufficient energy to break the hydrogen bonding through the crystalline structure of the molecule is provided. Cellulose is also soluble in concentrated acids; however severe degradation of the lignocellulosic structure takes place in this case. In alkaline solutions extensive swelling of cellulose is observed besides dissolution of the low molecular weight portions of the polymer (DP < 200) (Harmsen et al., 2010; Krassig and Schurz, 2002). Cellulose also does not liquefy with temperature, but its decomposition begins at a temperature of 180°C (Thermowood handbook, 2003; Harmsen et al., 2010).

1.2.3 Hemicellulose

Hemicelluloses are the second most abundant polysaccharides in nature after cellulose. They are plant cell wall polysaccharides which are insoluble in water. However, they can be hydrolyzed by dilute acids or separated by aqeous alkali. Hemicelluloses have a heterogeneous structure of several sugar units based on the type of plant. They can be categorized as xylans (β-1,4-linked ᴅ-xylose units), mannans (β-1,4-linked ᴅ-mannose units), galactans (β-1,3-linked ᴅ-galactose units) and arabinans (β-1,5-linked L-arabinose units) (Sipiridon and Popa, 2008). The overall structure of hemicelluloses depends on a sugar backbone substituted with side chains. The linkages between structural units and the nature of the monosaccharide define some properties of hemicelluloses, such as three-dimensional conformation and solubility.

The main hemicelluloses of hardwood and softwood are given in Table 1.3.

Table 1.3 The main hemicelluloses in wood (Sipiridon and Popa, 2008).

Type of hemicellulose	**Hardwood**	**Softwood**
Methylglucuronoxylan	80-90	5-15
Arabinomethylglucuronoxylan	0.1-1	15-30
Glucomannan	1-5	1-5
Galactoglucomannan	0.1-1	60-70
Arabinogalactan	0.1-1	1-15
Other galactans	0.1-1	0.1-1
Pectins	1-5	1-5

Xylan is one of the main constituents (25–35%) of lignocellulosic structures (Ebringerova and Heinze, 2000). They are the main hemicelluloses in hardwood and they are also the predominant hemicellulose in cereals and annual plants. Its structure has a linear backbone consisting of β-1,4-linked ᴅ-xylopyranose residues. These may be replaced with branches of acetyl, arabinosyl and glucuronosyl residues (Figure 1.3). Hardwood xylan (*O*-acetyl-4 methyl-glucuronoxylan) is substituted at irregular intervals (Fig. 1.4) with 4- *O* -methyl- α- ᴅ -glucuronic acid groups linked to xylose by α-1,2-glycosidic linkages. Mostly every tenth xylose unit includes an uronic acid group connected at C2 or C3 of the xylopyranose (Sipiridon and Popa, 2008).

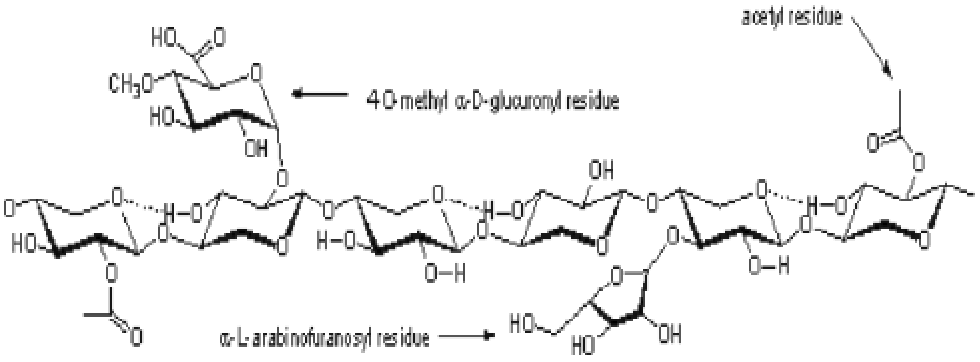

Figure 1.3 A schematic representation of the hemicellulose backbone of arborescent plants (Harmsen et al., 2010).

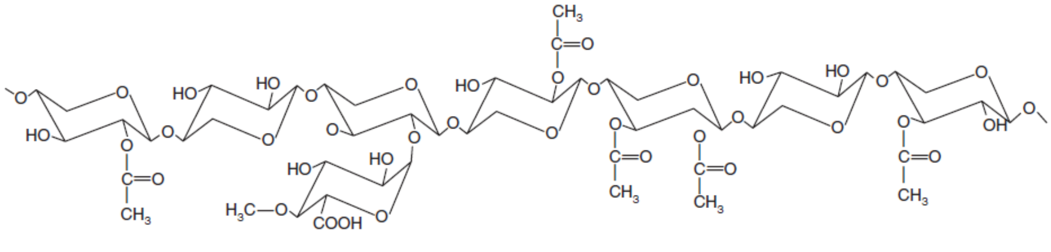

Figure 1.4 Structure of *O* -acetyl-(4-OMe-glucurono)xylan from hardwood (Sipiridon and Popa, 2008).

Arabinoxylans consist of α - L -arabinofuranose residues attached as branch-points to β-(1,4)-linked D–xylopyranose polymeric backbone chains (Figure 1.5). The arabinose residues may be attached to other groups, such as ferulic acid crosslinks and glucuronic acid residues (Smith and Hartley, 1983; Sipiridon and Popa, 2008).

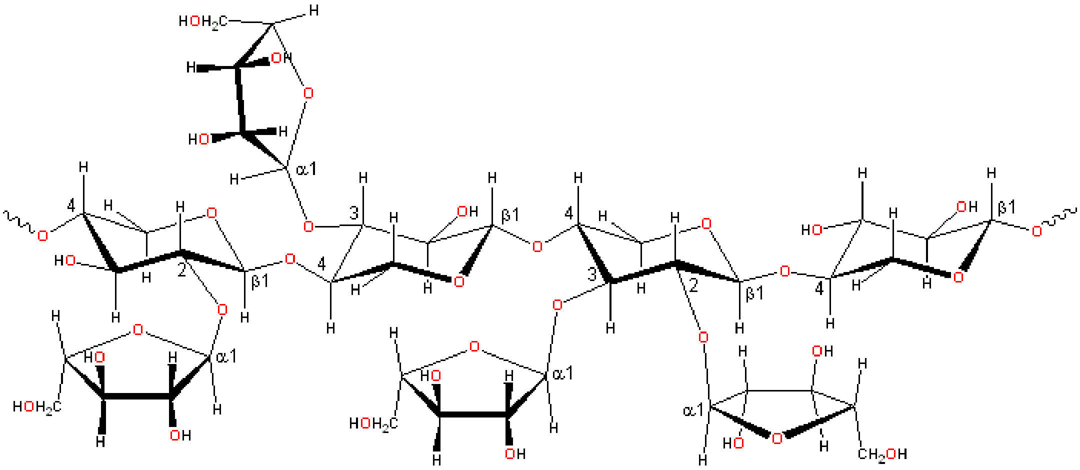

Figure 1.5 Structure of arabinoxylan: β-(1,4)-linked D–xylopyranose backbone substituted with α - L -arabinofuranose residues (www.lsbu.ac.uk).

The physicochemical features and functional characteristics of carbohydrate macromolecules depend on their degree of polymerization, molecular mass, macroscopic structure and branching.

Arabinoxylan or β-(1,4)-xylan polymers in an unsubstituted form have a tendency to aggregate into insoluble complexes which becomes stable through intermolecular hydrogen bonds (Andrewartha et al., 1979; Sipiridon and Popa, 2008). The polymer creates a three-fold, left-handed helix (Fincher and Stone, 1986; Sipiridon and Popa, 2008). Unsubstituted polymer parts show a tendency for interchain aggregation through H-bonding that is the most probable reason of low solubility of these polymers in water. The solubility of arabinoxylan macromolecules is enhanced through the existence of arabinose substituents which prevent intermolecular aggregation of unsubstituted xylose residues (Vinkx et al., 1996; Sipiridon and Popa, 2008).

Galactoglucomannan is the most abundant hemicellulose in softwood. Its backbone is established by β-1,4-linked D-glucopyranose D-mannopyranose units arbitrarily distributed in the main chain. Partial substitutions are possible with α -D-galactose side groups, which can be joined to both glucose and mannose units by an α-1,6-linkage (Sipiridon and Popa, 2008). Mannose/galactose ratio can differ from 1.0 to 5.3 based on the source of the polysaccharide (Dea and Morrison, 1975; Dey, 1978; Sipiridon and Popa, 2008).

Other hemicelluloses such as galactans, arabinans, arabinogalactans, etc are considered as pectic substances of plant cell walls. They are widely existed in the soft tissues of some fruit and sugar beet pulp, however less common in wood tissues (Sipiridon and Popa, 2008).

The important characteristic of hemicellulose is the lack of crystalline structure, essentially because of the highly branched structure, and the existence of acetyl groups linked to the polymer chain (Harmsen et al., 2010).

Hemicellulose is not soluble in water at low temperature but, the hydrolysis of hemicellulose begins at a lower temperature than that of cellulose, which makes it soluble at elevated temperatures (Harmsen et al., 2010; Thermowood handbook, 2003). The presence of acid enhances the solubility of hemicellulose in water (Harmsen et al., 2010).

1.2.4 Lignin

Lignin is one of the most abundant biopolymers exist in plants. Lignin builds up the cell wall together with cellulose and hemicelluloses, which is controlled on the nano-scale and leads to lignin–carbohydrate network structures. The molecular complexity of lignin makes all identification and isolation processes difficult and, so many structural queries still exist.

Lignin is a complex, amorphous and three dimensional molecular structure with cross-linked polymers of phenolic monomers. More specifically, p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Figure 1.6) are the ones generally encountered (Harmsen et al., 2010; Kumar et al., 2009).

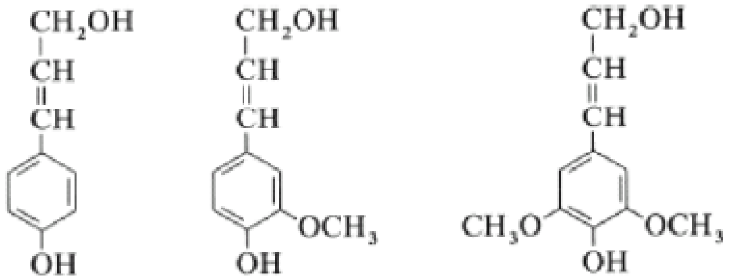

Figure 1.6 The dominant building blocks of the three-dimensional polymer lignin. From left to right: P-coumaryl-, coniferyl- and sinapyl alcohol, respectively (Harmsen et al., 2010).

The lignin from softwood is consisted of more than 90% of coniferyl alcohol with the residual being mostly p-coumaryl alcohol units. Unlike softwoods, the lignin from hardwood is consisted of changing ratios of coniferyl and sinapyl alcohol type of units (Harmsen et al., 2010).

Figure 1.7 represents a model structure of lignin from spruce pine.

Lignin in wood acts as an insoluble three-dimensional network. It plays a significant role in the cell's strength and development since it affects the transport of nutrients, water, and metabolites in the plant cell. Lignin is soluble in low molecular alcohols, acetone, pyridine, dioxane and dimethyl sulfoxide. Moreover, thermal softening of lignin occurs at elevated temperatures which accelerate depolymerization reactions (O’Connor et al., 2007; Harmsen et al., 2010).

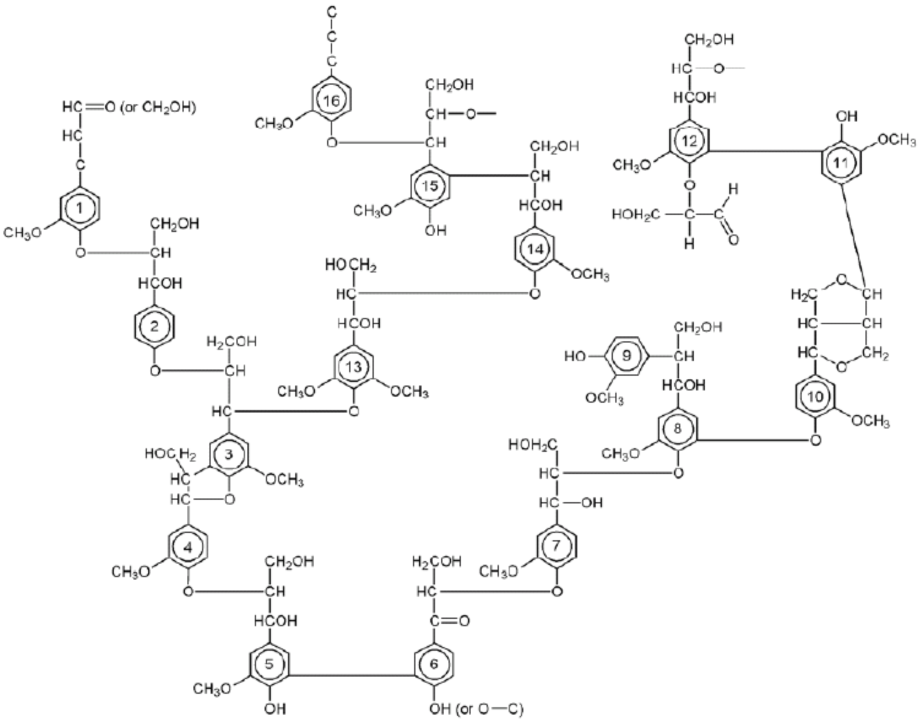

Figure 1.7 Model structure of spruce lignin (Harmsen et al., 2010).

1.3 Pretreatment Processes

Pretreatment is the key process in lignocellulose fractionation and utilization where it serves to enhance the product yields obtained via the subsequent processes by altering the lignocellulosic structure composed of cellulose, hemicellulose and lignin (Figure 1.8). Pretreatment has been acknowledged as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion (Carvalheiro et al., 2008; Taherzadeh and Karimi, 2008; Hendriks and Zeeman, 2009; Alvira et al., 20010; Harmsen et al., 2010). Several pretreatment schemes have been established in order to improve the reactivity of cellulose and increase the fermentable sugars yield (Brodeur et al., 2011). The aim of the pretreatment process is to separate cellulose, hemicellulose and lignin, reduce the crystallinity of cellulose and increase the porosity of the lignocellulosic structure. Other typical goals of pretreatment include (1) the formation of highly digestible solids to subsequently form sugars by enzymatic hydrolysis, (2) preventing the degradation of sugars together with those obtained from hemicellulose, (3) preventing the formation of inhibitors for succeeding enzymatic hydrolysis and fermentation processes, (4) recovery of lignin for conversion into value added products and (5) to be cost effective by minimizing heat and power requirements (Brodeur et al., 2011; Kumar et al., 2009).

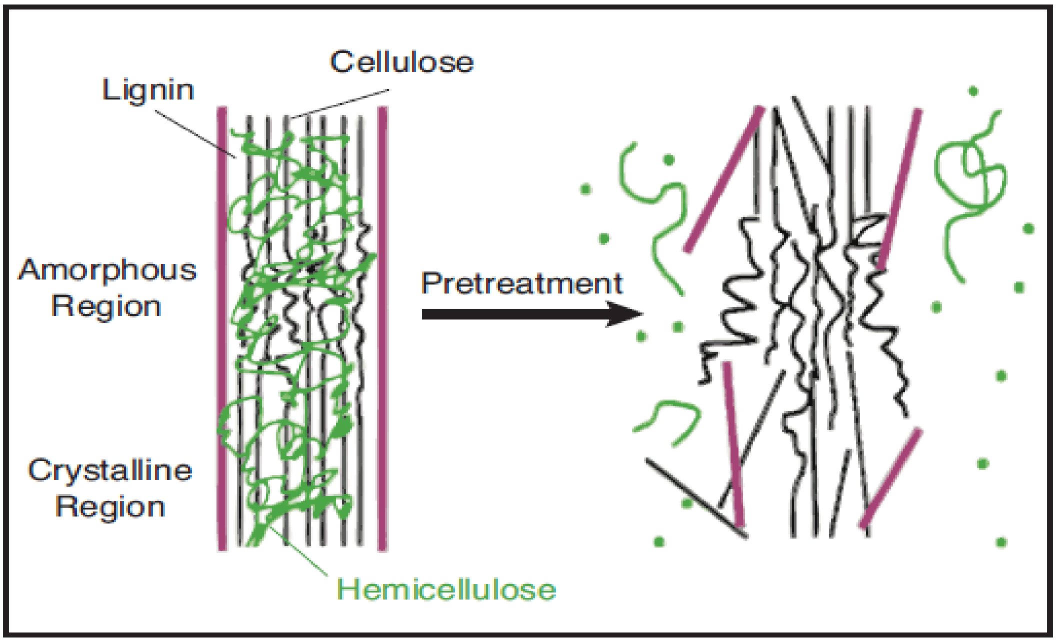

Figure 1.8 Schematic of goals of pretreatment on lignocellulosic biomass (Mosier et al., 2005).

Pretreatment processes are usually categorized into physical, chemical, physicochemical, and biological. Physical pretreatment includes the decrease of the size of biomass and crystallinity by grinding or milling. The reduction in crystallinity and enhanced mass transfer properties as a result of the decrease in particle size lead to improvement in enzymatic hydrolysis. The energy necessities for physical pretreatments are based on the degree of reduction in crystallinity and the final particle size of the lignocellulosic biomass. Physical pretreatment is an expensive method and so it will not be preferred in a full-scale process. Biological pretreatment includes the usage of microorganisms (mainly fungi) in order to degrade hemicellulose and lignin but leave the cellulose as undegraded (Shi et al., 2008; Sanchez, 2009; Brodeur et al., 2011). Lignin degradation takes place with the aid of the activity of lignin degradation enzymes that the fungi release. Despite the fact that biological pretreatments include mild process conditions and are of relatively low cost, the disadvantages are the low rates of enzymatic hydrolysis and long pretreatment times needed compared to other pretreatment processes (Sun and Cheng, 2002; Brodeur et al., 2011). Nowadays studies based on the development of effective processes which combine biological pretreatments with other pretreatments and novel microorganisms for fast hydrolysis are widely investigated (Sanchez and Cardona, 2008; Sanchez, 2009; Brodeur et al., 2011).

1.3.1 Physicochemical Pretreatments

1.3.1.1 Steam Explosion

One of the most widely preferred pretreatment methods is steam explosion pretreatment since it utilizes chemical and physical methods to disrupt the lignocellulosic structure (Brodeur et al., 2011). In this method, high-pressure saturated steam is applied to the biomass, and rapid reduction of the pressure follows, by the help of which the biomass undergoes an explosive decompression. Steam explosion is usually started at a temperature of 160-260°C (corresponding pressure, 0.69-4.83 MPa) for some seconds to a few minutes before the atmospheric pressure is applied to the biomass (Sun and Cheng, 2002; Kumar et al., 2009) The biomass/steam mixture is held for an amount of time to enhance hydrolysis of hemicellulose, and the process is completed by an explosive decompression. The process leads to lignin transformation and hemicellulose degradation because of high temperature, so it enhances the potential of cellulose hydrolysis. Hemicellulose is considered to be hydrolyzed by acetic and other acids released throughout the steam-explosion pretreatment process (Kumar et al., 2009). Water, itself, also acts as an acid at high temperatures (Baugh et al., 1988a,b; Weil et al., 1997; Mosier et al., 2005).

Steam is extremely useful in quickly heating the cellulosic materials up to the target temperature and at the same time, the resulting sugars are not diluted excessively. The release of rapid pressure leads to a reduction in temperature and terminates the reaction at the end of the pretreatment process. The rapid thermal expansion which is utilized to stop the reaction, leads to the opening up of the structure of biomass, however improvement in the digestibility of cellulose in the pretreated solid can only be faintly associated with this physical effect (Biermann et al., 1984; Brownell et al., 1986; Mosier et al., 2005).

1.3.1.2 Liquid Hot Water (LHW)

Similar to the steam explosion process, liquid hot water (LHW) pretreatment utilizes water at high pressures and elevated temperatures in order to keep its liquid form that stimulates separation and disintegration of the lignocellulosic structure. Liquid hot water pretreatment temperatures can be between 160°C to 240°C and pretreatment time can range from a few minutes to an hour. Temperature has a strong effect on the types of sugar obtained and pretreatment time is effective on the amount of sugar obtained (Yu et al., 2010a; Brodeur et al., 2011). The process mechanism of liquid hot water pretreatment is very similar to the mechanism of steam explosion pretreatment except the rapid decompression (Kobayashi et al., 2009; Ingram et al., 2009; Yu et al., 2010a; Brodeur et al., 2011). In this process, it was aimed to solubilize hemicellulose and separate it from the lignocellulosic structure while decreasing the amount of inhibitors formed. Reactive cellulose fibers are produced for the subsequent production of pentosans and the lignocellulosic structure is disrupted through the cell diffusion of the biomass by the water (Yu et al., 2010b; Kim et al., 2009; Brodeur et al., 2011).

Water pretreatment decreases the requirement for neutralization because acid is not supplemented. Size reduction of the lignocellulosic biomass is not required because the lignocellulosic structure is disrupted when heated in water (Kohlman et al., 1995; Weil et al., 1997). A highly digestible cellulose can be produced when enzyme is added (Weil et al., 1998a,b; Mosier et al., 2003a,b), and high sugar yields from hemicellulose can be obtained throughout this pretreatment process. (Mosier et al., 2003a,b, 2005).

1.3.1.3 Ammonia Fiber Explosion (AFEX)

The ammonia fiber/freeze explosion (AFEX) process is a very similar process to steam explosion pretreatment, in which the lignocellulosic material is exposed to liquid anhydrous ammonia under moderate temperatures and high pressures and is then depressurized rapidly. The temperatures (60°C to 100°C) are considerably less than that of the steam explosion pretreatment process. Therefore, less energy is required compared to steam explosion pretreatment process. The degree of disruption to the lignocellulosic structure is based on temperature since it will affect the speed of the ammonia vaporization inside the reactor for the period of depressurization. The pretreatment time mostly varies between 5 minutes to 30 minutes (Brodeur et al., 2011).

The ammonia freeze explosion pretreatment decreases the amount of lignin and discards some hemicellulose simultaneously while decrystallizing cellulose. Therefore it has a strong effect on the micro-and macro-accessibility of the cellulases to the cellulose. Liquid ammonia leads to cellulose swelling and it is thought that the carbohydrate is become more accessible by the ammonlolysis of glucuronic cross-links (Mosier et al., 2005).

1.3.1.4 Ammonia Recycle Percolation (ARP)

Ammonia recycle percolation (ARP) has been combined with the AFEX pretreatment process by many scientists; however it has some different features. Longer reactions times and reasonably high temperatures (140 °C to 210 °C) are required in this process as compared to AFEX process that results in higher energy costs (Kim et al., 2006; Brodeur et al., 2011).

The advantage with this process over AFEX is its capability to solubilize more than half of the hemicellulose (50–60%) and to discard most of the lignin (75–85%) without loss of cellulose content (Kim and Lee, 2005; Brodeur et al., 2011). This is achieved because of the selectivity of ammonia and its capability to disrupt lignin by ammonolysis while solubilizing hemicellulose above the longer pretreatment times. This process also decreases the production of inhibitors to some extent (Mes-Hartree et al., 1988; Brodeur et al., 2011). High liquid loadings and energy costs are some of the main disadvantages that need to be solved before this process is confirmed to be cost effective.

1.3.1.5 Supercritical Fluid (SCF) Pretreatment

A supercritical fluid is a substance which is neither liquid nor gas and utilized in a state above the critical pressure and temperature. It demonstrates exceptional properties which do not resemble the properties of gases or liquids under standard conditions. Since a supercritical fluid shows gas-like transport properties of viscosity and diffusivity and besides, a liquid like density, it is capable of entering the gaps within the crystalline structure of lignocellulosic biomass which overwhelms the mass transfer limitations faced with other pretreatments. Super critical carbon dioxide (T_c=31°C and P_c=7.4 MPa) is seen as an important solvent for the pretreatment of different types of lignocellulosic materials. When it is used together with water, it forms carbonic acid which enhances the hydrolysis of polymer. When the biomass is pretreated, explosive release of CO_2 breaks down the cellulose and hemicellulose structure, so increasing the accessible surface area for hydrolysis. The lower temperature utilized in the process supports the stability of the sugars and prevents degradation observed in other types of pretreatment processes (Brodeur et al., 2011).

1.3.2 Chemical Pretreatments

1.3.2.1 Wet Oxidation

Wet oxidation uses oxygen as an oxidizing agent for the compounds dissolved in water. There are two reactions that take place throughout this process. One is a high temperature oxidation reaction and the other is a low temperature hydrolysis reaction (Martin et al., 2007; Brodeur et al., 2011). Wet oxidation process includes drying and milling lignocellulosic material in order to get particles which are 2 mm in length, to which water is added at a ratio of 1 L to 6 g biomass. A compound which is usually Na_2CO_3, is added to the mixture reducing the formation of byproducts. Air is pumped into the pretreatment vessel until a pressure of 12 bars is obtained. This pretreatment process is conducted at 195 °C for a range of between 10 to 20 minutes (Bjerre et al., 1996; Martin et al., 2008; Pedersen and Meyer, 2009; Brodeur et al., 2011). Wet oxidation is utilized to fractionate biomass by means of removing lignin and solubilizing hemicellulose (Martin et al., 2007; Banerjee et al., 2009; Brodeur et al., 2011).

1.3.2.2 Acid

Acid pretreatment includes the use of acids for the breakdown of the lignocellulosic structure. Dilute sulphuric acid (H_2SO_4) is the most widely used acid and it has been commercially used for the pretreatment of various types of biomass such as switchgrass (Digman et al., 2010; Li et al., 2010), corn stover (Xu et al., 2009; Du et al., 2010), spruce (softwood) (Shuai et al., 2010), and poplar (Wyman et al., 2009). There are also some other acids which are being used for the pretreatment of lignocellulosic materials, such as phosphoric acid (H_3PO_4) (Zhang et al., 2007; Marzialetti et al., 2008), nitric acid (HNO_3) (Himmel et al., 1997) and hydrochloric acid (HCl) (Wang et al., 2010). Acid pretreatment has been applied as a part of the overall fractionation process because of its ability to discard hemicellulose effectively (Zhang et al., 2007). Combination of acid pretreatment with alkaline pretreatment leads to obtain relatively pure cellulose because of the effective removal of hemicellulose and lignin (Brodeur et al., 2011).

Acid pretreatment generally involves the addition of acid solution (typically between 0.2% to 2.5% w/w) to the biomass and then followed by a continuous mixing at a temperature range between 130 °C and 210 °C. The pretreatment time will be in the range of minutes to hours based on the pretreatment conditions (Esteghlalian et al., 1997; Nguyen et al., 2000; Saha et al., 2005; Sun and Cheng, 2005; Silverstein et al., 2007; Sassner et al., 2008; Brodeur et al., 2011).

The most important advantage of acid pretreatment is that a subsequent enzymatic hydrolysis step is occasionally not necessary since the acid itself hydrolyses the biomass to produce fermentable sugars. Minimum degradation takes place during the solubilization of hemicellulose and lignin (Carrasco et al., 1994; Brodeur et al., 2011), and fermentable sugars are obtained from the hydrolysis of hemicellulose with acid pretreatment. Nevertheless, extensive washing and/or a detoxification step (Saha et al., 2005; Brodeur et al., 2011) is needed in order to get rid of the acid before a fermentation process (Nguyen et al., 2000; Sassner et al., 2008; Brodeur et al., 2011). Another disadvantage is that fermentation inhibitors like furfural and hydroxymethyl furfural (HMF) are produced that decreases the efficiency of the pretreatment process and further processes (Saha et al., 2005; Sun and Cheng et al., 2005; Brodeur et al., 2011).

1.3.2.3 Ionic Liquids

Pretreatment of lignocellulosic materials with ionic liquids has become important in the last decade because of the capability of them to dissolve a wide range of biomass types and tunability of the solvent chemistry. Ionic liquids are liquid under normal conditions and they are consisted of a large organic cation and a small anion. Ionic liquids have very low vapor pressure. The chemistry of the anion and cation can be altered to produce a wide range of liquids which can dissolve various types of lignocellulosic materials such as corn stover (Cao et al., 2010), switchgrass (Samayam and Schall, 2010), cotton (Zhao et al., 2009), wheat straw (Li et al., 2009), bagasse (Wang et al., 2009), and woods (Fort et al., 2007). Ionic liquids and similar solvents are recoverable more than 99% because of their low vapor pressure that leads to a reduction in the solvent cost. They are defined as green solvents because no toxic products are obtained during the pretreatment process and they are recoverable.

Ionic liquid pretreatment involves the dissolution of lignocellulosic material in the solvent at ambient pressures and temperatures of between 90 ˚C and 130 ˚C for a time range of between 1 hour and 24 hours (Zhu et al., 2006; Samayam and Schall, 2010; Brodeur et al., 2011). The lignocellulosic material is then reprecipitated by the addition of water and washed before enzymatic hydrolysis. Hydrogen bonds are formed between the anion of the ionic liquid and cellulose in a 1:1 ratio and the hydrogen bonded crystalline structure of cellulose becomes disrupted. As a result of this, more amorphous cellulose is obtained which is more susceptible to enzymatic hydrolysis. Furthermore, the chemistry of the ionic liquids can be altered in order to dissolve lignin and hemicellulose so it can be said that ionic liquids are suitable to dissolve different constituents of lignocellulosic materials. It should also be stated that ionic liquids do not degrade the chains and reduce the degree of polymerization while dissolving cellulose. The structure of hemicellulose and lignin was also proved not to be altered after the pretreatment process conducted with ionic liquids (Zhu et al., 2006; Wyman et al., 2009; Samayam and Schall, 2010; Brodeur et al., 2011). However, there is such a limitation for the use of ionic liquids that they tend to inactivate cellulase. This inactivation was irreversible. Therefore, cellulose should be regenerated after the pretreatment process and all traces of the ionic liquid should be removed before the enzymatic hydrolysis step. This makes a regeneration and separation step as necessary and prevents the development of a single step continuous pretreatment process for the conversion of lignocellulosic materials with ionic liquids (Brodeur et al., 2011).

1.3.2.4 Alkaline Hydrolysis

Alkaline pretreatment comprises the usage of bases, such as calcium, potassium, sodium, and ammonium hydroxide, for the pretreatment of lignocellulosic materials. Sodium hydroxide has been widely investigated for many years, and it has been revealed to disrupt the lignin structure of the biomass and enhance the accessibility of enzymes to cellulose and hemicellulose (MacDonald et al., 1983; Soto et al., 1994; Zhao et al., 2008; Zhu et al., 2010; Brodeur et al., 2011). The major effect of alkaline pretreatment is the removal of lignin from the structure, so enhancing the reactivity of the residual polysaccharides (Chang and Holtzapple, 2000; Harmsen et al., 2010). The usage of an alkali causes the degradation of glycosidic and ester side chains which results in structural change of lignin, partial decrystallization of cellulose, cellulose swelling (Cheng et al., 2010; ; McIntosh and

Vancov, 2010; Brodeur et al., 2011; Ibrahim et al., 2011), and partial solvation of hemicellulose (McIntosh and Vancov, 2010; Brodeur et al., 2011; Sills et al., 2011). The alkaline hydrolysis conditions are generally less severe when compared to the other pretreatment types. Alkaline hydrolysis can take place at ambient conditions, however if it is conducted at ambient conditions, pretreatment times are prolonged when compared to the alkaline pretreatments that are carried on at higher temperatures.

In alkaline pretreatment one soaks the biomass in a solution and mixes the solution for a certain amount of time at a specified temperature. Before enzymatic hydrolysis, for the removal of lignin and inhibitors (salts, furfural, phenolic acids and aldehydes), a neutralization step is needed.

Lime is the least expensive alkali among other alkali types. For instance, the approximate cost of materials was \$70/ton hydrated lime compared to \$320/ton for 50 wt% NaOH and 45wt% KOH and \$270/ton fertilizer grade ammonia (Brodeur et al., 2011). Lime (calcium hydroxide) can also be recovered from water as insoluble calcium carbonate through the reaction with carbon dioxide (Mosier et al., 2005; Park et al., 2010). The carbonate can then be transformed to lime through conventional lime kiln technology (Chang et al., 1998; Mosier et al., 2005).

1.4 Biodegradable Polymer Films

Petrochemical based plastics such as polyolefins, polyesters, polyamides, etc. have been increasingly used for packaging purposes because of their abundance at low cost and good packaging properties such as good tensile strength and good oxygen barrier (Tharanathan, 2003). However, their usage leads to serious environmental problems concerning waste disposal because of their non-biodegradability (Tharanathan and Saroja, 2001, Tharanathan, 2003). Therefore, there is a growing interest for replacing petroleum-based commodity plastics with biodegradable materials in a cost effective manner. Biopolymers have been considered as the strongest candidate to be used for this purpose (Vieria et al., 2011).

Biodegradable polymers can be classified in four categories based on the synthesis and on the sources (Tharanathan, 2003, Averous and Boquillon, 2004, Bordes et al., 2009, Vieria et al., 2011):

(a) Polymers obtained from biomass such as the agro-polymers from agro-resources;

(i) Polysaccharides, e.g., starches, ligno-cellulosic products and others (pectins, chitosan);

(ii) Protein and lipids, e.g., animals (casein, whey, collagen/gelatin), and plants (soya, gluten);

(b) Polymers via microbial production, e.g., polyhydroxyalkanoates (PHA) such as poly (hydroxybutyrate) (PHB) and poly (hydroxybutyrate cohydroxyvalerate (PHBv);

(c) Polymers synthesized chemically using monomers obtained from agro-resources, e.g., poly (lactic acid) (PLA);

(d) polymers whose monomers and polymers are both generated by chemical processes from fossil resources, for example;, polyesteramides (PEA), polycaprolactones (PCL), aromatic co-polyesters (e.g., PBAT) and aliphatic co-polyesters (e.g., PBSA).

1.4.1 Polysaccharide Based Films

Polysaccharide-based films are mostly made from cellulose ethers, alginate, chitosan, starch, carrageenan and pectins (Tharanathan, 2003, Vieria et al., 2011).

Carboxymethyl cellulose (CMC), anionic cellulose ether, is water soluble and compatible with other (bio) molecules. The films obtained with CMC show excellent film formation properties. CMC films have good application potentials in the packaging of deep fat-fried foods because they are capable of reducing oil pick-up in such foods. The mechanical and barrier properties of CMC films are strongly dependent on the molecular weight of the cellulose and high molecular weight enhances the properties of CMC films (Krochta et al., 1994). There are some commercially available CMC based composite coating formulations such as TAL-Prolong, Sempefresh and Nature-Seal (Nisperos-Carriedo et al., 1992, Tharanathan, 2003). They are used as a coating material in order to prolong the shelf life of fruits.

Starch which is another raw material in abundance, especially from corn, has thermoplastic properties upon disruption of its molecular structure (Tharanathan, 1995, Tharanathan and Saroja, 2001). Research on starch based plastics began in the 1970s and technologies have been developed for continuous production of films containing 50% or more starch. While starch films with high amount of amylose show good film properties such as high strength and elongation, the branched structure of amylopectin leads to films with poor mechanical properties such as low strength and elongation (Tharanathan, 2003). Various plasticizers such as sorbitol, xylitol and glycerol are used to overcome the brittleness of starch based films (Vieria et al., 2011). Starch based films and coatings are widely used in the food industry in order to preserve the foods in a good condition without spoilage (Thomas and Atwell, 1997).

Pectin which is a heterogeneous branched polysaccharide is one of the major cell wall structural polysaccharides of higher plant cells. Pectin and starch blends are used to obtain films with good film properties (Coffin and Fishman, 1993). Plasticized blends of citrus and amylose starch give flexible, strong and thermally stable films up to 180°C. Laminated films of chitosan and pectin with glycerol or lactic acid have been produced. Pectin based films have some potential applications in detergents, insecticides, medical delivery systems and devices (Tharanathan, 2003).

Chitosan which is one of the most abundant polysaccharides on the earth is derived from chitin (Lezica and Quesada-Allue, 1990, Hoagland and Parris, 1996). Chitosan is commercially available from a renewable feedstock which is a waste from the shellfish industry (Knorr, 1991). Chitosan based films show good film properties. Biodegradable composite films with chitosan and cellulose have been obtained with the addition of plasticizer such as glycerol which showed good mechanical properties (Hosokawa et al., 1990; Nishiyama, 1993). Chemically modified chitosan membranes could be used for the purpose of separating ethanol from water by pervaporation (Lee, 1993). Chitosan membranes are also used for water purification (Muzzarelli et al., 1989). Chitosan films are used to prolong the shelf life of fruits and vegetables (Elghaouth et al., 1991). Studies concerning chitosan films have been investigated for the drug delivery (Bonvin and de Bertorello, 1993).

1.4.2 Protein Based Films

Proteins have a unique structure with 20 different monomer types which enable a wide range of functional properties such as high intermolecular binding potential (Cuq et al., 1997). Protein based films have a high potential to form numerous linkages because of their ability to form bonds at different positions (Ou et al., 2005; Bourtoom, 2009). Zein which is the prolamine of corn has been investigated as a raw material for packaging purposes (Santosa and Padua, 1999). Oleic and linoleic acids were used as plasticizers with protein based films which resulted in flexible films with low modulus, high elongation and toughness and low tensile strength. Linoleic acid was found as more effective than oleic acid concerning the decrease in the water absorption of films. On the other hand, oleic acid was found as more effective in terms of the toughness and water resistance of films. Therefore, zein films with oleic acid were thought to be as applicable in thermoformed packaging trays. Glycerol and polyethylene glycol plasticized β-lactoglobulin films have also been obtained with good mechanical properties (Sothornvit and Krochta, 2001; Vieria et al., 2011).

Edible films have been produced from sunflower proteins (Meixueir et al., 2000). Film formation in such proteins is accomplished through hydrogen bonds and intermolecular disulphide bridges besides surface dehydration. Flexible but brittle films have also been produced from whey proteins (20% of total milk proteins) (Kaya and Kaya, 2000; Tharanthan, 2003).

Collagen, which is a major constituent of skin, tendon and connective tissues, is the most dominant and widely distributed fibrous protein in the animals (Tharanathan, 2003). Collagen films have been used for preparing edible sausage casing (Hood, 1987). Gelatin is obtained from partial hydrolysis of collagen and it is used to produce flexible and tough films with the addition of sorbitol or glycerine as plasticizer (Tharanathan, 2003).

1.4.3 Lipid Based Films

Wax coatings naturally exist on fruit and vegetable surfaces where they help prevent moisture loss, especially in the dry humid season. The studies related with the preservation of fresh and dry fruits and nuts by wax coatings have been conducted for many years. Bees wax, paraffin wax and candelilla wax which is an oily exudate of the candelilla plant grown in the United States and Mexico are some of the wax preparations used in the packaging of foods. Wax coatings are also used as micro-encapsulation agents, especially for spice flavouring substances (Tharanathan, 2003).

Shellac, which is made up of complex mixtures of aliphatic, alicyclic hydroxy acid polymers, is a secretion of the insect *Laecifer lacca*. It is widely used for the purpose of confectioners glaze on candies. Free fatty acids have also been used in some composites. Composites produced by a mixture of hydrocolloid, emulsifiers and lipid molecules have shown significant potential for the extension of shelf-life of several agri-horticultural products (Krochta et al., 1994; Tharanthan, 2003).

Glycerol, ethylene glycol, diethylene glycol or propylene glycol added pig skin gelatin based films have also been produced with good thermal and functional properties (Van de Velde and Kiekens, 2002 ; Vieria et al., 2011).

1.5 Aim of the Study

The multi-product lignocellulosic biorefinery concept, which considers the co-production of various value added products from different components of lignocellulosic biomass, is a crucial issue related with lignocellulosic biomass research since such an approach is necessary in order to make lignocellulosic biorefineries economically feasible and applicable. Pretreatment applied to lignocellulosic biomass is a key step during the conversion of lignocellulosic materials to value added products. However despite the importance of a multi-product approach, a very significant portion of the current literature on pretreatment deals solely with the improvement of glucose yield obtained from lignocellulosic biomass via the enzymatic hydrolysis following the pretreatment with some others also considering the xylose yield and the fermentation of glucose, xylose or both to ethanol and thus lacking a multi-product approach. In other words, the pretreatment conditions are evaluated and optimized for a single product in a majority of the current pretreatment literature. This study considers the co-production of glucose and hemicellulose based films from lignocellulosic biomass, namely corn cobs, while investigating the effect of different pretreatment parameters such as alkaline type, alkaline concentration, boron compound addition and type and pretreatment time on a multi-product basis by evaluating each pretreatment parameter with respect to its effect both on the glucose yield and on the properties of hemicellulose based films.

CHAPTER 2

LITERATURE SURVEY

2.1 Alkaline Hydrolysis

Playne (1984) has treated sugarcane bagasse with lime for up to 192 h at ambient conditions in order to improve the enzymatic digestibility of cellulose from 20% before pretreatment to 72% after pretreatment. Shorter reaction times and higher temperatures were also shown to efficiently pretreat lignocellulosic materials with lime. Chang et al. (1998) found comparable digestibility results through the pretreatment of bagasse with lime at 120 °C for 1 h. Chang et al. (1997) also found that lime pretreatment was effective for the pretreatment of switchgrass. The reducing sugar yield, total sugar yield and glucose yield were enhanced 5, 7 and 21 times as compared to untreated switchgrass for the pretreatment conditions of 100°C for 2 hours, respectively.

The addition of air/oxygen to the reaction mixture significantly enhances the delignification of the lignocellulosic material, particularly extremely lignified materials such as poplar (Chang and Holtzapple, 2000). Oxidative lime pretreatment of poplar (Chang et al., 2001) at 150°C for 6 h was shown to discard 77.5% of the lignin from the wood chips and enhanced the yield of glucose obtained after the enzymatic hydrolysis from 7% (untreated) to 77% (treated) compared to the untreated and pretreated poplar wood, respectively (Mosier et al., 2005). For lesser lignified lignocellulosic materials such as corn stover, the usage of oxygen seems to only slightly enhance the digestibility of pretreated corn stover with lime at a

1:0.075 ratio (stover:lime) at 120˚C for times up to 6 h, (Karr and Holtzapple, 1998; Mosier et al., 2005). Corn stover pretreated for 4 hours (optimum time) at 120˚C loses 32% of the lignin. Glucose yield was obtained as 88% after a 7 day enzymatic hydrolysis of the biomass. Kaar and Holtzapple (2000) also showed that pretreatment with lime enhanced the enzymatic hydrolysis of corn stover nine times as compared to untreated corn stover under the pretreatment conditions of 120 °C and 4 hours.

An approach to lime pretreatment was suggested by Park and coworkers (2010) to discard the solid-liquid separation step after neutralization through neutralizing the lime with carbon dioxide before hydrolysis resulting in 89% glucose yield from rice straw.

Dilute NaOH treatment has been determined to cause swelling which results in an increase in the area of internal surface, a decrease in crystallinity, a separation of structural bonds between lignin and carbohydrates, a decrease in the degree of polymerization, and a disruption of the lignin structure. Digestibility of NaOH-treated hardwood increased from 14% to 55% with a decrease of lignin content from 24-55% to 20%. Nevertheless, no effect of dilute NaOH pretreatment was detected for softwoods with lignin content which is greater than 26% (Millet et al., 1976).

Macdonald et al. (1983) has conducted a study at which it was showed that alkali pretreatment of corn stover enhanced the enzymatic hydrolysis of cellulose and hemicellulose. The pretreatment process was carried out for 1 to 60 min at a temperature of between 100 and 150°C and NaOH concentrations of between 0 and 2%. They found that 65% of the original stover was dissolved after 5 min and 52% saccharification (g sugar/g stover) of the residue and dissolved solids through enzymatic hydrolysis was obtained compared to 20% for untreated corn stover at 150°C and 2% NaOH concentration.

Dilute NaOH pretreatment was also determined as effective for the hydrolysis of straws with comparatively low lignin contents of 10-18% (Bjerre et al., 1996; Kumar et al., 2009). Chosdu et al. (1993) applied a combination of irradiation and 2% NaOH for pretreatment of corn stalk, cassava bark, and peanut husk. The glucose yield of corn stalk was obtained as 20% for untreated samples and 43% after handling with electron beam irradiation and 2% NaOH, however the glucose yields were obtained as only 3.5% and 2.5%, for cassava bark and peanut husk, respectively (Kumar et al., 2009).

Sun and coworkers (1995) investigated the effectiveness of various alkaline solutions and found that the optimum results was achieved by using 1.5% sodium hydroxide during pretreatment of wheat straw that resulted in 80% release of hemicellulose and 60% release of lignin.

Lawther at al. (1996) conducted a study at which various alkaline regimes were used in order to obtain the optimum process conditions for the extraction and isolation of hemicellulose and cellulose from wheat straw. Within this study, the effect of potassium hydroxide concentration, alkaline type (calcium hydroxide, sodium hydroxide, lithium hydroxide, and liquid ammonia), pretreatment temperature and pretreatment time on the extraction process was examined. The optimum time for extracting hemicellulose with 10% KOH and 2% H_3B0_3 at 20°C was determined as between 21 and 26 h. The optimum concentrations of KOH for extracting hemicellulose at 20°C for 2 h were obtained as between 20 and 30% and the optimum extraction concentration of H_3B0_3 with 24% KOH at 20°C for 2 h was found as either 2 or 5%.

Varga and coworkers (2002) studied with dilute H_2SO_4, HCI or NaOH solutions during pretreatment in order to see their effect on the enzymatic hydrolysis of corn stover. Pretreatment with 10% NaOH enhanced the enzymatic conversion more than four times, yielded a 79.4% enzymatic conversion and decreased the lignin fraction >95%. Moreover, it was found that the enzymatic degradability can be increased using a less concentrated base in the case of longer pretreatment times.

Silverstein et al. (2007) has showed that the most noteworthy effect of sodium hydroxide pretreatment during the pretreatment of cotton stalks was on delignification with concentration of sodium hydroxide being the major factor. Lignin reduction increased linearly with increase in sodium hydroxide concentration used during pretreatment.

Zhao and coworkers (2008) showed that sodium hydroxide is an effective alkali for wheat straw, softwoods, hardwoods and switchgrass with less than 26% lignin content (Menon and Rao, 2012). Sodium hydroxide pretreatment has also been found as effective to increase biogas production from corn stover by 37% compared to untreated biomass (Zhu et al., 2010).

In a study of Xu et al. (2010), it was showed that NaOH pretreatment was an effective way to enhance the enzymatic digestibility of switchgrass at all the temperatures investigated which were 121, 50, and 21˚C. The total reducing sugar yields were enhanced at least 3.39 times as compared to untreated biomass for the optimum process conditions. The best conditions for switchgrass pretreatment were obtained as 50˚C, 12 h, and 1.0% NaOH based on total reducing sugar yield. NaOH pretreatment demonstrated exceptional capacity of delignification and the removal of the lignin barrier were strictly associated with the severity of the pretreatment applied. Nevertheless, reduction of the lignin should not be viewed as a suitable indicator for pretreatment efficiency because of greater carbohydrate loss at further severe pretreatment conditions. It was also shown that NaOH has an excellent potential to work at lower temperatures.

Cheng and coworkers (2010) also conducted experiments to measure the effects of alkaline loading and pretreatment time on delignification and sugar yield obtained after enzymatic hydrolysis of rice straw. The range of delignification was obtained as 8.6% to 23.1% for NaOH pretreatments and was 13.1% to 27.0% for lime pretreatments. Both reaction time and alkaline loading had significant positive effects ($p<0.001$) on delignification, but only alkaline loading had a significant positive effect on enzymatic hydrolysis.

There are several combinations of alkaline pretreatment with other pretreatment types, such as steam explosion, ammonia fiber explosion, wet oxidation and ammonia recycled percolation. Hu and Wen (2008) used different concentrations of NaOH solutions during the pretreatment of switchgrass and then applied microwave or conventional heating to the pretreated samples. Microwave pretreatment gave higher sugar yields compared to conventional heating with alkali loadings of 0.05-0.3 g of alkali/g of biomass. The highest yield which is 90% of maximum potential sugars was obtained at an alkali loading of 0.1 g/g. These results show that microwave-assisted alkali treatment is an effective way to enhance the enzymatic hydrolysis of switchgrass. (Kumar et al., 2009) Zhao and coworkers (2008) found that radiofrequency-based dielectric heating could be used with alkaline pretreatment to enhance sugar yield from switchgrass. The advantage of this combined method is that it enables uniform temperature profile in the pretreated material and pretreatment of high solid content. It is suggested that the utilization of radiofrequency-based dielectric heating disrupts the lignocellulosic structure to a higher extent through an explosion effect among the particles that resulted in higher sugar yields compared to the pretreatment methods conducted with alkali and conventional heating (Brodeur et al., 2011).

2.2 Enzymatic Hydrolysis of Cellulose

The potential importance of cellulose hydrolysis regarding the conversion of lignocellulosic biomass to fuels and chemicals is extensively acknowledged (Lynd et al., 1991, 1999; Himmel et al., 1999; Zhang and Lynd, 2004). Effective conversion of recalcitrant lignocellulosic structure to fermentable sugars necessitates three consecutive steps which are size reduction, pretreatment/fractionation, and enzymatic hydrolysis (Wyman, 1999; Zhang and Lynd, 2004).

Cellulose is a linear polymer made up of D-anhydroglucopyranose linked together by β-1,4- glycosidic bonds with a degree of polymerization (DP) from 100 to 20,000 (Tomme et al., 1995; O'Sullivan, 1997; Zhang and Lynd, 2004; Percival Zhang et al., 2006). Coupling of adjacent cellulose chains and sheets of cellulose with the aid of hydrogen bonds and van der Waal's forces lead to parallel alignment of cellulose and a crystalline structure with straight, stable fibers that have low accessibility and great tensile strength (Zhbankov, 1992; Nishiyama et al., 2003; Notley et al., 2004;

Zhang and Lynd, 2004; Demain et al., 2005; Percival Zhang et al., 2006). Cellulose is a material that shows a great deal of stability; it has a half-life of 5–8 million years for β-glucosidic bond cleavage at 25 °C (Wolfenden and Snider, 2001; Percival Zhang et al., 2006) and so faster enzyme-driven cellulose biodegradation process is needed to return the carbon in residues to the atmosphere (Berner, 2003; Cox et al., 2000; Falkowski et al., 2000; Schlamadinger and Marland, 1996; Percival Zhang et al., 2006).

Experimental results show that the heterogeneous structure of cellulose leads to a rapid decrease in rate as hydrolysis continues, even when the effects of product inhibition and cellulase deactivation are considered (Zhang et al., 1999; Valjamae et al., 1999). It is thought that fewer accessible chain ends, less surface area and/or adsorption of inactive cellulase on the cellulose (or lignocellulose) surfaces which block the subsequent hydrolysis are the reasons of the decrease in residual cellulose reactivity during enzymatic hydrolysis.

The widely recognized mechanism for enzymatic hydrolysis of cellulose involves synergistic action of endoglucanase (EC 3.2.1.4), exoglucanase or cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Henrissat, 1994; Knowles et al., 1987; Lynd et al., 2002; Teeri, 1997; Wood and Garica- Campayo, 1990; Zhang and Lynd, 2004; Percival Zhang et al., 2006). Endoglucanases hydrolyze accessible intramolecular β-1,4- glucosidic bonds of cellulose chains randomly to produce new chain ends (Percival Zhang et al., 2006). The accessible ends of cellulose molecules are cleaved by exoglucanases in order to release soluble cellobiose or glucose. *T. reesei* cellobiohydrolase (CBH) I and II act on the reducing and non-reducing cellulose chain ends, respectively (Teeri, 1997; Teeri et al., 1998; Zhang and Lynd, 2004; Percival Zhang et al., 2006). β-glucosidases hydrolyze cellobiose to glucose in order to exclude cellobiose inhibition. These three hydrolysis processes take place simultaneously in such a process : i) chemical and physical changes of the cellulose in the solid phase; ii) primary hydrolysis, comprising the release of soluble intermediates from the reacting cellulose molecule surfaces; and iii) secondary hydrolysis, comprising the hydrolysis of soluble intermediates to intermediates with lower molecular weight, and finally to glucose (Zhang and Lynd, 2004) (Figure 2.1).

In the solid phase, chemical and physical changes take place. Chemical changes in residual cellulose are expressed as chain end concentration and changes in the degree of polymerization (DP). Primary hydrolysis takes place on the surface of solid substrates and soluble sugars are released into the liquid phase upon hydrolysis by endoglucanases and exoglucanases. This step is regarded as the rate-limiting step for the whole cellulose hydrolysis process because the rate of primary hydrolysis is much slower than the rate of secondary hydrolysis. Endoglucanase increases the concentration of chain ends and considerably decreases DP through attacking interior parts of cellulose particles. Exoglucanases shorten DP incrementally and only sometimes decrease the chain ends concentration. Hence, endoglucanase activity is assumed to be essentially responsible for the chemical changes of cellulose in solid-phase which take place over the course of hydrolysis; however it is not so effective in solubilization as compared to exoglucanase. On the other hand, exoglucanase activity is supposed to be mainly responsible for solubilization but it is not so effective for changing the chemical properties of residual cellulose. Physical changes in solid phase cellulose are expressed as changes in accessible surface area because of geometrical changes which are caused by the enlargement or consumption of accessible cellulose surface due to increasing solubilization. It is also thought that soluble cellodextrins are the major portion of the instantaneous products produced through primary hydrolysis but they cannot be detected because of the faster rate of secondary hydrolysis compared to the rate of primary hydrolysis. Throughout the enzymatic hydrolysis process, cellodextrins with a polymerization degree value of greater than 4 exist in the solid phase which is associated with crystalline cellulose (Kleman-Leyer et al., 1994, 1996; Srisodsuk et al., 1998), and it has been proposed that this association hinders the release of cellodextrins to the solution. Nevertheless, cellodextrins with a polymerization degree value of greater than 4 are not observed associated with amorphous cellulose. Therefore, it is believed that enzymatic hydrolysis of cellodextrins with a length of 4–6 related with the solid phase will be a significant part of the solubilization process for only crystalline substrates, not for amorphous substrates (Zhang and Lynd, 2004). Secondary hydrolysis takes place in the liquid phase and involves mainly the hydrolysis of cellobiose to glucose by β-glucosidases (Zhang and Lynd, 2004; Percival Zhang et al., 2006). The solid substrate characteristics also change throughout the cellulose hydrolysis because of the changes in the cellulose chain end number resulting from generation by endoglucanases and consumption by exoglucanases (Kleman-Leyer et al., 1992, 1994, 1996; Srisodsuk et al., 1998; Kongruang et al., 2004; Zhang and Lynd, 2005; Percival Zhang et al., 2006) and the changes in cellulose accessibility due to substrate consumption and cellulose fragmentation (Halliwell and Riaz, 1970; Chanzy et al., 1983; Walker et al., 1990, 1992; Din et al., 1991, 1994; Woodward et al., 1992; Lee et al., 1996, 2000; Banka et al., 1998; Boisset et al., 2000; Saloheimo et al., 2002; Wang et al., 2003; Percival Zhang et al., 2006).

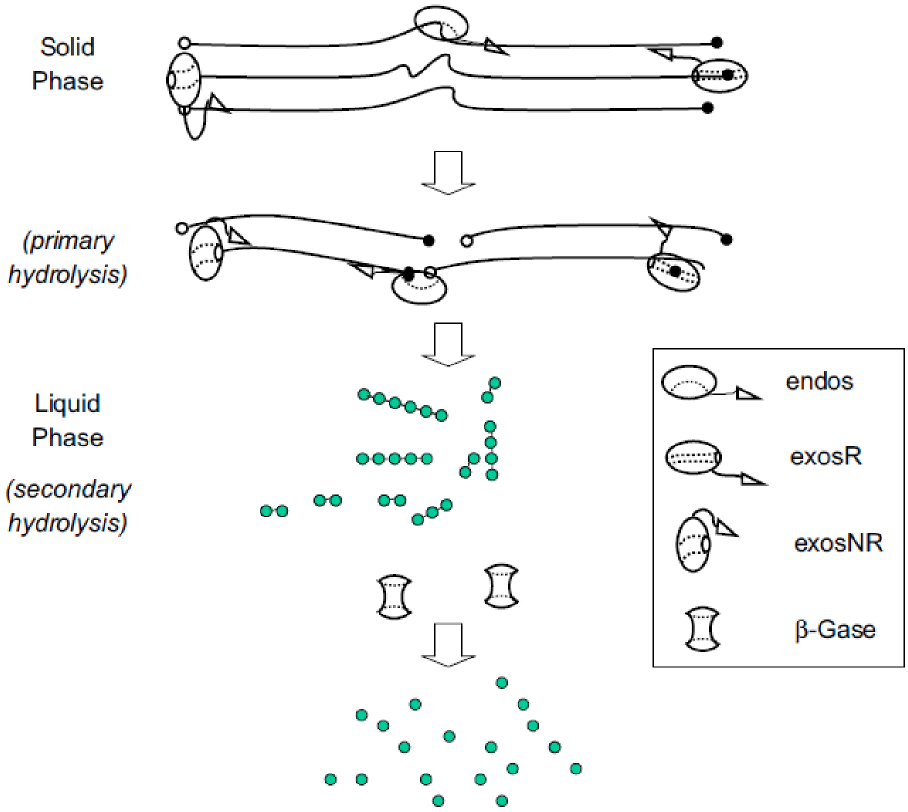

Figure 2.1 Mechanistic scheme of enzymatic cellulose hydrolysis by *Trichoderma* non-complexed cellulase system (Percival Zhang et al., 2006).

2.3 Hemicellulose Based Films

The targeted uses of hemicelluloses have generally been packaging films for the food industry (Davis and Song, 2006, Kale et al., 2007). The oxygen barrier property of the hemicellulose films besides their mechanical properties was seen as an important parameter for food packaging and the oxygen permeability values of hemicellulose films was found as comparable to other biopolymers such as amylopectin and amylose (Hansen and Plackett, 2008).

Fredon et al. (2002) has produced hemicellulose films from maize bran. The obtained films had a Young's modulus value of 118 and 87 MPa, ultimate tensile strength value of 10 and 11 MPa and elongation at break value of 8 and 22 with degree of substitutions of 0.7 and 1.1, respectively. The casting method affected the film properties as seen by comparison of solution-cast and tape-cast films with a degree of substitution of 1.1. The tape-cast film demonstrated increased elasticity with Young's modulus of 57 MPa and an elongation of 38%.

Another study which focused on the production of hemicellulose films from maize bran was conducted by Peroval et al. (2002). Composite arabinoxylan-based films were obtained by emulsifying a fat: palmitic acid, oleic acid, triolein, or a hydrogenated palm oil (OK35) in addition to glycerol. The elastic modulus was obtained as 72.4±35.2 MPa, the tensile strength was obtained as 26.5±4.1 MPa and elongation was obtained as 7.4±2.9% for the arabinoxylan based films. The films with triolein showed the best mechanical properties in terms of tensile strength and elongation (%).

Edible composite films from the blends of wheat gluten and xylan from grass, corn cob and birchwood were produced by Kayserilioglu et al. (2003). The xylan was isolated from grass and corn cob by alkaline extraction and birchwood xylan was commercially available. Birchwood xylan with weight fractions of 0-40% were mixed with wheat gluten and films were cast under alkaline and acidic conditions with a pH of 11 and 4, respectively. The films were dried at ambient and elevated temperature of 80 °C. The tensile strength of films was found as independent from the composition and the tensile strength of the films dried at alkaline conditions was found as 2-3 times higher than those of the films dried at acidic conditions. This could be because of the more uniform films obtained during casting at high pH. Besides, drying at ambient conditions gave better results in terms of the tensile properties of the films obtained. However, elastic modulus and percent elongation of the films increased with lower pH and elevated drying temperature. For comparison, xylan films (20 wt%) from corn cob and grass were cast from alkaline solution at ambient conditions. The tensile strengths of the xylan films from grass and corn cob (1.5-3 MPa) were lower than the tensile strength of the films from birchwood (≈8 MPa) which is nearly the same value obtained for pure gluten. Corn cob xylan films were found as the most stretchable with a Young's modulus of approximately 10 MPa and elongation of 600%. The other two films had an elongation value of approximately 50%. Furthermore, corn cob-xylan containing films were determined as the most homogeneous by SEM analysis.

Gröndahl et al. (2004) has examined the oxygen barrier properties of glucuronoxylan from aspen wood. The hemicellulose films obtained from unmodified xylan were brittle (Tg≈180°C) and so, sorbitol and xylitol were used as plasticizers in concentrations of 20, 35, and 50 wt%. All the films were found as semicrystalline without regard to its plasticizer content. The films with 20 wt% plasticizer have obtained with ultimate tensile strength of more than 40 MPa while the elongation at break was only 2 %. The ultimate tensile strength values of the films decreased with an increase in the plasticizer content. This decrease was accompanied with an increase in the elongation at break values. Sorbitol was determined as the most effective plasticizer regarding elongation. The oxygen permeability of the films with 35 wt% sorbitol was 0.21 cm^3 μm m^{-2} d^{-1} kPa^{-1} at 50% RH and this value was equal to the oxygen permeability value obtained with poly(vinyl alcohol) (PVOH) film under the same conditions. PVOH is a distinguished water soluble and biodegradable barrier material with good mechanical and thermal properties (Sapalidis et al., 2007).

Zhang and Whistler (2004) has produced edible films from corn hull arabinoxylan as the major component with M_w = 50600 g mol^{-1}. Propylene glycol, sorbitol or glycerol (0-22 wt %) was used as plasticizer and films were obtained with a thickness of 22-32 μm. The propylene glycol plasticized arabinoxylan films were found as brittle and tensile strengths were changing from 53 to 61 MPa, elastic moduli was between 1290 and 1314 MPa and elongations were between 6 and 8 %, which was noted to be similar to the results for propylene glycol plasticized cellulose films (Hansen and Plackett, 2008). The properties changed much more with plasticizer content for the sorbitol and glycerol plasticized films with tensile strengths of 20–48 MPa and 10–47 MPa, respectively and elongations varied from 6 to 9% and 6 to 12%, respectively. The best moisture barrier properties were found for sorbitol-plasticized films (13%) and propylene glycol-plasticized films (16%) and glycerol-plasticized films (5%) with a descending order. A high amount of glycerol (higher than 10%) resulted in an increase in permeability because of the hygroscopic nature of the plasticizer. The reduction of moisture loss from grapes was analyzed with arabinoxylan coatings. The obtained films were smooth, stable and transparent and showed good moisture barrier properties over a period of seven days with a reduction of weight loss from 82 to 59%. Furthermore, the obtained coatings were more effective as moisture barriers than carboxymethyl cellulose films and coatings of corn amylopectin which were produced for comparison.

Höije et al. (2005) has aimed to produce oxygen barrier films or coatings while investigating the effect of pretreatment on the isolation of hemicellulosic portion from barley husks. Four different types of pretreatment which are acidic pretreatment, enzyme pretreatment or acidic pretreatment combined with either chlorite or organosolv (ethanol) delignification were applied before alkaline extraction. The aim of the prehydrolysis was to remove proteins and it was shown that delignification process was necessary to accomplish this objective. It was found that the type of the pretreatment was important in terms of the amount of the arabinoxylan obtained as well as the molecular weight of the films. Four types of films were obtained as a result of different pretreatments applied and the chlorite delignification was regarded as the most efficient method (57 % arabinoxylan yield).

The hemicellulose films have been obtained with an ultimate tensile strength of more than 50 MPa, an elongation at break of 2.5 %, and a Young's modulus of 2900 MPa. Moreover, the hemicellulose films were found as highly hygroscopic with predominantly amorphous structure.

Gröndahl et al. (2006) has proposed surface fluorination of arabinoxylan films from barley husks for the purpose of obtaining hydrophobic films which will be used as a food packaging material. After casting, the films were subjected to gas-phase fluorination with trifluoroacetic anhydride. As a result of the modification, the equilibrium moisture content decreased from 18 to 12 %.

The hemicellulose *O*-acetylgalactoglucomannan (AcGGM) from wood was examined by Hartman et al. (2006a) to obtain films with oxygen barrier properties. AcGGM was obtained by the process water from thermomechanical pulping. The isolation of water soluble product was achieved by ultrafiltration. The AcGGM films with a thickness of 30-60 μm were cast with plasticizers such as sorbitol, glycerol or sorbitol with 21-25 wt%. Furthermore, alginate or carboxymethylcellulose (CMC) was used with 2:1 on weight basis for film formation. The storage modulus values of plasticized films decreased with increasing humidity especially for the AcGGM film with glycerol. The films with alginate and CMC showed better mechanical behavior even at high humidity as well as mostly having larger storage moduli than the films with plasticizer. A blend film of alginate and AcGGM was mixed with glycerol and exhibited intermediate mechanical properties compared to the equivalent two-component films. The percentage elongation of the films after DMA measurements reflected the mechanical stability and the highest elongation was found as 195% with glycerol plasticized AcGGM film. On the other hand, the hybrid films with CMC or alginate showed elongations of 3 and 4%, respectively. The lowest oxygen permeabilities were found for the AcGGM-CMC and AcGGM-alginate films. The oxygen permeability of sorbitol plasticized AcGGM film was 2.0 cm^3 μm m^{-2} d^{-1} kPa^{-1}, which was considerably lower than the value of 4.4 cm^3 μm m^{-2} d^{-1} kPa^{-1} found for the xylitol plasticized AcGGM film. The highest oxygen permeability, 4.6 cm^3 µm m^{-2} d^{-1} kPa^{-1}., was determined with the alginate-AcGGM film containing glycerol as plasticizer (17.5 wt %).It was not possible to conduct analysis on the glycerol plasticized AcGGM film.

Another study of AcGGM was conducted by Hartman et al. (2006b) at which AcGGM was blended with CMC or alginate at a ratio of 7:3 (weight basis) and then they were cast as films. Either plasma treatment followed by styrene grafting or vapor phase grafting with styrene was applied in order to increase the hydrophobicity of the films. Benzylation of AcGGM was done with benzyl chloride in NaOH solution of varying concentrations and so, ester groups were deacetylated which enables them susceptible to benzylation. The benzylated AcGGM (BnGGM) formed films were strong, flexible and transparent. The AcGGM-alginate was benzylated and then cast as film. BnGGM was laminated on to an AcGGM-alginate film by putting the latter in a solution of the former constituent. Lamination is regarded as an innovative approach to obtain hemicellulose based multilayer films. The blend films showed better oxygen barrier properties compared to the BnGGM films. The oxygen permeabilities of the BnGGM films were significantly higher than those of the blend films. An excellent oxygen permeability value of 8 cm^3 µm m^{-2} d^{-1} kPa^{-1} was determined for BnGGM-laminated AcGGM-alginate film.

The oxygen barrier property of hemicellulose based materials is very promising in the field. Amylose and amylopectin based films which have good oxygen barrier properties exhibit oxygen permeability values in the same range with those of the hemicellulose based films. Ethylene vinyl alcohol (EVOH), poly vinyl alcohol (PVOH) and chitosan films show remarkable oxygen barrier properties with values below 0.5 cm^3 µm m^{-2} d^{-1} kPa^{-1} and this was comparable with the value of the xylan film produced by Gröndahl and coworkers (2004). The oxygen permeabilities of AcGGM films and hybrid films of AcGGM with alginate and CMC with and without grafted styrene were slightly higher than those of the above mentioned films.

Cotton stalk-xylan films were produced for food packaging applications by Goksu et al. (2007). This is the first example in the literature of the production of films from xylan without any additive or plasticizer. Delignification was found as detrimental in terms of film forming properties and complete lignin removal from xylan was resulted in cracked films. Commercially available birchwood xylan was used to determine the lower limit of lignin required for producing films. Minimum 1% (w/w) lignin in birchwood xylan solution was required in order to obtain films without any crack. Cotton stalk xylan containing aforementioned amount of lignin with solutions of 8, 10, 12 and 14% (w/w) was cast to produce films with thicknesses of 0.29–0.38 mm. Tensile strength, strain at break, elastic modulus and film thickness generally increased with the xylan concentration. Glycerol plasticized films (2 % w/w) were produced from a 10% xylan solution. Goksu et al. (2007) suggested that the free volume within the film was increased and water evaporation was enhanced as a result of the plasticizer addition and which leads to a decrease in the film thicknesses. The tensile strength was reduced from 1.34 to 0.76 MPa with the addition of plasticizer. All the xylan films with or without plasticizer were water soluble with 98-99 % solubility.

Enzymatic treatment of rye arabinoxylan has been designed and performed to prepare arabinoxylans with a gradient of arabinose content by Höije et al. (2008). The initial arabinose to xylose ratio was 0.50 (AX-0.50). All the obtained films were strong and relatively stiff, but showed some deviations with strain at break values. The film with an arabinose to xylose ratio of 0.37 (AX-0.37) had the highest strain at break among other films and it was stiff and strong.

The first extensive study focusing on film-forming properties of an agrobiomass byproduct, specifically, oat spelt arabinoxylan (osAX) was conducted by Mikkonen et al., (2009). Glycerol or sorbitol was used to obtain cohesive films. The mechanical properties changed with the type and amount of plasticizer. The tensile strength of the glycerol plasticized films containing glycerol were higher than the sorbitol plasticized films with a 10 % (w/w) plasticizer content. However, sorbitol plasticized films preserved their tensile properties better than glycerol plasticized films with a 40% plasticizer content during 5 months of storage.

Saxena et al. (2009) produced nanocellulosic-xylan films from oat spelt xylan, cellulose whiskers and plasticizer. The addition of sulfonated cellulose whiskers resulted in a considerable improvement in tensile properties. The tensile strength of the film was increased by 141 % with the addition of 7 wt% of nanocellulose whiskers produced with sulfuric acid (sulfonated whiskers). Moreover, sulfonated whiskers were better at increasing the strength of the films than cellulose whiskers produced by hydrochloric acid hydrolysis of cellulosic fibers (HCl whisker)

Edlund et al. (2010) has produced thin films and coatings from spruce wood hydrolysate which was recovered from the wastewater after hydrothermal treatment of spruce wood. The wood hydrolysate contained 10 % lignin with respect to dry mass. Blend mixtures of wood hydrolysate with chitosan or CMC were used to cast films which were applied on PET films. The films with chitosan or CMC were obtained as transparent and shiny with very low oxygen permeabilities and good mechanical properties. As expected, the addition of chitosan and CMC enhanced the mechanical properties of wood hydrolysate films and when the relative amount of chitosan or CMC increased, the tensile strength and strain values increased too. Chitosan co-component films showed higher strength and strain at break than CMC co-component films. Coatings of wood hydrolysate/co-component blend mixtures were applied onto commercially used PET films in order to observe the applicability of wood hydrolysate films in packaging. The Young's modulus and strain at break values of coated PET films were similar to those of uncoated PET. The tensile strength values of coated PET films were decreased compared with those of uncoated PET. However, when the load at break values which were very similar for both uncoated and coated PET were compared, it was determined that the decrease in the strength values were caused by increase in the sample thickness (stress=force/(thickness width)). Consequently, it could be said that there was not such a significant effect of coatings on the mechanical properties of PET films that would hinder the physical integrity of coated PET films in packaging applications. Low oxygen permeability is an important property for food packaging. Wood-hydrolysate-based coatings dramatically decreased the oxygen permeabilities of PET backing compared with uncoated PET films. Furthermore, oxygen permeabilities of wood hydrolysate coatings containing CMC were lower than those of coatings containing chitosan. The obtained coatings showed promising oxygen barrier properties compared to the commercially used packaging materials.

Spruce galactoglucomannans (GGM) and microfibrillated spruce wood cellulose (MFC) were mixed to produce composite films (Mikkonen et al., 2011). Glycerol was used as plasticizer and the films were produced even with low glycerol contents. The addition of MFC significantly affected the mechanical properties of the films by increasing the tensile strength and Young's modulus of the films. High amount of glycerol within the films resulted in a decrease in strength and stiffness but increase in elongation at break. The production of cohesive self-standing films of GGM and MFC with glycerol content as low as 10 %, which was not successful without MFC could be possible.

Peng et al. (2011) described an effective approach to produce nanocomposite films with enhanced mechanical properties by adding cellulose nanofibers (CNFs) into xylan rich hemicelluloses (XH). Aqueous solutions of XH (64-75 wt %), sorbitol (16-25 wt %), and CNF (0-20 wt %) were cast to produce nanocomposite films. XH film with 25 wt % plasticizer (sorbitol, based on dry XH weight) was obtained with poor mechanical properties, on the other hand, addition of CNF (5-20 wt %, based on the total dry mixture) into the film enhanced the mechanical properties of the film because of mechanical strength of CNF and high aspect ratio and strong interactions between CNF and XH matrix.

Escalante et al. (2012) extracted arabinoglucuronoxylan from Norway spruce and films were prepared by casting from aqueous solution. Transparent and flexible films were obtained with a Young's Modulus 2735 MPa, an average stress at break of 55 MPa and a strain at break of 2.7%. Addition of sorbitol to the casting solution as plasticizer resulted in less strong but more flexible films (strain at break of 5%). Moreover, the films were obtained with low oxygen permeability and so, they have a promising application potential in food packaging.

Bahcegul and coworkers (2012) have recently evaluated the effect of alkaline pretreatment temperature on a multi-product basis for the co-production of glucose and hemicellulose based films from cotton stalk. The hemicellulosic part of the process was much more responsive to the changes in the pretreatment temperature compared to the cellulosic part, which accounts for the production of glucose. Increasing pretreatment temperature to 90°C was determined as detrimental on the properties of hemicellulose based films. The hemicellulosic part of the process was found to be decisive on the pretreatment temperature rather than the cellulosic part.

Table 2.1 Summary of the properties of the hemicellulose based films in the literature.

Biomass*	Matrix	Additive	E (MPa)	UTS (MPa)	e (%)	Water content (%)	Crosshead Speed (mm/min)	RH/T (%,°C)	References
Maize bran (AX)	-	-	57-118	10-11	8-38	-	500	-/19	Fredon et al.,2002
Maize bran (AX)	-	glycerol	72.4 ± 35.2	26.5 ± 4.1	7.4 ± 2.9	-	100	54/25	Peroval et al.,2002
Birchwood (X)	wheat gluten (60-90%(w/w))	glycerol (2%(w/w))	~20-200	~2-8	~20-380	-	20	60/20	Kayserilioglu et al.,2003
Corn cob (X)	wheat gluten (80%(w/w))	-	~10	~1	~620	-			
Grass (X)	wheat gluten (80%(w/w))	-	~35	~3	~40	-			
Aspen wood (GX)	-	xylitol	~100-900	5-40	2-8	~12	5	50/23	Gröndahl et al.,2004
		sorbitol (20-50%(w/w))	200-5000	6-40	2-11	-			
Corn hull (AX)	-	-	1316	53.8	6.2	13-18	10	54/22	Zhang&Whistler,2004
		glycerol (0-22%(w/w))	365-1148	9.7-46.5	5.6-12.1				
		propylene glycol (0-22%(w/w))	1225-1320	52.6-60.7	5.9-7.9				
		sorbitol (0-22%(w/w))	463-1161	19.7-47.5	5.5-8.9				
Barley husk (AX)	-	-	2930±300	50	2.5	35.5	5	50/23	Höije et al.,2005
Cotton stalk (X-L)	-	-	0.11-0.49	1.08-1.39	45.56-56.76	17-20	10	40/20	Goksu et al.,2007
	-	glycerol (2%(w/w))	0.08	0.76	88.89	-			
Rye (AX)	-	-	630-1750	36.8-57.7	4.7-10.4	11-13	5	50/23	Höije et al.,2008
Oat spelt (AX)	-	glycerol (10-40%(w/w))	~100-1100	~4-28	~5-11	11	5	50/23	Mikkonen et al.,2009
	-	sorbitol (10-40%(w/w))	~300-700	~9-17	~4-9	10-13			
Oat spelt (X)	-	sorbitol (50%(w/w))	-	2	22	-	-	50/23	Saxena et al.,2009
	sulfonated whisker (2.5-10%(w/w))	sorbitol (50%(w/w))	-	2.5-6	18-30	-			
	HCl whisker (2.5-10%(w/w))	sorbitol (50%(w/w))	-	3-4.5	10-24	-			

Table 2.1 ***(Continued)*** Summary of the properties of the hemicellulose based films in the literature.

Biomass*	Matrix	Additive	E (MPa)	UTS (MPa)	e (%)	Water content (%)	Crosshead Speed (mm/min)	RH/T (%,°C)	References
Softwood hydrolysate	CMC (20-50%(w/w))	-	1624-2432	9.8-50.6	0.9-3.1	-	5	50/23	Edlund et al.,2010
	chitosan (40-50%(w/w))	-	1915-2365	47.5-62.4	5.6-7.2	-			
Spruce (GGM)	-	glycerol (40%(w/w))	~ 750	~ 14	~ 4.3	-	5	50/23	Mikkonen et al.,2011
	MFC (5%(w/w))		~ 800	~ 13	~ 4.8	-			
	MFC (15%(w/w))		~ 900	~ 19	~ 3.7	-			
Bamboo (X)	-	sorbitol (25%(w/w))	735	11.9	3.4	12.6	4	50/23	Peng et al.,2011
	cellulose nanofiber (5-20%(w/w))		1322-3404	15.5-39.5	1.4-2.9	11-12			
Norway spruce (AGX)	-	-	2735	55	2.7	-	5	50/25	Escalante et al.,2012
	-	sorbitol (5-25%(w/w))	1163-2612	26-57	3.1-4.3	-			

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[*] AX= Arabinoxylan; X= Xylan; GX= Glucuronoxylan; X-L= Xylan with lignin; GGM= Galactoglucomannan; AGX= Arabinoglucuronoxylan.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Air dried corn cobs were obtained from Hatay, Turkey and milled to pass a 10 mesh (2 mm) screen. Potassium hydroxide, sodium hydroxide, acetic acid, tri-sodium citrate dihydrate, citric acid monohydrate, boric acid, sodium borohydride and ᴅ-glucose were purchased from Merck (Darmstadt, Germany). Calcium hydroxide, Potassium sodium tartrate, 3,5-dinitro salicylic acid, phenol, sodium sulfate and alkali lignin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cellulase (Celluclast 1.5 L) and β-glucosidase (Novozyme 188) were obtained from Novozymes (Bagsvaerd, Denmark).

3.2 Methods

3.2.1 Pretreatment of Corn Cobs and Isolation of Cellulosic Portion

Alkaline pretreatment procedure applied to the milled corn cobs was mainly derived from the previously reported procedures by Lawther et al. (1996) and Zilliox and Debeire (1998). Ten grams of dry corn cob powder were swollen in 200 ml water for 1 hour at room temperature with magnetic stirring. The suspension was filtered and the corn cobs were incubated with 85 ml of 24% (w/v) KOH and 1% (w/v) $NaBH_4$ solution for 3 hours. At the end of pretreatment the alkaline insoluble fraction, which is regarded as the cellulosic portion throughout the work, was obtained by filtration through cloth. The samples of cellulosic portion were washed with 200 ml of de-ionized water for three times and during the final wash the pH of the suspension was adjusted to 4.8 via the addition of acetic acid. The purpose of the wash step after pretreatment is to remove the alkali residues and inhibitors formed during pretreatment that might hinder enzymatic hydrolysis and microbial fermentation (Mes-Hartree *et al.,* 1983; Larsson *et al.,* 1999; Cheng *et al.,* 2010). Finally the cellulosic portion was rinsed with de-ionized water and dried in an incubator at 60°C overnight and weighted. Pretreatments were conducted in duplicates for each of the designated condition. Cellulosic portion recovery (R_c) (%) was defined and calculated using the following formula:

$$R_C(\%) = \frac{\begin{array}{c}\mathit{Weight\ of\ alkaline\ insoluble}\\ \mathit{biomass\ recovered\ after\ pretreatment}\end{array}}{\begin{array}{c}\mathit{Weight\ of\ untreated\ corn\ cobs}\\ \mathit{subjected\ to\ pretreatment}\end{array}} \times 100 \qquad (3.1)$$

Within the scope of this study, the effect of different pretreatment parameters which were alkaline type (KOH, $Ca(OH)_2$, NaOH), alkaline concentration (5,10,24 % (w/v)), addition of boron compound and type ($NaBH_4$ and H_3BO_3) and pretreatment time (1, 2, 3 hour(s)) were investigated on the co-production of glucose and hemicellulose based films from corn cobs. Since the solubility of $Ca(OH)_2$ is very low in water (1.6 g/L at 20°C and 0.71 g/L at 100°C) the experiments were conducted with saturated $Ca(OH)_2$ solution (Oates, 2007; Cheng et al., 2010).

3.2.2 Isolation of Hemicellulosic Portion

Following the removal of the alkaline insoluble portion from the pretreatment suspension, the solution was centrifuged at 8000g for 10 min in order to obtain a clear solution free of any insoluble particles. Following the centrifugation, 250 ml of precipitation solution, which composed of 1:10 volumetric ratio of acetic acid to ethanol, was added to the alkaline solution as suggested by Zilliox and Debeire (1998). The precipitated material, which is regarded as the hemicellulosic portion, was collected by filtration through cloth and dried at ambient conditions and weighted upon drying. Hemicellulosic portion recovery is defined as the ratio of precipitated alkaline soluble biomass recovered after pretreatment to untreated corn cobs subjected to pretreatment.

Hemicellulosic portion recovery (R_H) (%) was defined and calculated using the following formula:

$$R_H(\%) = \frac{\begin{array}{c} Weight\ of\ precipitated\ alkaline\ soluble\ biomass \\ recovered\ after\ pretreatment \end{array}}{\begin{array}{c} Weight\ of\ untreated\ corn\ cobs \\ subjected\ to\ pretreatment \end{array}} \times 100 \qquad (3.2)$$

3.2.3 Enzymatic Hydrolysis

Enzymatic hydrolysis of the cellulosic portion obtained after pretreatment was conducted at 50 °C in a shaking incubator (Minitron, Infors AG, Bottmingen, Switzerland) at 150 rpm together with untreated corn cobs for 48 h. Enzymatic hydrolysis was conducted in 30 ml of 0.05 M sodium citrate buffer with a pH of 4.8. The detailed description of the preparation of 0.05 M sodium citrate buffer are given in Appendix A. 0.9 g of biomass were hydrolyzed under the indicated conditions via the addition of cellulases (Celluclast 1.5 L, Novozymes, Bagsvaerd, Denmark) and β-glucosidase (Novozyme 188, Novozymes, Bagsvaerd, Denmark) at a loading of 50 filter paper units/g biomass and 60 cellobiose units/g biomass, respectively, based on the enzyme activities reported by Yang and Wyman (2006). One filter paper unit (FPU) is defined as the enzyme amount that releases 1 µmol of glucose equivalents from Whatman no. 1 filter paper in 1 min under the reaction conditions used. One unit of β-glucosidase activity is defined as the enzyme amount that converts 1 µmol of cellobiose in 1 min under the reaction conditions used (Ghose, 1987; Hu et al., 2008). The enzyme loading was chosen on the basis that further increase in the enzyme loadings did not cause any significant improvement on the enzymatic hydrolysis. Samples of 500 µl were withdrawn from the hydrolysis reactions at the 1st, 5th, 24th and 48th hour of enzymatic hydrolysis and immediately placed in boiling water for 5 min in order to stop the enzymatic reaction. Finally the samples were centrifuged at 10,000g for 5 min and supernatants were used for reducing sugar analysis.

3.2.4 Reducing Sugar Analysis

Enzymatic cellulose hydrolysis was followed by measuring reducing sugar concentration with respect to reaction time period. During the enzymatic hydrolysis, samples were withdrawn from the reaction mixtures at certain time intervals and analyzed for reducing sugars via 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The detailed description of the preparation of 3,5-dinitrosalicylic acid (DNS) solution are given in Appendix A. The samples were centrifuged at 10,000g for 5 min prior to DNS analysis. The clear hydrolysate obtained after centrifugation was diluted with distilled water in order to get readings within the glucose concentration range used to obtain the calibration curve. Then, 1.5 ml of DNS reagent was added to the 1 ml of the diluted solution and the solution was vortexed for about 10 s. This mixture was then placed in a boiling water bath for about 5 min and cooled. The absorbance at 540 nm of the reacted mixture was measured by using a double beam UV-Vis spectrophotometer (Nicolet Evolution 100, Thermo Electron Corporation, WI, USA) and converted to reducing sugar concentration with a calibration curve prepared using glucose standard solutions. The details of the calibration curve are given in Appendix B.

3.2.5 Glucose Analysis using Blood Glucose Monitor (BGM)

The samples withdrawn from the reaction mixtures at the 48th hour of enzymatic hydrolysis were analyzed for glucose concentration by using a blood glucose monitor (OneTouch Select , LifeScan Inc., Milpitas, CA, USA) based on a recently reported procedure (Bahcegul et al., 2011). According to the producer's specifications, OneTouch Select (LifeScan Inc., Milpitas, CA, USA) uses test strips with glucose oxidase (GO). The measurement principle of BGM is based on amperometry, where the sample is automatically withdrawn into the test strip upon the contact of the strip's tip with the sample. BGM was able to conduct measurements within 5–8 s with sample volumes of 0.6–1 µl according to the producer's specifications. Prior to the measurements, the device was first coded based on the directions of the producer for its own LOT# of strips via entering the code manually to the device (One Touch Select). Standard solutions of glucose in

0.05 M sodium citrate buffer at pH 4.8 were prepared to obtain the response of the BGM to glucose at a concentration range of 0.5–4.0 g/l. Afterwards, the calibration curves were prepared and used to calculate glucose concentrations in the enzymatic hydrolysates of biomass samples. The details of the calibration curve are given in Appendix B. The samples from the enzymatic hydrolysis systems were diluted to get 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 curves in order to discard the effect of pH, which might affect the readings measured with the BGM (FitzGerald and Vermerris, 2005; Choy et al., 2007; Bahcegul et al., 2011). Each analysis was conducted in duplicates. D-glucose was used as a standard.

The weight of glucose obtained after enzymatic hydrolysis was calculated first through the value read from BGM and then theoretically maximum amount of glucose was calculated by multiplying the amount of cellulose in the untreated corn cob by 1.11 which is the factor that corresponds to the mass balance of the transfer of cellulose to glucose. The glucose yield (G (%)) was calculated on the basis of cellulose content of untreated corn cobs using the formula given below:

$$G(\%) = \frac{Weight\ of\ glucose\ obtained\ after\ enzymatic\ hydrolysis}{\begin{array}{c}Weight\ of\ theoretically\ maximum\ glucose\ that\ could\ be\\ obtained\ from\ the\ cellulosic\ content\ of\ untreated\ corn\ cob\end{array}} \times 100 \qquad (3.3)$$

3.2.6 Compositional Analysis of Corn Cobs

The composition of corn cob used during the experiments conducted was obtained according to the National Renewable Energy Laboratory (NREL, Golden, CO) analytical methods for biomass (NREL, 1996; 2011). This method utilizes a two-step acid hydrolysis to fractionate the biomass into forms that are more easily measured. The lignin is separated into acid soluble and acid insoluble material. The acid insoluble material is measured by gravimetric analysis. The acid soluble lignin is measured by UV-Vis spectroscopy. During the hydrolysis process, the polymeric carbohydrates are hydrolyzed into the monomeric forms being soluble in the hydrolysis liquid which are then measured by HPLC. The first stage of acid hydrolysis was conducted by adding 300.0 ± 10.0 mg of the sample to the solution of 72 %

(w/w) H_2SO_4 at 30 °C for 60 minutes. In the second stage, the reaction mixture was diluted to 4 % (w/w) H_2SO_4 by adding 84.00 ± 0.04 mL deionized water and autoclaved at 121 °C for 1 h. The autoclaved hydrolysis solution was filtered with a vacuum through the weighed filtering crucibles. An aliquot of approximately 50 mL was transferred into a bottle. This sample was used to analyze acid soluble lignin from absorbance data at 205 nm and sugar content by HPLC. All the remaining solids were transferred out of the pressure tube into the filtering crucible by using deionized water. The crucible and acid insoluble residue was then dried at 105 °C until they reached a constant weight. The crucible and acid insoluble residue was put into a furnace at 575 °C for 24 hours and then weighted for the calculation of % Acid Insoluble Lignin (AIL) content of the biomass. The following equations were used to determine the acid insoluble lignin content of the films and acid soluble lignin (ASL) content of the films, respectively:

$$\% AIL = \frac{DW_{crucible\ plus\ AIL} - DW_{crucible}}{DW} \tag{3.4}$$

where $DW_{crucible\ plus\ AIL}$ is the dry weight of the crucible and acid insoluble lignin in a furnace, $DW_{crucible}$ is the dry weight of the crucible in a furnace (575 °C) and DW is the dry weight of the biomass sample (0.3 grams).

$$\% ASL = \frac{\frac{A}{b \times a} \times df \times V \times \frac{L}{1000\ mL}}{DW} \times 100 \tag{3.5}$$

where A is the absorbance at 205 nm, df is the dilution factor, b is the cell path length, which is 1 cm, a is the absorptivity, which is 110 L/g-cm, V is the filtrate volume which is 87 mL, DW is the initial dry biomass sample weight in grams.

The cellulose and hemicellulose content of the biomass is calculated by measuring monosaccharide concentration through high performance liquid chromatography (HPLC) with a Shimadzu LC-20A HPLC system (Kyoto, Japan) equipped with a BIORAD Aminex HPX-87H column (Hercules, CA, USA) at 55 °C with a flow rate of 0.6 ml/min using 5 mM H_2SO_4 as the mobile phase where D-glucose, D-xylose and D-arabinose were used as standards. The glucose, xylose and arabinose concentrations were calculated with a calibration curve prepared using D-glucose, D-xylose and D-arabinose standard solutions. Then, the cellulose content of the biomass was calculated using the following formula:

$$\% \, Cellulose \; content = \frac{C_G \times V \times \frac{L}{1000 \, ml}}{DW \times 1.11} \times 100 \tag{3.6}$$

where C_G is the glucose concentration measured with a calibration curve through HPLC, V is the volume which is 87 ml and DW is the initial dry biomass sample weight in grams .

The hemicellulose content was calculated using the formula given below:

$$\% \, Hemicellulose \; content = \frac{C_x \times V \times \frac{L}{1000 \, ml}}{DW \times 0.88} \times 100 \tag{3.7}$$

where C_x is the xylose concentration measured with a calibration curve through HPLC, V is the volume which is 87 ml and DW is the initial dry biomass sample weight in grams.

3.2.7 Film casting

0.5 g of dry hemicellulosic portion was dissolved in 15 ml of de-ionized water at 121 °C in a period of 20 minutes in an autoclave and then stirred for 24 hours at ambient conditions on a magnetic stirrer. After the dissolution, the solutions were poured into petri dishes with a diameter of 9 cm. Films were dried and aged for 3 days upon drying with a temperature of 23 °C at 50% relative humidity at controlled cabinet (Climacell, MMM Group, Munich, 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 ((Biliaderis et al., 1999, Skendi et al., 2003, Wang et al., 2008). The following equation was used to determine the water content of the films:

$$Water \; content(\%) = \frac{w_0 - w_1}{w_0} \times 100 \tag{3.8}$$

where w_0 and w_1 are the weight of films maintained at controlled cabinet (23°C, 50% RH) for 24 h, and in an incubator (105°C) to a constant weight, respectively. All samples were analyzed in duplicate.

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. The details of the lignin calibration curve are given in Appendix B.

3.2.8 Tensile testing

Prior to tensile testing, dog bone shaped specimens (Figure 2.1) were cut from the films with a manual cutting press (ZCP 020, Zwick GmbH & Co., Ulm, Germany). Specimens were tested for their mechanical properties with a universal testing machine (Zwick/ Roell Z250, Zwick GmbH & Co., Ulm, Germany) equipped with a 100 N load cell which are calibrated regularly once a year and pneumatic grips. The test was conducted at a crosshead speed of 5 mm/min in an environment with approximately 50% relative humidity at a temperature of 23 °C as determined by a thermo-hygrometer. At least five samples were analyzed. The test results including ultimate tensile strength (UTS), percent elongation at break (e_B), Young's modulus (E) and tensile energy to break (TEB), were obtained via the software (testXpert2) accompanying the universal testing machine. Ultmate tensile strength (UTS) was determined as the maximum value of the stress values obtained from tensile testing. Percent elongation at break was expressed as the percentage of the change of the original length of a specimen between grips at break. The Young's modulus (E) was determined as the slope of the linear part of the stress-strain curve. Tensile energy to break (TEB) value was expressed as the area under the stress-strain curve up to the point of fracture. The thickness of the test specimens were measured prior to testing by a digital micrometer (40 EXL, Mahr GmbH, Esslingen, Germany) with a resolution of 1 µm.

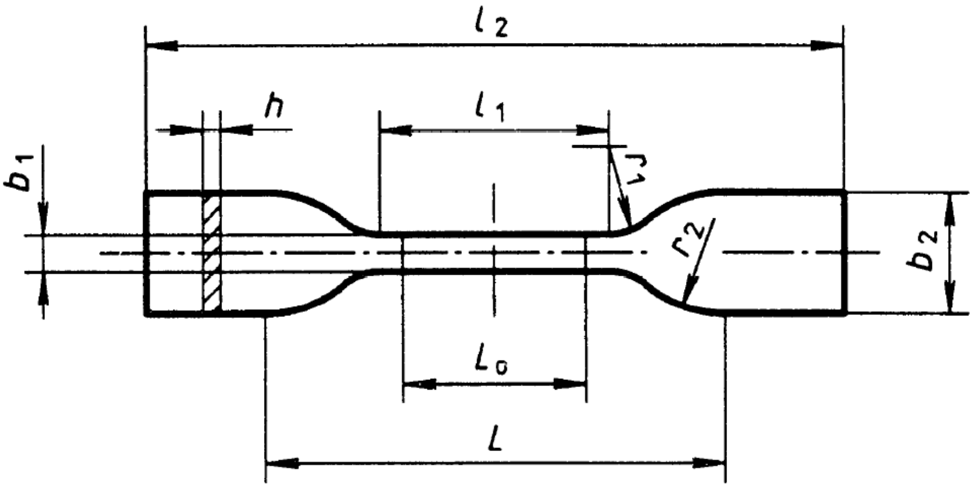

Figure 3.1 Shape and dimensions of the specimen used in mechanical testing procedures where l1 is the length of narrow parallel sided portion (25 mm), l2 is the overall length (75 mm), b1 is the width of narrow parallel sided portion (4 mm), b2 is the width at ends (12.5 mm), r1 is the small radius (8 mm), r2 is the large radius (12.5 mm), L is the initial distance between grips (50 mm), L0 is the gauge length (20 mm) and h is the thickness.

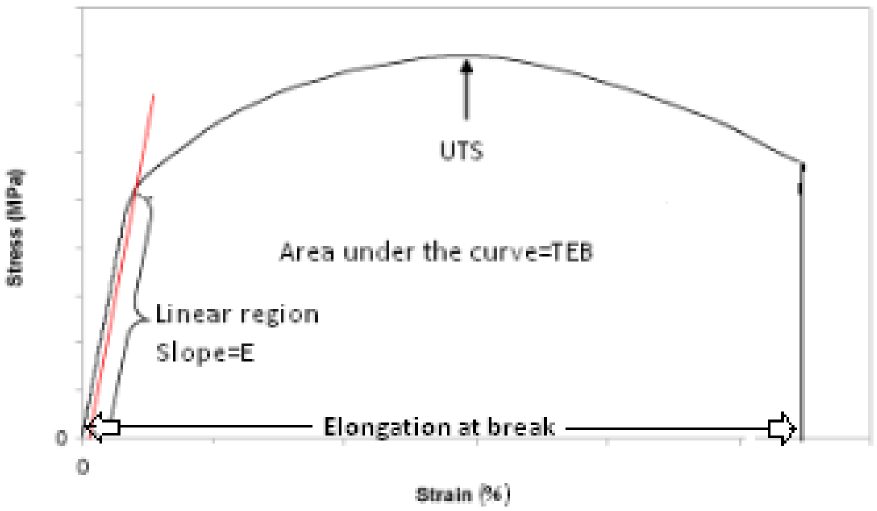

Figure 3.2 Stress-strain curve

3.2.9 Statistical analysis

One-way analysis of variance (ANOVA) was used for the statistical analysis of mechanical properties of the films. ANOVA is a statistical procedure for evaluating the influence of a categorical variable on the variance of a dependent variable. It compares the difference of each subgroup mean from the overall mean with the difference of each observation from the subgroup mean. If there are more variation between-groups differences, then the categorical variable is effective on the dependent variable. One-way ANOVA measure the effects of one factor (Güntürkün, 2007; www.chem.agilent.com). All statistical analyses were performed with MINITAB (Release 13.2). The confidence level of this study was taken as 95%.

CHAPTER 4

RESULTS AND DISCUSSION

In this study, the alkaline pretreatment method was utilized to isolate cellulose and hemicellulose from corn cobs. The effect of different pretreatment parameters which were alkaline type and concentration, boron compound utilization and type and pretreatment time on the co-production of glucose and hemicellulose based films were investigated. In the first stage of this study, corn cob's lignocellulosic structure was separated into cellulose rich and hemicellulose rich portions and the amount of cellulose and hemicellulose obtained as a result of different pretreatment conditions were determined. Among the fractionated components, cellulosic portion was used for the production of glucose while hemicellulosic portion was used for the production of films. In the second stage of this study, reducing sugars were produced from cellulose via enzymatic hydrolysis while hemicellulose based films were produced from hemicellulose by solvent casting. The properties of the products obtained were examined in relation with the pretreatment parameters. At this stage, the amount of total sugars and glucose obtained from cellulose were determined together with the mechanical properties of the hemicellulose based films produced from hemicellulose. In the final stage, optimum pretreatment conditions were determined based on the properties of the products obtained with different pretreatment parameters.

4.1 Corn Cob Composition

The cellulose, hemicellulose and lignin composition of the corn cobs were analyzed according to NREL LAP procedures (NREL 1996; 2011). As shown in Table 4.1, the composition of the corn cobs was found to be approximately 33% cellulose, 36% hemicellulose, 24% lignin by dry weight. In the literature, the corn cob composition was given as 45% cellulose, 35% hemicellulose, 15% lignin and others by dry weight (Jorgensen et al., 2007; Kumar et al., 2009). However, the composition may change depending on the type of the corn. The cellulose content was lower and lignin content was higher than the values in the literature (Jorgensen et al., 2007; Kumar et al., 2009).

Table 4.1 Composition of corn cob

Components	**Content (g dry weight/100 g dry corn cob)**
Cellulose	32.8
Hemicellulose	35.5
Acid soluble lignin	4.7
Acid insoluble lignin	19.1
Others (Protein, acetyl, uronic acids, etc.)	7.9

4.2 Effect of Alkaline Type and Concentration during the Co-Production of Glucose and Hemicellulose Based Films from Corn Cobs

Various alkaline regimes with different alkaline types and concentrations were used during the pretreatment process in order to determine the optimum conditions required for the production of glucose and hemicellulose based films from corn cobs simultaneously. Three different types of alkaline compounds which are KOH, $Ca(OH)_2$ and NaOH were chosen for the pretreatment process. $Ca(OH)_2$ was determined as an ineffective alkaline compound for the process. The low solubility of $Ca(OH)_2$ resulted in insufficient amount of hemicellulosic portion obtained after the pretreatment process. Therefore, $Ca(OH)_2$ as an alkaline compound was not used further in this study. KOH and NaOH were used as alkaline compounds in the pretreatment process for further evaluation.

4.2.1 Effect of Alkaline Type and Concentration on the Enzymatic Hydrolysis of Corn Cobs

At the end of the alkaline pretreatment conducted at different alkaline concentrations with KOH or NaOH, pretreated corn cob samples were subjected to enzymatic hydrolysis in order to assess their hydrolyzability in terms of reducing sugars released, which were detected by DNS method.

As shown in Figure 4.1, the amount of reducing sugars released did not change considerably with increasing KOH concentration. The concentrations of reducing sugars at the end of 48 h of enzymatic hydrolysis were enhanced approximately 7 times as compared to untreated corn cobs for KOH concentrations of 5%, 10% and 24% (w/v). The amount of reducing sugars released decreased slightly with increasing NaOH concentration for 5 and 10 % NaOH cases. However, as seen in Figure 4.2, the enzymatic cellulose hydrolysis was enhanced about 7-9 times as compared to untreated corn cobs using NaOH. These results showed that alkaline pretreatment improves the enzymatic hydrolysis in accordance with other studies investigating alkaline pretreatment of lignocellulosic feedstocks (Cheng *et al.,* 2010, Saha *et al.,* 2007, Rabelo *et al.,* 2008). Within the light of these results, utilization of both KOH and NaOH as an alkaline compound in the pretreatment process enhances the enzymatic hydrolysis in terms of the amount of the reducing sugars. In addition, utilization of NaOH seems better than the others based on the amount of reducing sugars obtained after enzymatic hydrolysis. The decrease in reducing sugar concentration for the case containing 24 % NaOH may be because of microbial contamination which resulted in sugar utilization.

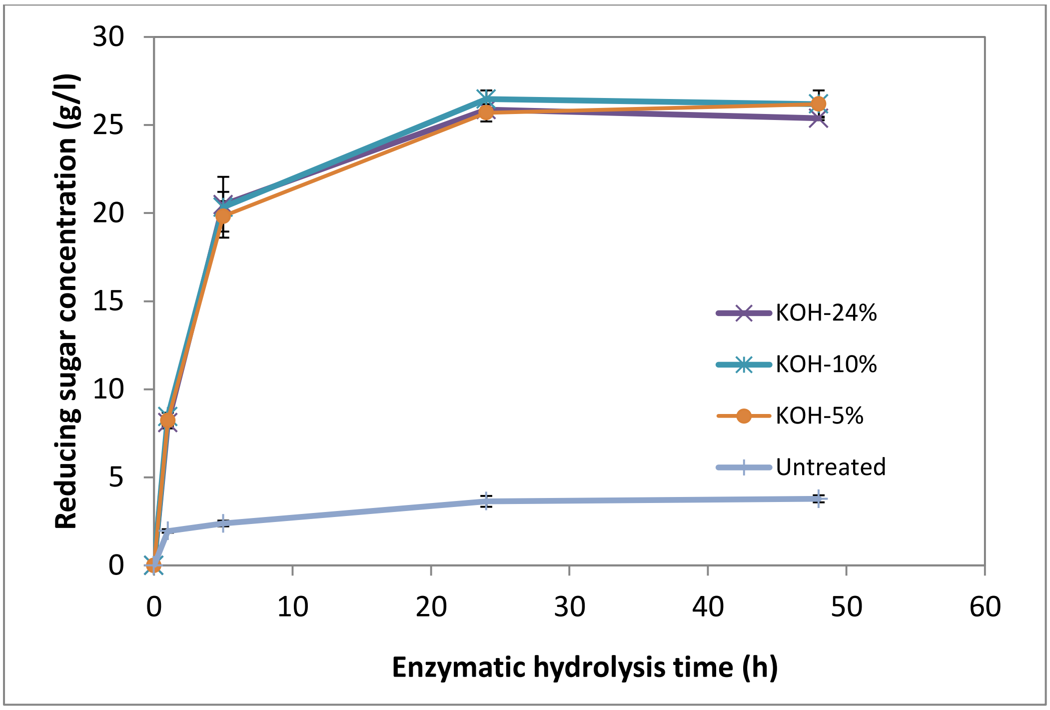

Figure 4.1 Effect of KOH concentration on the enzymatic hydrolysis of corn cobs. Error bars represent standard deviations.

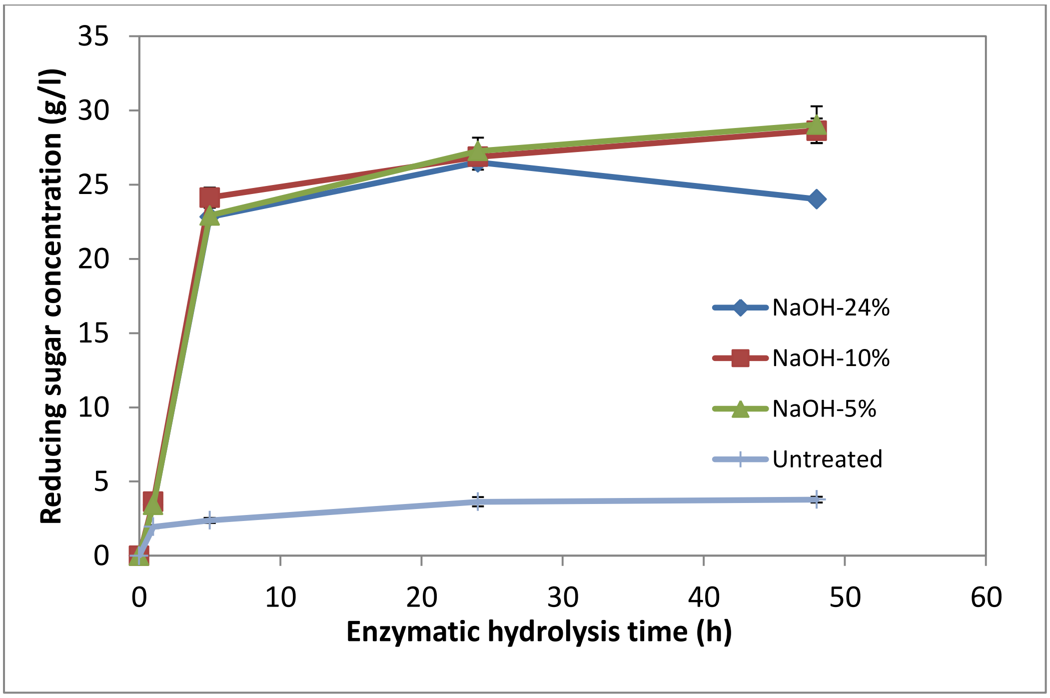

Figure 4.2 Effect of NaOH concentration on the enzymatic hydrolysis of corn cobs. Error bars represent standard deviations.

As shown in Figure 4.3, with increasing alkaline concentration the amount of cellulosic portion recovered after pretreatment decreased. This decrease was accompanied by an increase in the recovered hemicellulosic portion (Figure 4.4) in agreement with the previously reported results by Lawther *et al.* (1996). In other words, increasing the alkaline concentration increased the amount of hemicellulosic portion extracted from corn cobs. In accordance with the alkaline concentration used, a hemicellulosic portion recovery was in the range of 8 - 33% and 21- 26 % for KOH and NaOH, respectively. The “hemicellulose dissolving power” of KOH and NaOH varied with different alkaline concentrations. When the alkaline concentration was low (5%), use of NaOH gave higher yields in terms of the hemicellulosic portion compared to KOH which was in accordance with the results of Lawther *et al.* (1996). However, when the alkaline concentration increased, KOH became a more effective alkali in terms of the capability of the process to provide higher amount of hemicellulosic portion. When the cellulosic recoveries obtained with 10% KOH compared with the results obtained in another study using cotton stalk having the same amount of cellulosic content, it was seen that approximately 10% lower cellulosic recovery obtained through this study (Bahcegul et al., 2012).

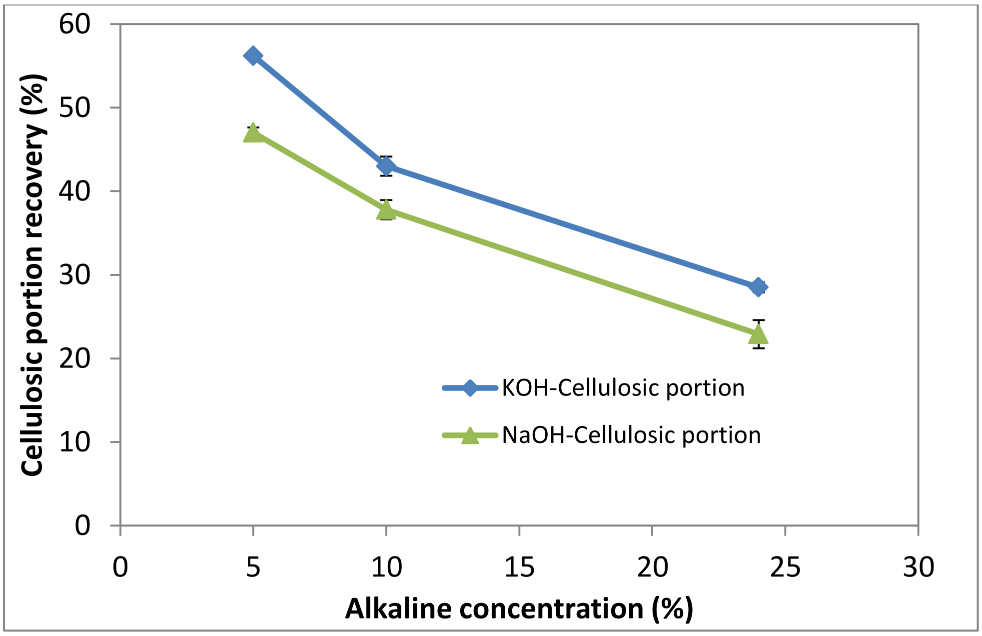

Figure 4.3 Effect of alkaline type and concentration on the amount of cellulosic portions recovered after pretreatment. Error bars represent standard deviations.

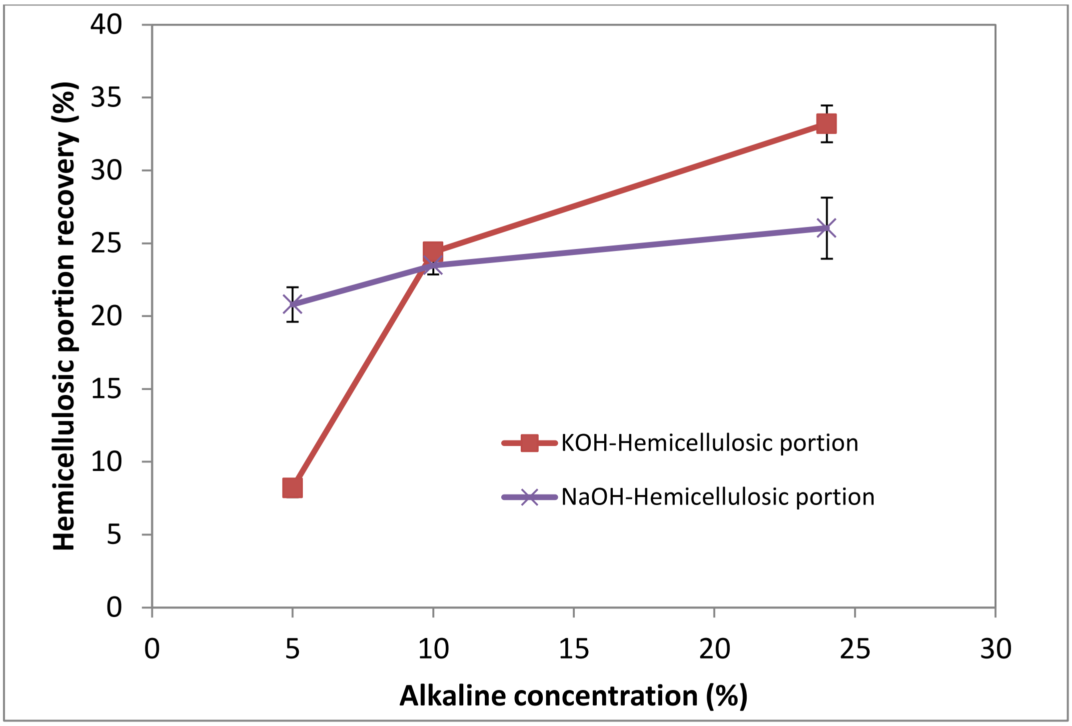

Figure 4.4 Effect of alkaline type and concentration on the amount of hemicellulosic portions recovered after pretreatment. Error bars represent standard deviations.

4.2.2 Effect of Alkaline Type and Concentration on the Properties of Hemicellulose Based Films

In this study, following the pretreatment the alkaline insoluble fraction was removed from the suspension and then the hemicellulosic portion was precipitated from the solution by adding ethanol and acetic acid. Upon the recovery of the hemicellulosic portion obtained after pretreatment at different alkaline concentrations and types, cohesive and self-standing films were obtained via solvent casting without any addition of plasticizer.

As shown in Figures 4.5 and 4.6, the colors of the films obtained from the corresponding hemicellulosic portions were not considerably different from each other with a change in alkaline type and concentration. Another important observation was that the change in alkaline type and concentration did not cause any problem regarding the film formation properties of the hemicellulosic portions obtained after pretreatment.

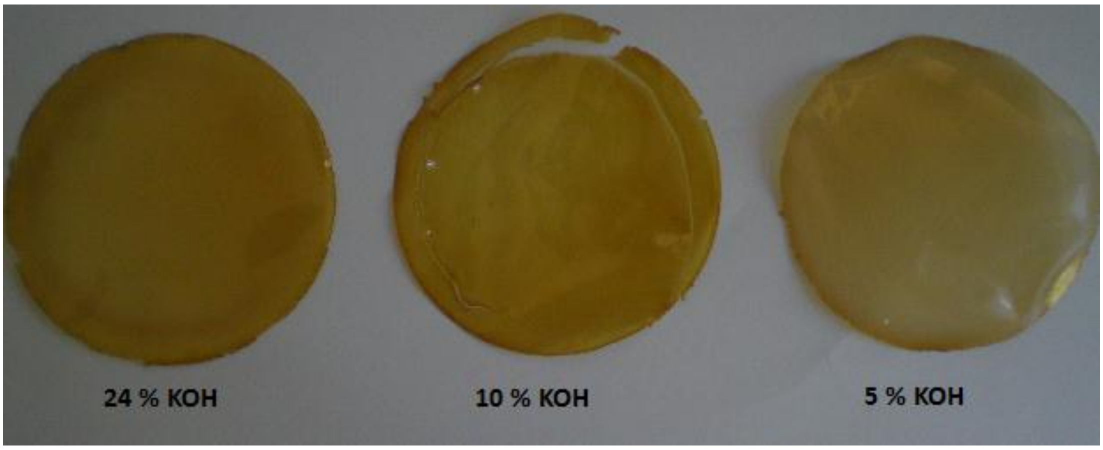

Figure 4.5 Effect of alkaline concentration on the visual appearances of hemicellulose based films. From left to right: Films obtained from hemicelluloses isolated at the end of pretreatments conducted with KOH concentration of 24%, 10% and 5%.

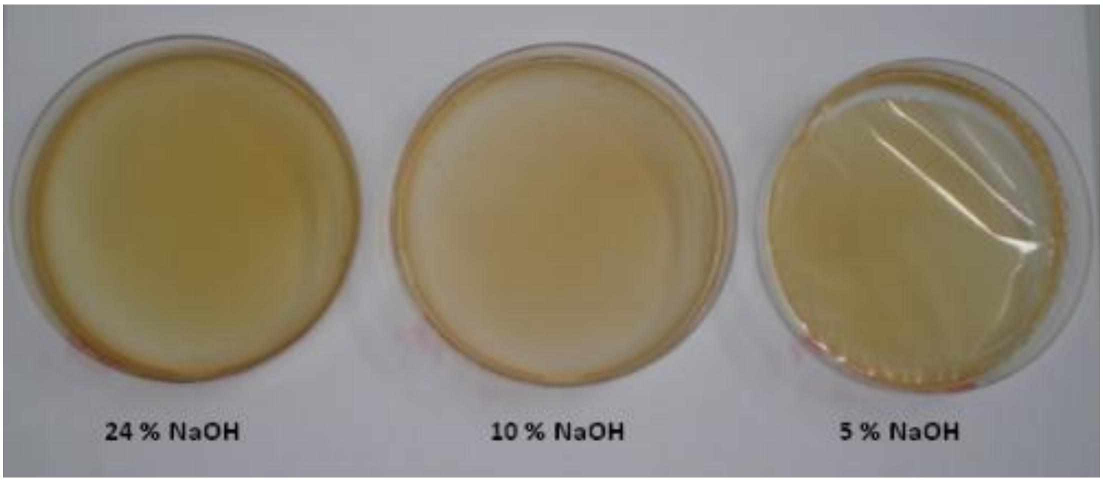

Figure 4.6 Effect of alkaline concentration on the visual appearances of hemicellulose based films. From left to right: Films obtained from hemicelluloses isolated at the end of pretreatments conducted with NaOH concentration of 24 %, 10 % and 5 %.

In addition to visual and film formation properties, another important aspect related to hemicellulose based films is their mechanical properties as a function of alkaline type and concentration. A tensile test was applied in order to analyze the mechanical properties of the films through their ultimate tensile strength (UTS), percent elongation at break (e_B) and Young's modulus (E) values. The effect of alkaline concentration on the stress-strain curves of the can be seen in Appendix C. As shown in Tables 4.2 and 4.3, the alkaline concentration has a notable effect on the mechanical properties of hemicellulose based polymer films. The tensile properties obtained with 10 % KOH solution was also consistent with the values obtained through the study of Bahcegul et al. (2012) at which the same amount of KOH used during pretreatment of cotton stalks.

Increasing the alkaline concentration caused a decrease in the UTS and E values of the films and increase in the e_b values of the films. As it is seen in Tables 4.2 and 4.3, the highest percent elongation at break values were obtained with the use of 24% NaOH during the pretreatment and the highest ultimate tensile strength and Young's modulus values were obtained with the use of 5% KOH during pretreatment. The tensile strengths of the films were enhanced at least 10 times compared to the values obtained from the study of Goksu et al. (2007) that could be seen in Table 2.2 which gives brief information about the properties of the hemicellulose based films reported in the literature.

The higher water content of the polymer films obtained through the pretreatment conducted with an alkaline concentration of 24% could be seen as the causative factor for the highest elongation value obtained because water is acting as a plasticizer for the hemicellulose based polymer films (Salme´n and Olsson, 1998, Gröndahl et al., 2004; Höije et al., 2005; Bahcegul et al., 2012; Escalante et al., 2012). On the other hand, while e_B values of the films which were obtained as a result of the pretreatment conducted with high alkaline solutions (24%) were higher, UTS values of these films were significantly lower than the others ($p<0.05$).

Table 4.2 Effect of KOH concentration on the mechanical properties, water contents and lignin contents of hemicellulose based films

KOH concentration (%)	UTS (MPa)	e_B (%)	E (MPa)	Water content (%)	Lignin content (%)
24	30.2±8.1	7.9±2.5	1846.3±411.8	12.7±0.2	10.9±0.3
10	48.1±6.6	4.1±1.8	2428.8±436.2	11.8±0.5	12.0±0.0
5	60.6±7.3	3.1±0.4	2942.5±318.4	11.2±0.2	17.0±1.3

Table 4.3 Effect of NaOH concentration on the mechanical properties, water contents and lignin contents of hemicellulose based films.

NaOH concentration (%)	UTS (MPa)	e_B (%)	E (MPa)	Water content (%)	Lignin content (%)
24	14.3±2.0	12.3±1.8	554.4±176.4	16.5±0.3	11.6±0.1
10	45.0±6.2	7.6±1.7	2174.6±304.8	14.4±0.1	11.8±0.0
5	46.6±11.9	4.7±1.2	2118.3±492.4	14.8±0.6	15.8±0.8

The presence of lignin together with hemicellulose was shown to promote the agglomeration of hemicellulose-lignin complexes (Westbye et al., 2007), which would affect the mechanical properties of hemicellulose based films (Bahcegul et al., 2012). Therefore, taking into account the different lignin contents of the films, the higher lignin content of the film obtained after the pretreatment conducted at 5 % alkaline concentration might also be responsible for the lower elongation values based on the agglomeration mechanism reported by Westbye et al. (2007).

The second important aspect of lignin associated with hemicellulose based films is related to the oxygen barrier property of the films. As recently shown by Ryberg et al. (2011) the presence of lignin in hemicellulose based coatings applied on polyethylene terephthalate films reduces the oxygen permeability up to more than 5-fold as compared with the coatings obtained with delignified hemicellulose. The approach demonstrated by Ryberg et al. (2011) is important since it provides a new application area for lignin as an oxygen barrier enhancer upon the utilization of hemicelluloses without delignification (Bahcegul et al., 2012). In this sense low alkaline concentration might be thought to be more advantageous compared to the pretreatment conducted at higher alkaline concentrations since the lignin contents of these films were enhanced approximately 60 and 36% as compared to the lignin contents of the films obtained after a pretreatment process conducted with a 24% concentration of corresponding alkaline solution (KOH or NaOH) for the pretreatment processes applied with 5% KOH and 5% NaOH solutions, respectively. The remarkably higher lignin content of the films obtained through low alkaline consumption during the pretreatment process is likely to cause better oxygen barrier properties on the basis of the results reported by Ryberg et al. (2011).

4.2.3 Evaluation of Alkaline Type and Concentration with a Multi-Product Perspective

In order to evaluate the alkaline types and concentrations employed during the pretreatment of corn cobs with a multi-product perspective, the glucose yield was considered together with the tensile energy to break (TEB) or toughness values of the films. TEB value takes into account both the UTS and e_B values of the films, providing an overall measure of film mechanical properties. The glucose yields were calculated on the basis of the cellulosic content of untreated corn cobs, which was determined as 33 % by dry weight. A blood glucose monitor was used to determine the glucose yields at the 48th hour of enzymatic hydrolysis.

In a study by Bahcegul and coworkers (2012), cotton stalks were subjected to alkaline pretreatment for the co-production of glucose and hemicellulose based films with a multi-product approach. To our knowledge this is the first work in the literature that experimentally considers the co-production of glucose and hemicellulose based films by providing a unique multiproduct framework as well as the first work in the literature that investigates the effect of alkaline pretreatment temperature on the properties of hemicellulose based films, which accounts for the novelties of this study. Three pretreatment temperatures (25, 60 and 90°C) were evaluated for their effects both on the glucose yield and on the properties of hemicellulose based films. Compared to untreated cotton stalks, the glucose yields were enhanced 3.9, 4.1 and 4.2 times for pretreatments conducted at 25, 60 and

90°C, respectively. The pretreatment temperature of 90°C was found as detrimental in terms of film formation. Tensile energy to break values of the films obtained after pretreatments conducted at 25, 60 and 90°C were 1.1, 0.8, and 0.4 MJ/m^3, respectively. The hemicellulosic part of the process, which considers the production of hemicellulose based films, were thought to govern the pretreatment temperature since it was more responsive to the changes in the pretreatment temperature compared to the cellulosic part that accounts for glucose production.

In our study, glucose yields were enhanced 3.8, 3.5 and 2.6 times as compared to the untreated corn cobs using KOH concentrations of 5, 10 and 24 %, respectively. When NaOH was used as an alkaline for pretreatment, glucose yields were enhanced 4.3, 3.7 and 2.3 times as compared to untreated corn cobs for the concentrations of 5, 10 and 24%, respectively. Investigation of Figure 4.7 revealed that there was a sharp decrease in terms of the glucose yields with an increase in alkaline concentration. There are some studies in the literature showing a similar decrease in glucose yields because of solid loss with increased alkaline strength (Wang et al., 2008; Hu and Wen, 2008; Varga el al., 2002). Wang et al., (2008) have reported that pretreatment of coastal bermuda grass, which has a composition of 25.6% glucan, 19.4% hemicellulose (mostly xylan), 19.3% lignin with 1%(w/v) concentration of NaOH solution yielded significantly higher reducing sugars compared to the case when 2%(w/v) or 3%(w/v) NaOH solution was used during pretreatment with a temperature of 121 °C and so it was concluded that higher NaOH concentrations used during pretreatment gave higher total solid loss as in our case, thus led to less reducing sugars yield. Another study of Hu and Wen (2008) showed that switchgrass, which has a composition of 33.6% glucan, 19.3% xylan, 21.4% lignin and others, responded unfavorably (significantly less total recoverable sugars) to NaOH loading above 10%. Varga et al. (2002) have also reported in accordance with the trend of change in glucose yield values of our results that after a 48 hour enzymatic hydrolysis of corn stover, the amount of released glucose was 20.7, 19.2, and 16.8 g per 100 g of untreated material with 1, 5, and 10 % NaOH solutions used during pretreatment, respectively.

In terms of the tensile energy to break values of hemicellulose based films, usage of NaOH solution instead of KOH solution during pretreatment caused relatively higher tensile energy to break values except for the case at which a high amount of alkaline (24 %) used as it can be seen in Figure 4.8.

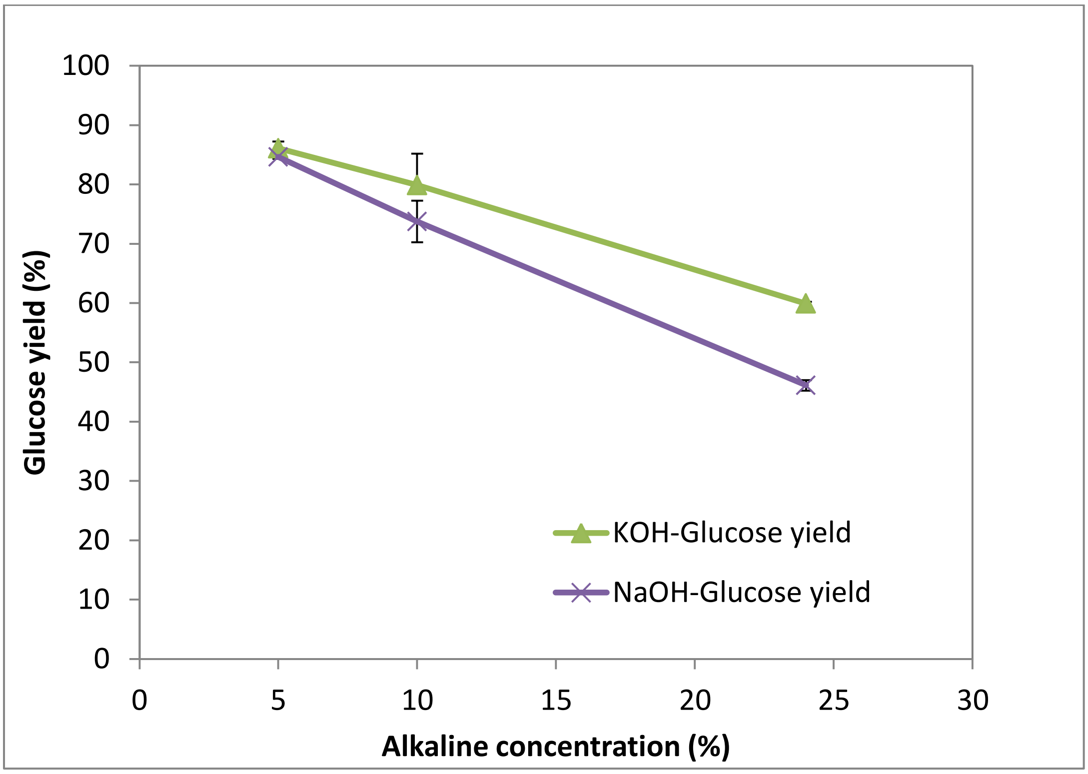

Figure 4.7 Effect of alkaline type and concentration on the glucose yield. Glucose yield is calculated on the basis of cellulose content of untreated corn cobs. Error bars represent standard deviations.

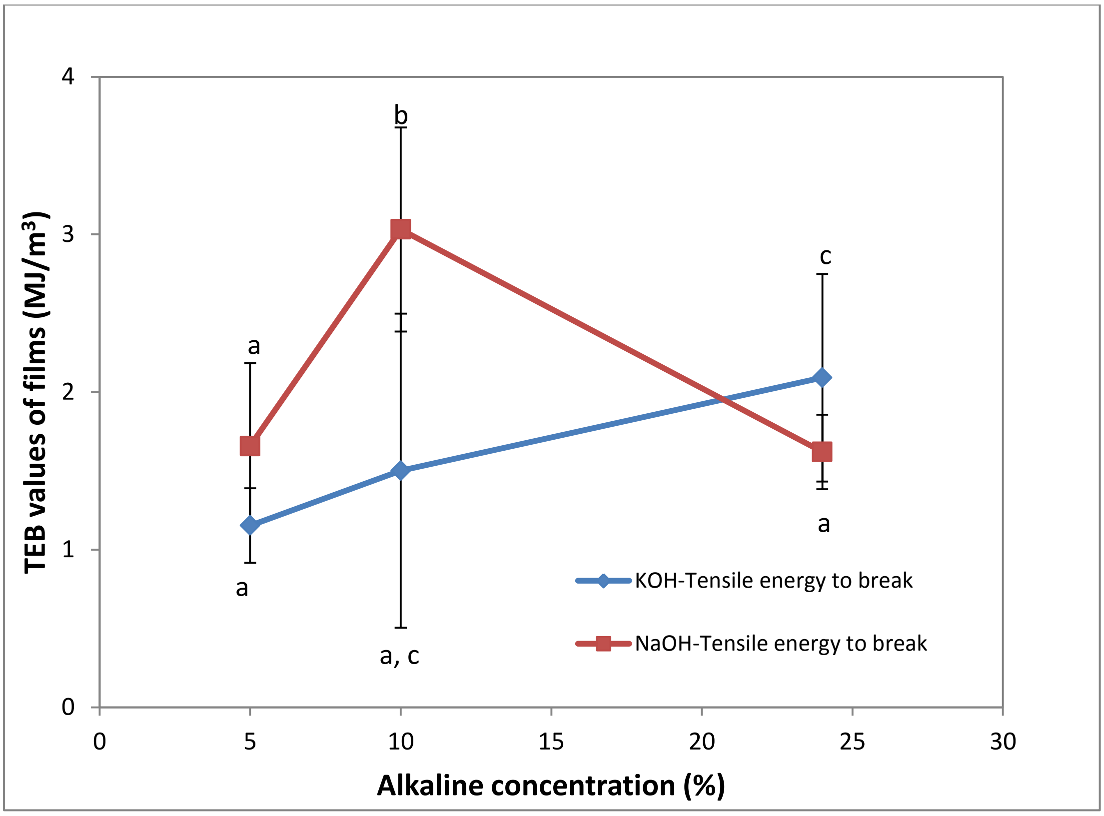

Figure 4.8 Effect of alkaline type and concentration on the tensile energy to break (TEB) values of hemicellulose based films. Error bars represent standard deviations. The small letters a, b and c mean significantly ($p<0.05$) different TEB values of films. Detailed results of one-way ANOVA tests are given in Appendix D.

Finally the amount of products (glucose and hemicellulosic fraction) obtained from corn cobs are given in Figures 4.9 and 4.10 in order to provide an overall evaluation of the multi-product process. At this point the hemicellulosic portion obtained from corn cobs is regarded as a product since it can be entirely converted to hemicellulose based films and the glucose obtained can be utilized for bioethanol production. The results in Figures 3.9 and 3.10 show that the pretreatment applied to corn cobs with a multi-product perspective resulted with an increase of the products by at least approximately 5 times as compared to untreated corn cobs. Although the highest total product yield, 55%, was obtained when 24% KOH solution was used during pretreatment (Figure 4.11), this value is only approximately 3% higher than the second highest total product yield which was

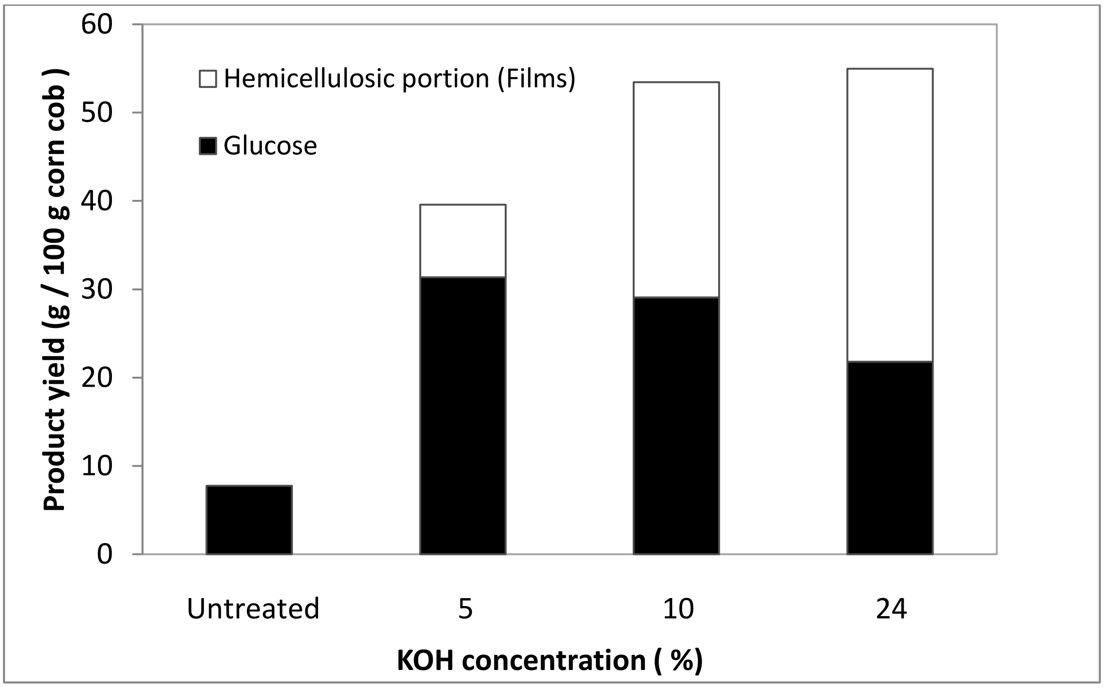

Figure 4.9 Effect of KOH concentration on the total amount of products obtained from corn cobs.

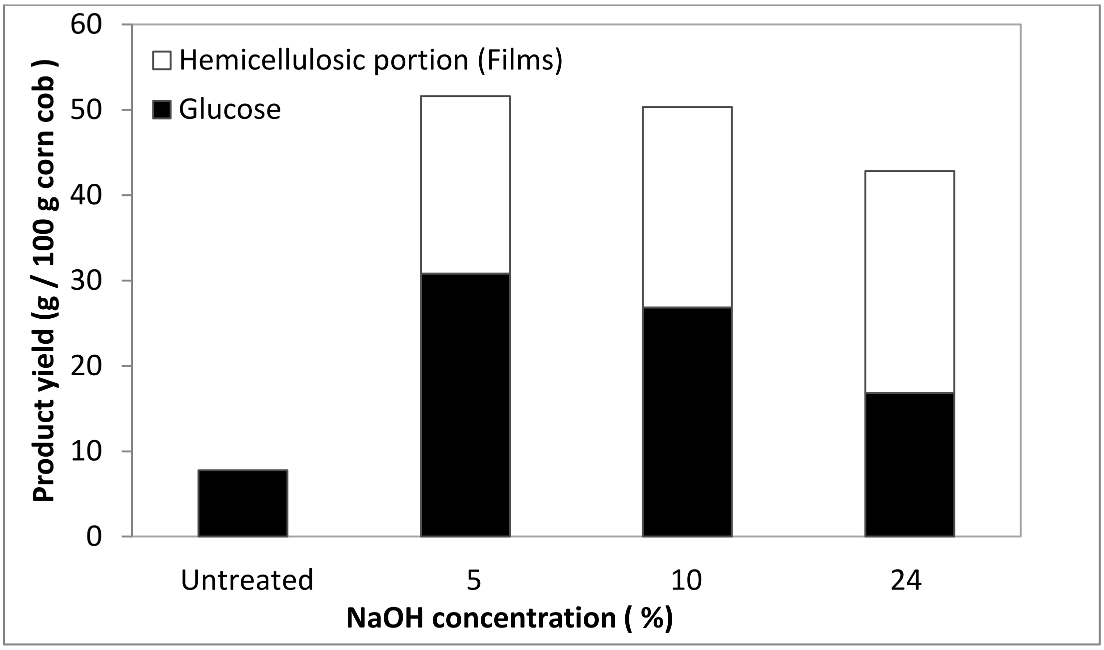

Figure 4.10 Effect of NaOH concentration on the total amount of products obtained from corn cobs.

obtained when 10% KOH solution was used during pretreatment and together with such a slight increase, there were some problems related with the properties of the films such as low ultimate tensile strength and low lignin content values. The lowest total product yields were obtained when 5% KOH or 24% NaOH solutions were used during pretreatment which were 39.6% and 42.8%, respectively. Therefore, 5% KOH will not be a suitable preference because of this low total product yield despite the fact that the properties of the films obtained at this condition showed better mechanical properties and had higher lignin content. Using 24% NaOH solution during pretreatment will not be a good choice too because of low total product yield besides low ultimate tensile strength and low lignin content values. The total product yields obtained when 5% NaOH and 10% NaOH were used during pretreatment were very close to each other which were 51.6 and 50.3%, respectively but, the lignin contents of the films obtained with 5% NaOH concentration were approximately 34% higher than the other case and ultimate tensile strength values of the films obtained at 5% NaOH were slightly higher than the ones obtained at 10% NaOH with an increasement value of 3.6%. The total product yield values of the cases which were obtained when 10% KOH or 5% NaOH solutions were used during pretreatment were also very close to each other and there is only a 1.8% difference from each other. When the mechanical properties of the films are compared, it is seen that ultimate tensile strength values and percent elongation values were not statistically different from each other ($p>0.05$). The lignin content of the films obtained with 5% NaOH solution is 31.6% higher than the ones obtained with 10% KOH solution during pretreatment. Taking into consideration all these factors besides the consumption amount of the alkaline solutions during pretreatment, it appears that the optimum results were obtained when 5% NaOH solution was used during pretreatment. The mechanical properties of the films obtained with 5% NaOH is also comparable with the results of other studies in the literature at which an additive and/or plasticizer used (Saxena et al., 2009, Peng et al., 2011). Saxena et al., (2009) obtained nanocellulosic-xylan films from oat spelt xylan, cellulose whiskers and sorbitol as plasticizer. The tensile strengths of the films even with the addition of cellulose whiskers in addition to sorbitol were very low compared to the values obtained in our study although the e_B values were remarkably higher (Table 3.3). Peng et al. (2011) also produced sorbitol plasticized xylan based films which had a Young's modulus of 735 MPa, tensile strength of 11.9 MPa and elongation at break of 3.4%. The tensile strength and percent elongation at break values obtained in our study were thought to be as comparable compared to the values of the plasticized films obtained by Peng et al. (2011).

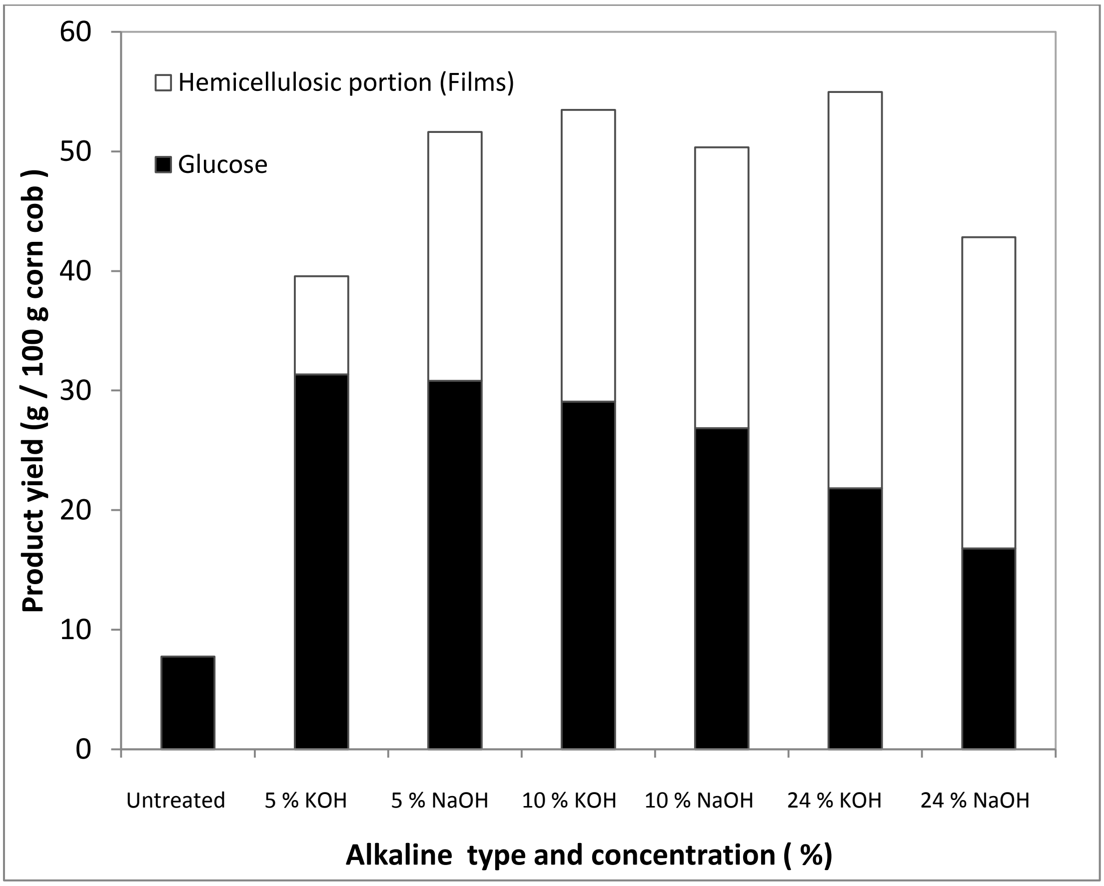

Figure 4.11 Effect of alkaline type and concentration on the total amount of products obtained from corn cobs.

4.3 Effect of Boron Compound Addition and Type during the Co-Production of Glucose and Hemicellulose Based Films from Corn Cobs

It was determined that the optimum conditions could be obtained when 5% NaOH solution used during pretreatment. $NaBH_4$ was used as a boron compound with a 1% concentration within the pretreatment solution during the investigations regarding the effect of alkaline type and concentration on the properties of the products were being conducted. The reason for using H_3BO_3 or $NaBH_4$ is that hemicelluloses can be extracted from biomass by an alkaline pretreatment; however a portion may be extremely resistant to extraction and the addition of H_3BO_3 or $NaBH_4$ to KOH or NaOH increases the dissolving power (Gonzalez et al., 1986; Lawther et al., 1996). In the following part, the experiments were done with H_3BO_3 which is a cheaper and less toxic chemical and without any boron compound to understand the effect of boron compound on the amount of glucose obtained and the properties of the films.

4.3.1 Effect of Boron Compound Addition and Type on the Enzymatic Hydrolysis of Corn Cobs

As observed in Figure 4.12, the reducing sugar yields at the end of 48 hour of enzymatic hydrolysis were obtained as 29.0, 26.4 and 26.3 g/l for the cases containing 1% $NaBH_4$, 1% H_3BO_3 and no boron compound, respectively. Use of 1% H_3BO_3 within the pretreatment solution does not have a remarkable effect on the amount of reducing sugars released following an enzymatic hydrolysis since approximately same amount of reducing sugar was obtained compared to the case when there was no boron compound used during pretreatment. The use of 1% $NaBH_4$ solution during pretreatment was shown to increase the reducing sugar production slightly compared to the other two cases. The effect of boron compound on reducing sugar concentration could also be compared with the results of Bahcegul et al. (2012) which were determined through enzymatic hydrolysis of cellulosic portion obtained after pretreatment of cotton stalk (cellulose content is 33%) without any boron compound. The reducing sugar concentration at the end of

48 hour of enzymatic hydrolysis was approximately 12 g/l which was notably lower than the reducing sugar concentration values obtained in this study.

As seen in Figure 4.13 that the utilization of boron compound during pretreatment has a positive effect on the cellulosic portion recovery. The cellulosic portion recoveries were obtained as 45.3, 47.0 and 52.0% for no boron compound, $NaBH_4$ and H_3BO_3 cases, respectively. There was an approximately 7% increase in the amount of cellulosic portion recovery when H_3BO_3 was used as a boron compound instead of $NaBH_4$ during pretreatment. For hemicellulosic portion recoveries, utilization of H_3BO_3 instead of $NaBH_4$ during pretreatment caused an approximately 27% loss and the hemicellulosic portion recovery decreased to the value of 15.1% from 20.8% as observed in Figure 4.14. In the case of that there was no boron compound used during pretreatment, hemicellulosic portion recovery remained almost constant compared to the case when 1% $NaBH_4$ was used during pretreatment.

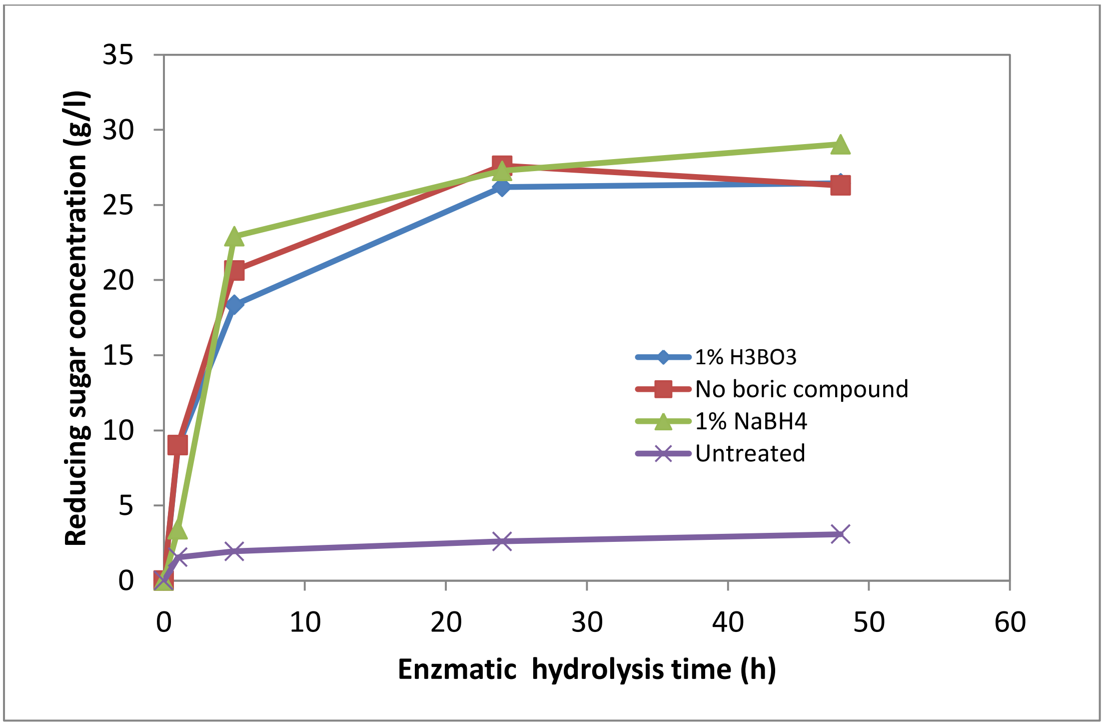

Figure 4.12 Effect of boron compound addition and type on the enzymatic hydrolysis of corn cobs. Error bars represent standard deviations.

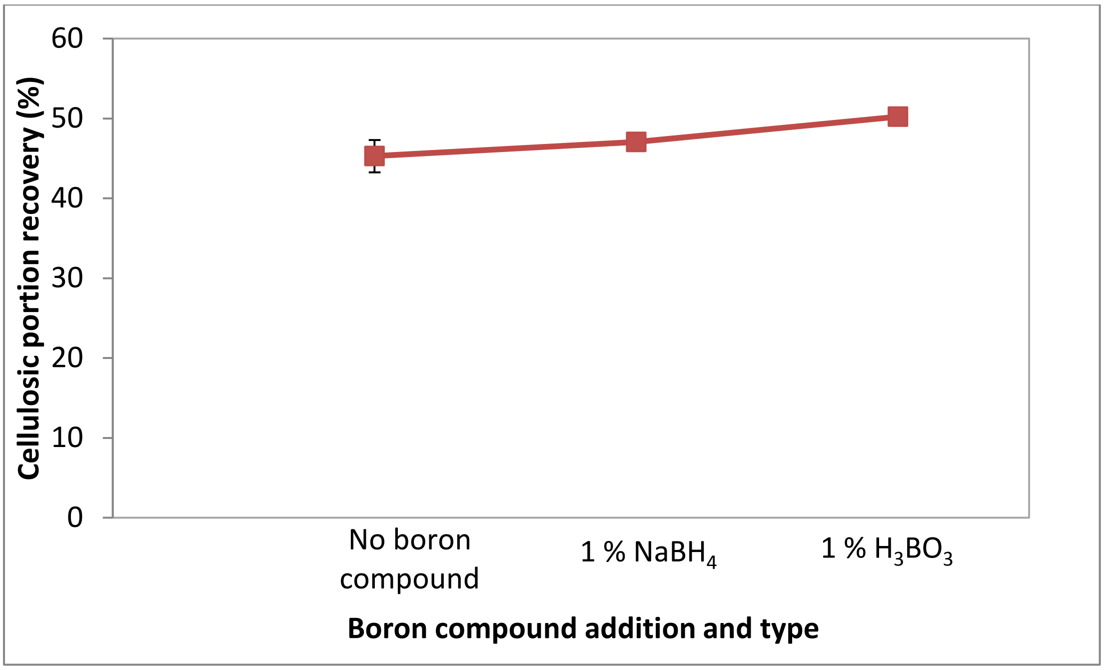

Figure 4.13 Effect of boron compound addition and type on the amount of cellulosic portions recovered after pretreatment. Error bars represent standard deviations.

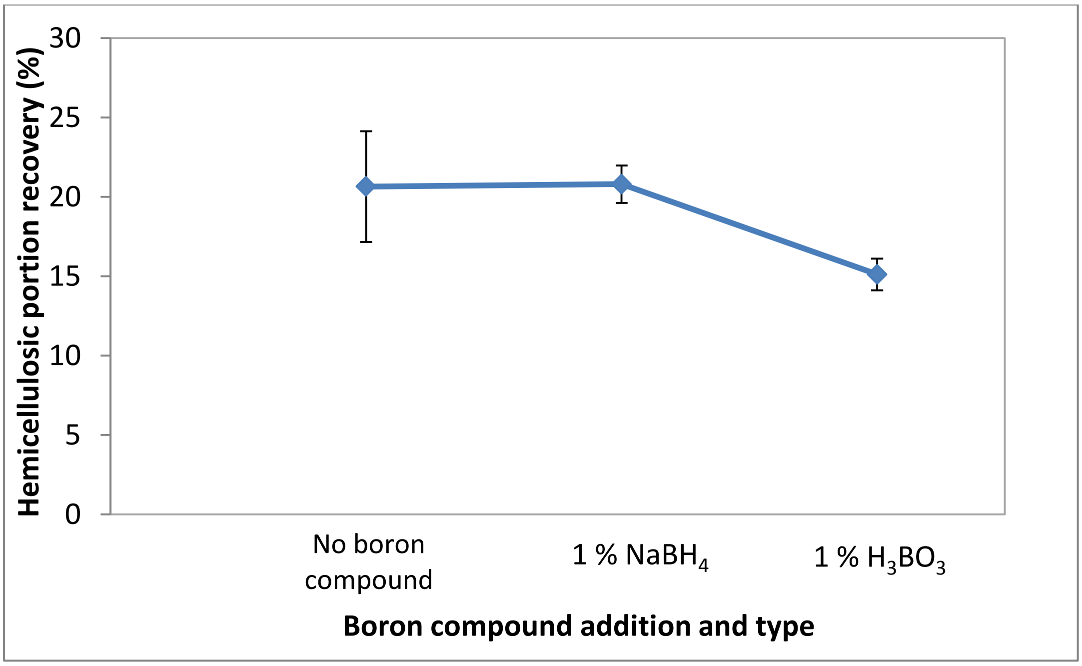

Figure 4.14 Effect of boron compound addition and type on the amount of hemicellulosic portions recovered after pretreatment. Error bars represent standard deviations.

4.3.2 Effect of Boron Compound Addition and Type on the Properties of Hemicellulose Based Films

As observed in Figure 4.15, there was not seen a significant difference between the visual properties of the films in all the cases with or without boron compounds. It was also observed that addition and type of the boron compound did not lead to any problem concerning the film formation properties.

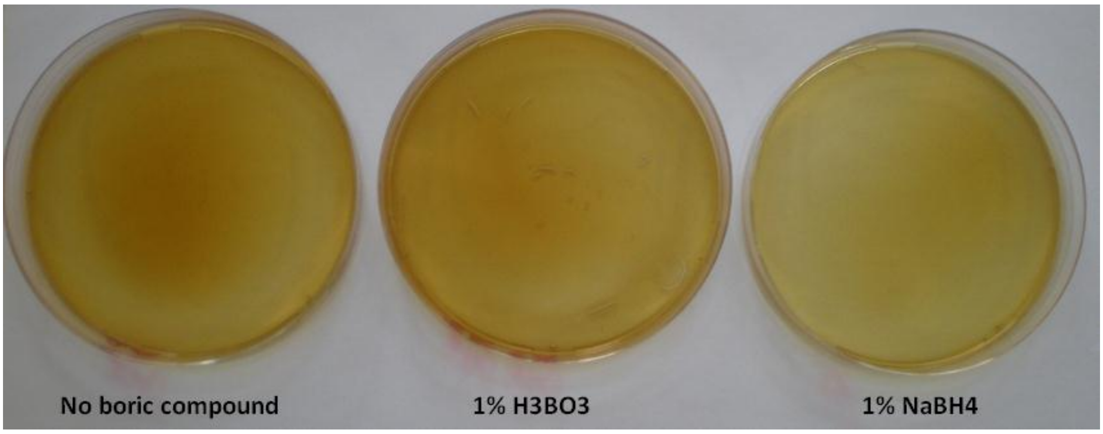

Figure 4.15 Effect of boron compound addition and type on the visual appearances of hemicellulose based films. From left to right: Films obtained from hemicelluloses isolated at the end of pretreatments conducted without boron compound and with 1% H_3BO_3 and 1% $NaBH_4$.

The effect of boron compound addition and type on the mechanical properties, water contents and lignin contents of hemicellulose based films can be seen in Table 4.4 and the effect of boron compound addition and type on the stress-strain curves of the films can be seen in Appendix C. In the light of the data from Table 4.4 a notable improvement in the mechanical properties of the films was observed when $NaBH_4$ was used compared to the cases containing boron acid or no boron compound. The ultimate tensile strength values of the films were obtained as 9.7, 8.6 and 38.8 MPa with 0% for the cases of no boron compound, H_3BO_3 and $NaBH_4$, respectively. The increase in the values of the strength of the films with the use of

$NaBH_4$ during pretreatment could be seen as noteworthy in terms of the applicability of the films in packaging applications. The elongation at break values which were 14.4, 18.5 and 5.5 % with no boron compound, 1% H_3BO_3 and 1% $NaBH_4$, respectively were changing in accordance with the water content of the films due to its plasticizer effect as it can be seen in Table 3.4 (Salme´n and Olsson, 1998; Gröndahl et al., 2004; Höije et al., 2005; Bahcegul et al., 2012; Escalante et al., 2012). The lowest elongation at break value, 5.5 %, was obtained with 1 % $NaBH_4$ and this could be related with the water content value of the film which was the lowest value among the others.

The lignin contents were taken into account in terms of their effect on the oxygen barrier properties of the films based on the studies of Ryberg *et al.* (2011) while evaluating the effect of boron compound addition and type during the production of glucose and hemicellulose based films from corn cobs simultaneously. The lignin contents of the films were obtained as 16.5, 17.6 and 15.8 % with no boron compound, H_3BO_3 and $NaBH_4$, respectively. Despite the fact that the films produced with no boron compound and 1 % H_3BO_3 would most probably have good oxygen barrier properties because of the higher lignin contents based on the study of Ryberg et al. (2011), the obtained films were not strong and this could be considered as unfavorable in terms of applicability. Therefore, taking into account the factors mentioned above, it could be said that optimum conditions in terms of film properties could be obtained with 1% $NaBH_4$.

Table 4.4 Effect of boron compound addition and type on the mechanical properties, water contents and lignin contents of hemicellulose based films.

Boron compound addition and type	**UTS (MPa)**	**e_B (%)**	**E (MPa)**	**Water content (%)**	**Lignin content (%)**
No boron compound	9.7±6.0	14.4±4.0	364.2±315.2	16.4±1.6	16.5±0.5
1% H_3BO_3	8.6±4.6	18.5±6.3	246.1±188.6	17.3±0.6	17.6±0.3
1% $NaBH_4$	38.8±11.4	5.5±1.3	1742.5±517.1	13.6±0.1	15.8±0.8

4.3.3 Evaluation of Boron Compound Addition and Type with a Multi-Product Perspective

As seen in Figure 4.16 there was not a sharp decrease in the glucose yield when 1% H_3BO_3 was used instead of 1% $NaBH_4$ during pretreatment and the glucose yields were obtained as 84.6% and 80.9% with 1%$NaBH_4$ and 1% H_3BO_3 solutions, respectively. Similarly, there was not seen a sharp decrease in glucose yield when the pretreatment was conducted without any boron compound and the glucose yield was obtained as 79.2%. Taking into consideration all these results, it could be easily said that there was not a substantial effect of addition and type of boron compound being used during pretreatment on the glucose yields. However, a significant effect on the mechanical properties of the films was observed. The highest value of tensile energy to break was obtained with 1% $NaBH_4$ and a slight decreasing trend with the use of no boron compound and boric acid (Figure 4.17).

When the results obtained for the glucose production and film properties are considered together, it appears that it is the hemicellulosic part of the process that should influence the decision made on the addition of boron compound and type rather than the cellulosic part of the process.

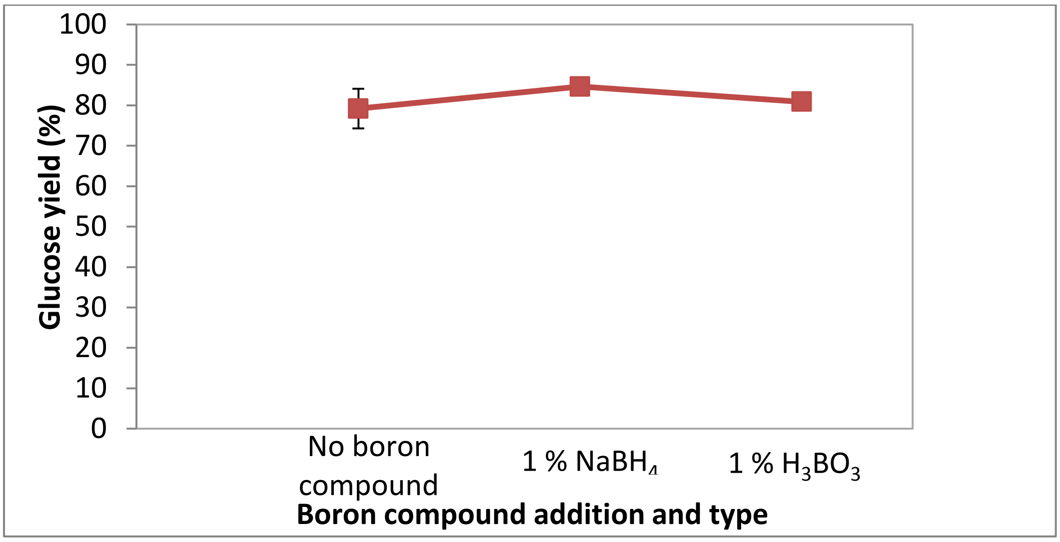

Figure 4.16 Effect of boron compound addition and type on the glucose yield. Glucose yield is calculated on the basis of cellulose content of untreated corn cobs. Error bars represent standard deviations.

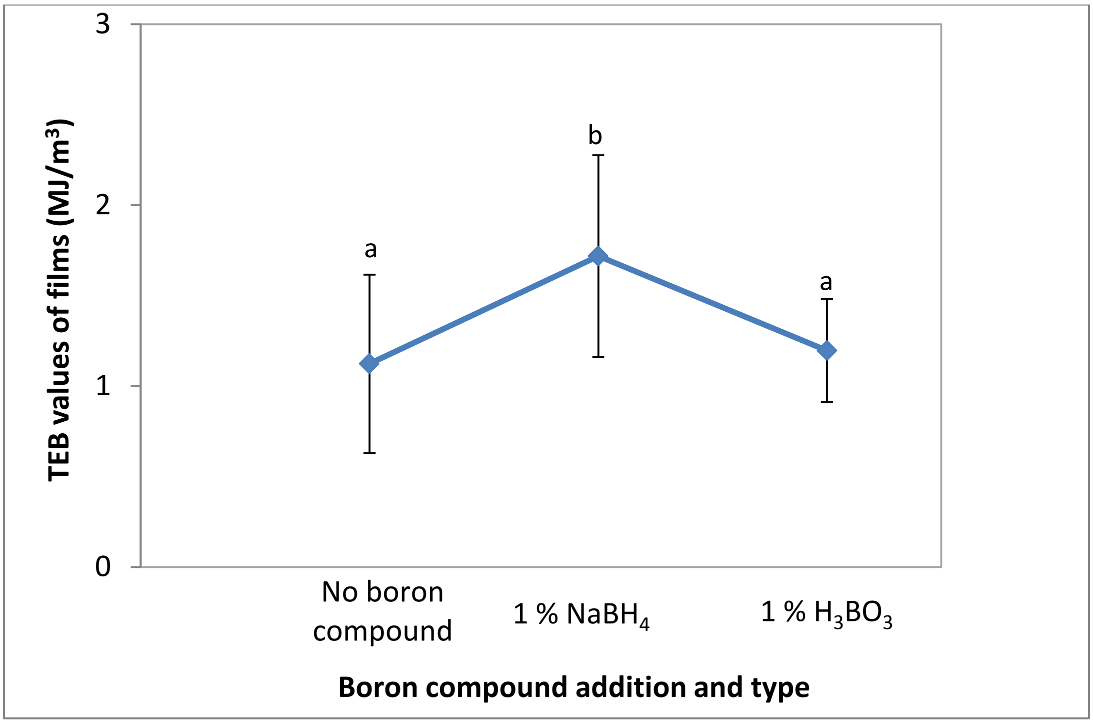

Figure 4.17 Effect of boron compound addition and type on the tensile energy to break (TEB) values of hemicellulose based films. Error bars represent standard deviations. The small letters a and b mean significantly ($p<0.05$) different TEB values of films. Detailed results of one-way ANOVA tests are given in Appendix D.

In the final stage, Figure 4.18 was used to get an overall evaluation regarding the effect of boron compound addition and type on the total amount of the products obtained. The total product yields were enhanced 7.7, 8.0 and 6.9 times as compared to untreated corn cobs. The low amount of hemicellulosic portion obtained with 1% H_3BO_3 was seen as the reason of relatively lower total product yield obtained with 1% H_3BO_3 than the others. The amounts of hemicellulosic portions were 20.7, 20.8 and 15.1 grams per 100 grams of corn cob for no boron compound, 1% $NaBH_4$ and 1% H_3BO_3 containing pretreatments, respectively. There was approximately 4% increase in the total amount of the products with 1% $NaBH_4$ solution used during pretreatment as compared to the case when there is no boron compound used during pretreatment besides the good mechanical properties of the films discussed before. Considering all the aspects discussed so far, the most suitable conditions could be attained with the use of 1% $NaBH_4$ solution during pretreatment on a multi-product basis under the aforementioned conditions.

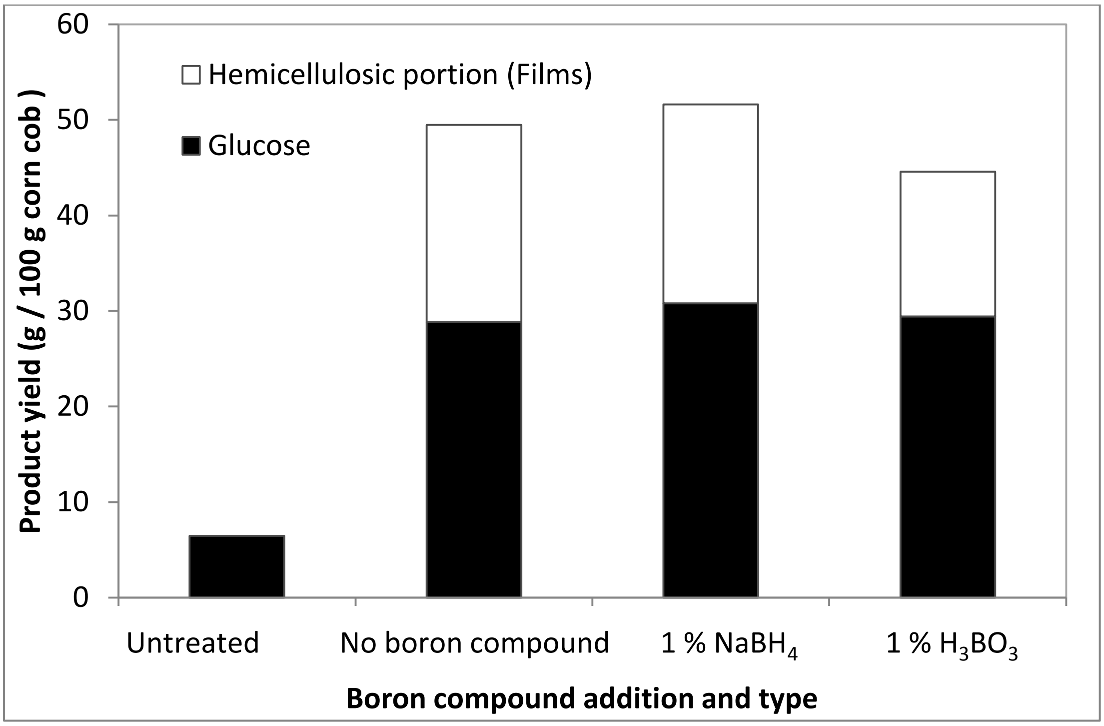

Figure 4.18 Effect of boron compound addition and type on the total amount of products obtained from corn cobs.

4.4 Effect of Pretreatment Time during the Co-Production of Glucose and Hemicellulose Based Films

As a result of the experiments conducted in order to understand the effect of alkaline type and concentration and boron compound addition and type on the properties of the products, it was found that the most suitable conditions could be obtained with the use of 5 % NaOH and 1 % $NaBH_4$ solution during pretreatment. Pretreatment time which has been reported to influence the severity of the process, and consequently the effectiveness of biomass pretreatment and hydrolysis was the final parameter to be investigated within the scope of this study (Overend *et al*., 1987; Kaar *et al*., 2000; Mosier *et al*., 2005; Hu and Wen, 2008). The optimum conditions in terms of alkaline type and concentration and boron compound addition and type were determined using a pretreatment time in the range of 1-3 hours for the purpose of getting the optimum conditions with a multi-product perspective.

4.4.1 Effect of Pretreatment Time on the Enzymatic Hydrolysis of Corn Cobs

As seen in Figure 4.19, the concentrations of reducing sugars at the end of 48 hour of enzymatic hydrolysis were enhanced 8.7, 9.5 and 9.7 times as compared to untreated corn cobs for pretreatment times of 3 hours, 2 hours and 1 hour, respectively. Approximately 10% increase in the amount of reducing sugars was observed by decreasing pretreatment time from 3 hours to 2 hours and 1 hour.

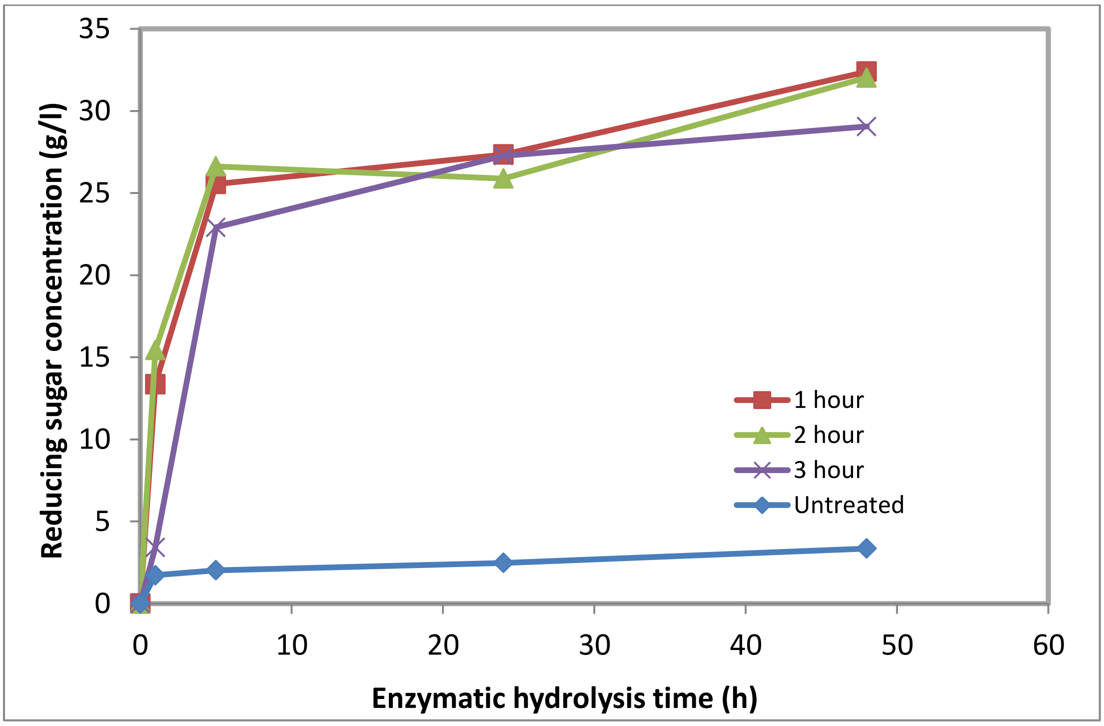

Figure 4.19 Effect of pretreatment time on the enzymatic hydrolysis of corn cobs. Error bars represent standard deviations.

As shown in Figure 4.20, the hemicellulosic portion recovery increased from 20.8% to 30.1% when the pretreatment time shortened to the value of 2 hours from 3 hours. On the other hand, there was a significant decrease in the hemicellulosic portion recovery when the pretreatment time decreased to 1 hour from 3 hours and the hemicellulosic portion recovery decreased from 30.1% to 24.4%. For the cellulosic portion recoveries, the pretreatment time was not so effective compared to its effect on the amount of hemicellulosic portion. As it can be seen in Figure 4.21, the cellulosic portion recoveries were obtained as 47.0, 45.2 and 50.1% with pretreatment times of 3 hours, 2 hours and 1 hour, respectively.

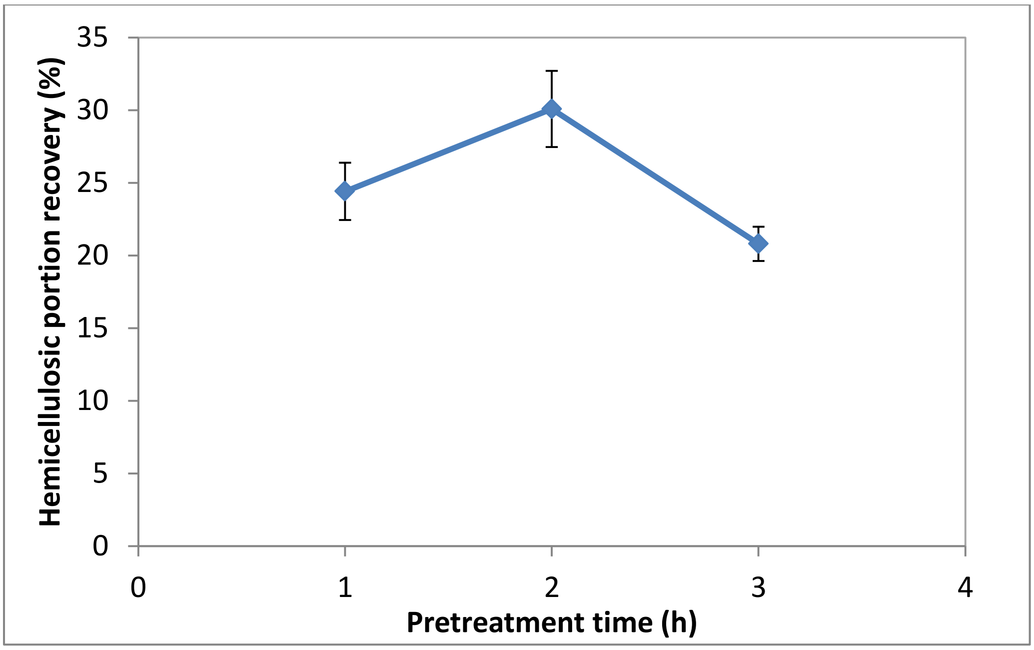

Figure 4.20 Effect of pretreatment time on the amount of hemicellulosic portions recovered after pretreatment. Error bars represent standard deviations.

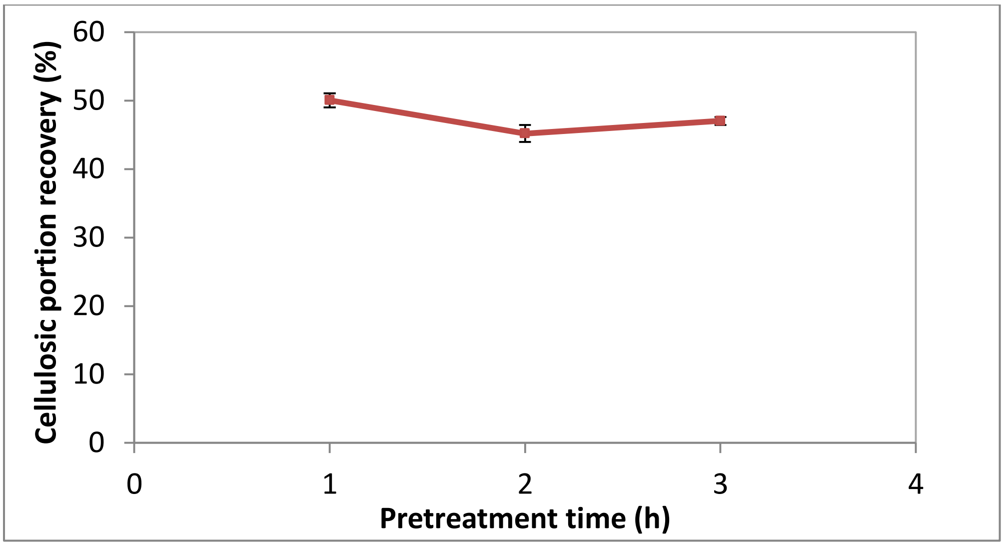

Figure 4.21 Effect of pretreatment time on the amount of cellulosic portions recovered after pretreatment. Error bars represent standard deviations.

4.4.2 Effect of Pretreatment Time on the Properties of Hemicellulose Based Films

As shown in Figure 4.22, no major visual difference was observed between the films obtained with different pretreatment times. It was also determined that the change in the pretreatment time did not give rise to any problem about the film formation properties of the hemicellulosic portions obtained after pretreatment.

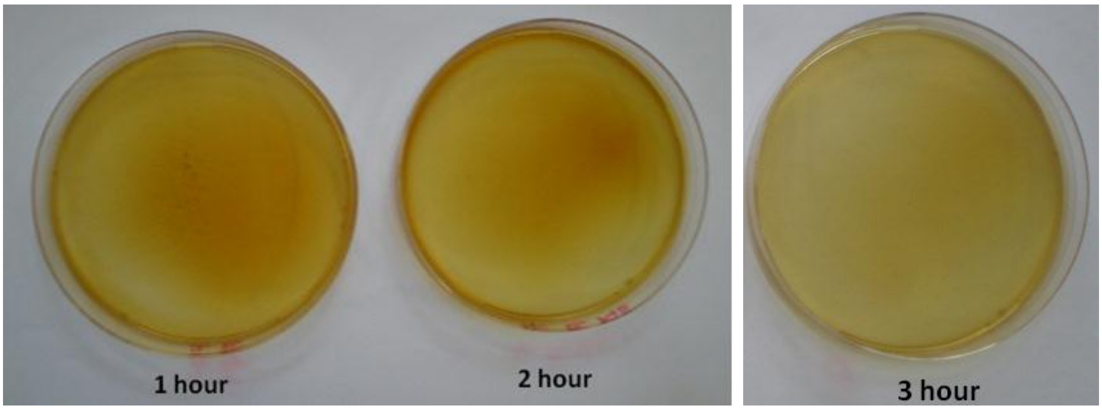

Figure 4.22 Effect of pretreatment time on the visual appearances of hemicellulose based films. From left to right: Films obtained from hemicelluloses isolated at the end of pretreatments conducted with 1, 2 and 3 hour(s).

According to the data given in Table 4.5, it was deduced that the mechanical properties of the films were strongly based on pretreatment time. The effect of pretreatment time on the stress-strain curves of the films can also be seen in Appendix C. Sharp decreases in the ultimate tensile strength, Young’s modulus and lignin content values of the films were observed in the case of shorter pretreatment times as compared to a 3 hour pretreatment time. However, a sharp increase in elongation at break values with shorter pretreatment times compared to the pretreatment time of 3 hours. These relatively higher elongation at break values obtained with shorter pretreatment times could be related with the relatively higher amount of water content of the films since water is acting as a plasticizer (Salme´n and Olsson, 1998; Gröndahl et al., 2004; Höije et al., 2005; Bahcegul et al., 2012; Escalante et al., 2012). The highest lignin content, 15.8%, was also obtained with a 3 hour pretreatment and so, it would most probably show better oxygen barrier properties than the others according to the study of Ryberg *et al.* (2011).

Table 4.5 Effect of pretreatment time on the mechanical properties, water contents and lignin contents of hemicellulose based films.

Pretreatment time (h)	**UTS(MPa)**	**e_B (%)**	**E(MPa)**	**Water content (%)**	**Lignin content (%)**
1	7.3±4.7	20.2±3.4	192.0±160.9	18.0±0.8	8.7±0.7
2	5.4±2.7	22.0±6.6	123.3±88.0	17.9±0.6	7.6±0.8
3	38.8±11.4	5.5±1.3	1742.5±517.1	13.6±0.1	15.8± 0.8

4.4.3 Evaluation of Pretreatment Time with a Multi-Product Perspective

As seen in Figures 3.23 and 3.24, the changes in the glucose yields and tensile energy to break values of the films with different pretreatment times show similar trends. The glucose yield decreased approximately 12% to the value of 74.6% from 84.6% by shortening the pretreatment time from 3 hours to 2 hours. In the case of further shortening of pretreatment time from 3 hours to 1 hour, the glucose yield decreased to the value of 77.3 % from 84.6 %. The shorter pretreatment times also had a detrimental effect on the mechanical properties of the films except elongation at break as seen in Figure 4.24. A sharp decrease in tensile energy to break values of the films was observed with decreasing pretreatment time. The tensile energy to break values were obtained as 1.72, 1.04, 1.23 MJ/m^3 with pretreatment times of 3 hours, 2 hours and 1 hour, respectively.

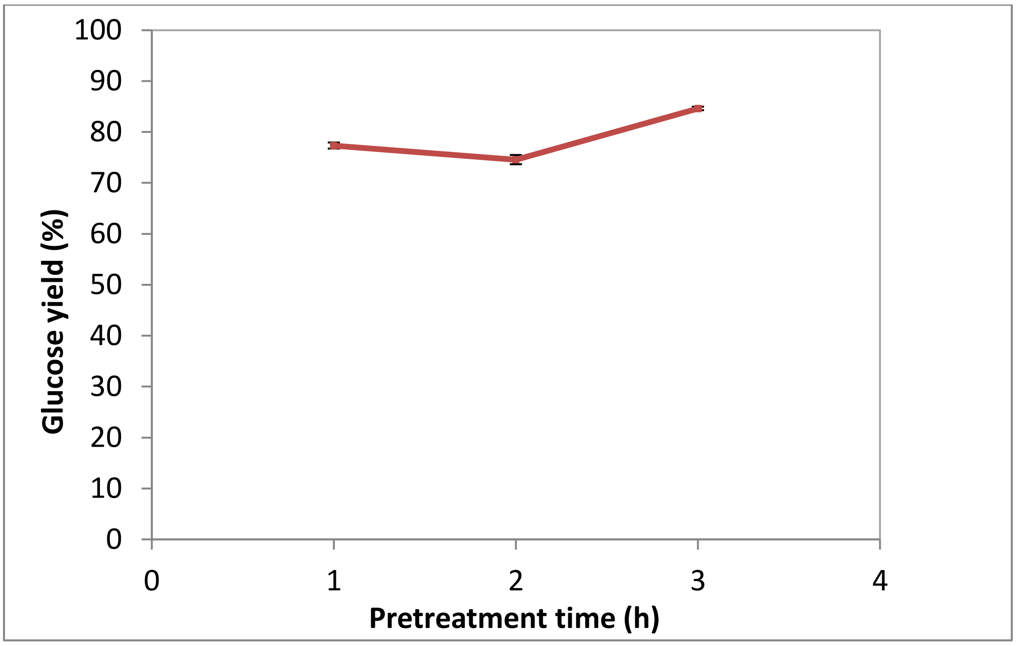

Figure 4.23 Effect of pretreatment time on glucose yield. Glucose yield is calculated on the basis of cellulose content of untreated corn cobs. Error bars represent standard deviations.

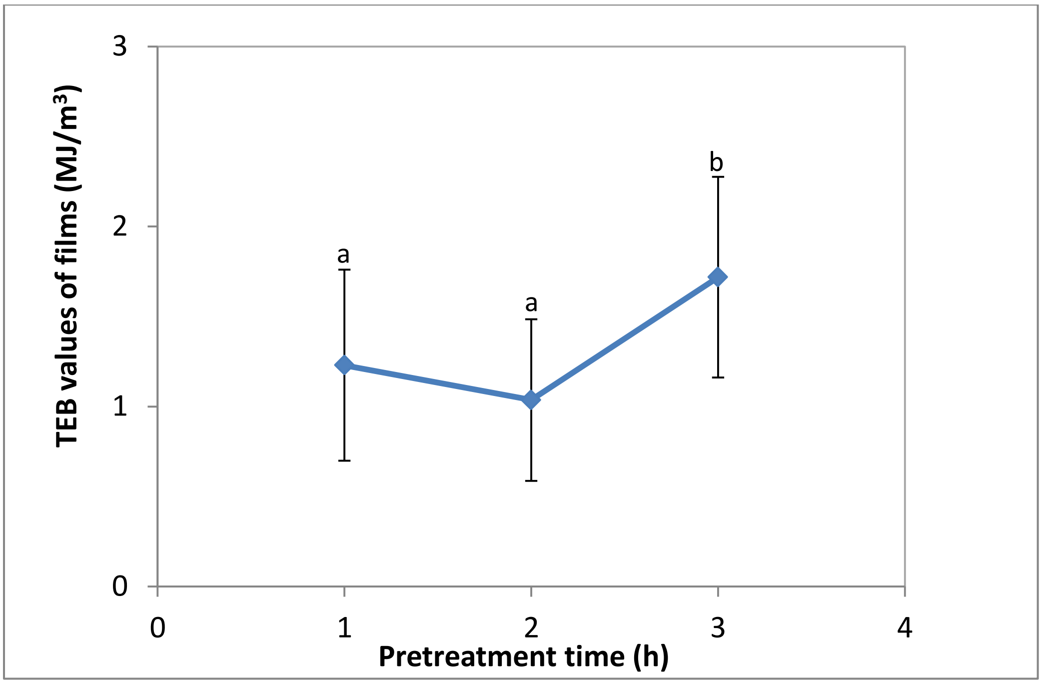

Figure 4.24 Effect of pretreatment time on tensile energy to break (TEB) values of hemicellulose based films. Error bars represent standard deviations. The small letters a and b mean significantly ($p<0.05$) different TEB values of films. Detailed results of one-way ANOVA tests are given in Appendix D.

Finally the amount of the products which were glucose and hemicellulosic fraction were taken into account in order to investigate the feasibility of the process in terms of the effect of pretreatment time on the properties of the products obtained with a multi-product perspective. According to Figure 4.25, the total product yield was enhanced 8.4, 9.1 and 8.2 times as compared to untreated corn cobs with pretreatment times of 1 hour, 2 hours and 3 hours, respectively. Therefore, it can be deduced that there was an increase in the amount of products by at least 8.2 times compared to untreated corn cobs with indicated conditions. Although the highest total product yield was obtained with a 2 hour pretreatment, the properties of the hemicellulose based films were not as good as the properties of the films obtained with a 3 hour pretreatment as discussed before. In the light of all these factors together, the optimum process conditions could be given as 5% NaOH, 1% $NaBH_4$ and 3 hours of pretreatment time (Figure 4.26).

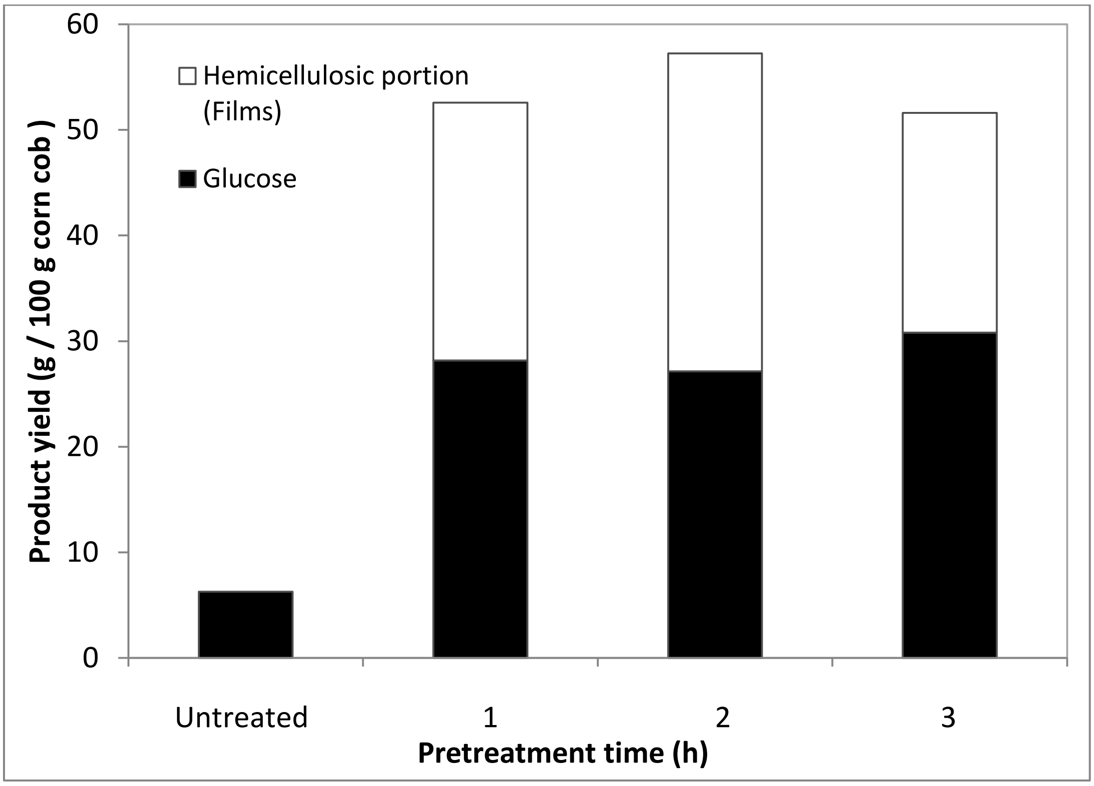

Figure 4.25 Effect of pretreatment time on the total amount of products obtained from corn cobs.

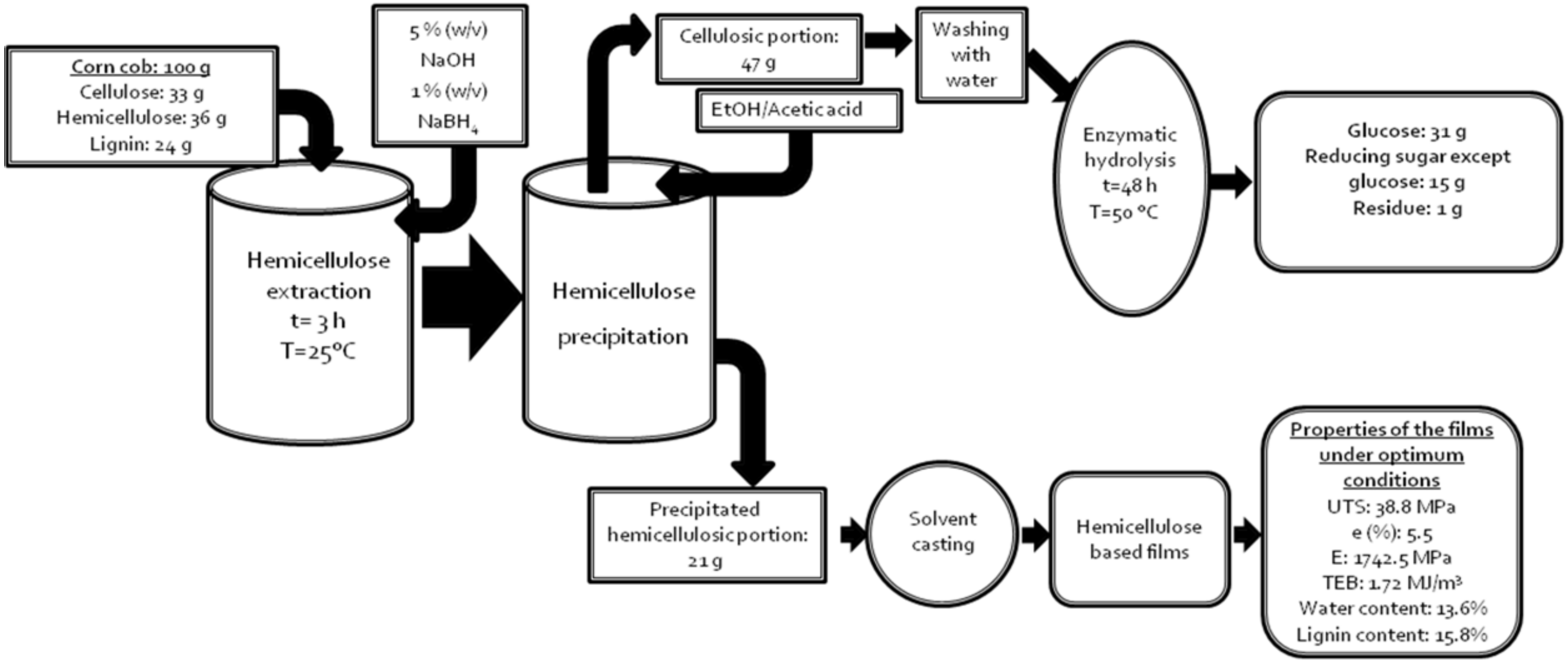

Figure 4.26 Schematic presentation of the bi-product process with optimum process conditions.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The alkaline pretreatment parameters which were alkaline compound type and concentration, boron compound utilization and type and pretreatment time were evaluated on a multi-product basis in terms of their effect on the co-production of cellulose based glucose and hemicellulose based films from corn cobs. The optimal process conditions were determined as 5% NaOH, 1% $NaBH_4$ and a 3 hour pretreatment time. The utilization of boron compound, especially $NaBH_4$, within the pretreatment solution significantly enhanced the mechanical properties of the films and there was not such a remarkable effect on the enzymatic glucose production. As a result of the optimization process, the amount of alkaline consumption was decreased and converted to a relatively cheaper alkaline compound. Thus, the pretreatment conditions were enhanced in terms of the co-production of cellulose based glucose and hemicellulose based films from corn cobs with a multi-product perspective. Moreover, a broad range of knowledge has been obtained for the development of multiproduct biorefinery concept through this study.

For further studies, it will be focused on the improvement of the process by using lower alkaline and boron compound concentrations and the effect of pretreatment temperature will also be investigated. Different agricultural wastes such as corn stalk, sunflower stalk and wheat bran may also be studied to develop the current process knowledge in terms of the multiproduct biorefinery concept.

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APPENDIX A

PREPARATION OF DNS SOLUTION AND BUFFER

A.1 Preparation of 3,5-Dinitrosalicylic Acid (DNS) Solution

1 % (w/v) DNS solution is prepared by dissolving 10 grams of NaOH, 361.5 grams of sodium potassium tartrate, 10 grams of 3, 5-dinitrosalicylic acid, 2 grams of phenol and 0.5 gram of sodium sulfite in 1 liter of distilled water. The solution is stored in a dark colored bottle.

A.2 Preparation of 0.05 M Sodium Citrate Buffer

1.47 grams of trisodium citrate dihydrate and 1.05 grams of citric acid monohydrate are dissolved in distilled water separately until the final volumes of the solutions are 100 mililiters. Then, the citric acid monohydrate solution is poured into the 100 mililiters of trisodium citrate dihydrate solution until the final pH is adjusted to 4.8.

APPENDIX B

CALIBRATION CURVES

B.1 Reducing Sugar Calibration Curve (DNS Method)

The reducing sugar concentration (C_R) was calculated by using the following formula:

$$C_R\left(\frac{mg}{ml}\right) = \frac{Absorbance - b}{a} \times Dilution\ factor \times \frac{1\ mg}{1000\ \mu g} \qquad (B.1)$$

where a is the slope and b is the y-intercept of the calibration curve.

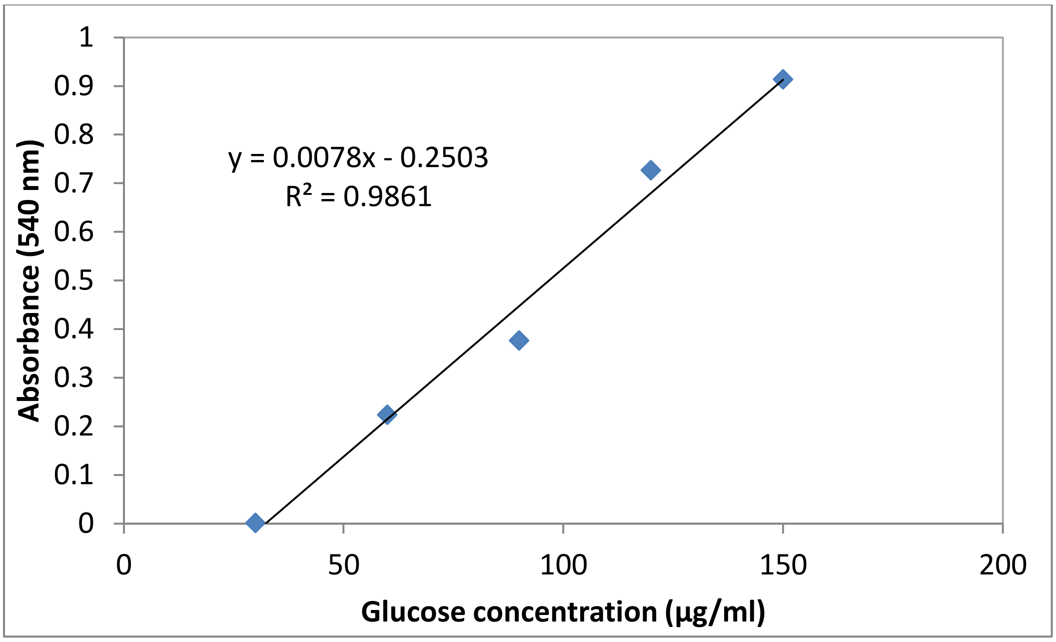

Figure B.1 Glucose calibration curve used in DNS method

B.2 Glucose Calibration Curve (Blood Glucose Monitor (BGM) Method)

The glucose concentration (C_G) was calculated by using the following formula:

$$C_G\left(\frac{mg}{ml}\right) = \left(\frac{BGM\ reading - b}{a} \times Dilution\ factor\right) \times \frac{1\ dl}{100\ ml} \qquad (B.2)$$

where BGM reading (mg/dl) is the value read from the blood glucose monitor, a is the slope and b is the y-intercept of the calibration curve.

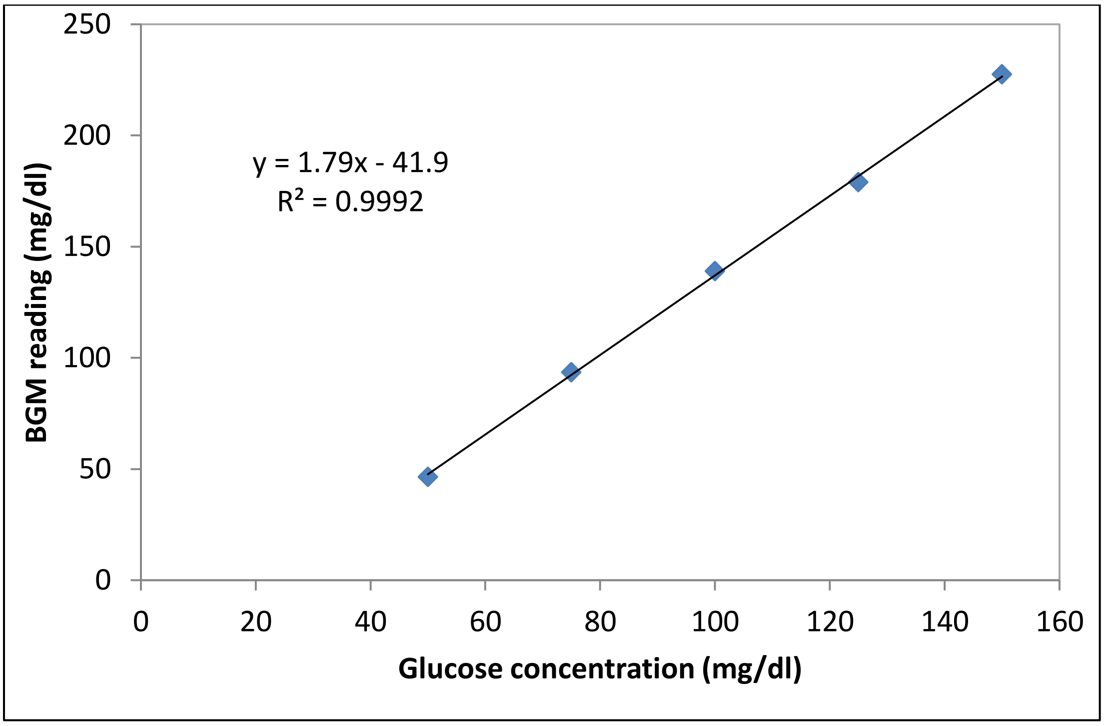

Figure B.2 Glucose calibration curve used in blood glucose monitor (BGM) method

B.3 Lignin Calibration Curve

The lignin concentration (C_L) was calculated by using the following formula:

$$C_L\left(\frac{mg}{ml}\right) = \left(\frac{Absorbance - b}{a} \times Dilution\ factor\right) \times \left(\frac{1\ mg}{1000\ \mu g}\right) \qquad (B.3)$$

where a is the slope, b is the y-intercept of the calibration curve.

The lignin content was calculated using the formula given below:

$$Lignin\ content\ (\%) = \frac{C_L \times volume\ of\ the\ solution}{weight\ of\ the\ sample\ used} \times 100 \qquad (B.4)$$

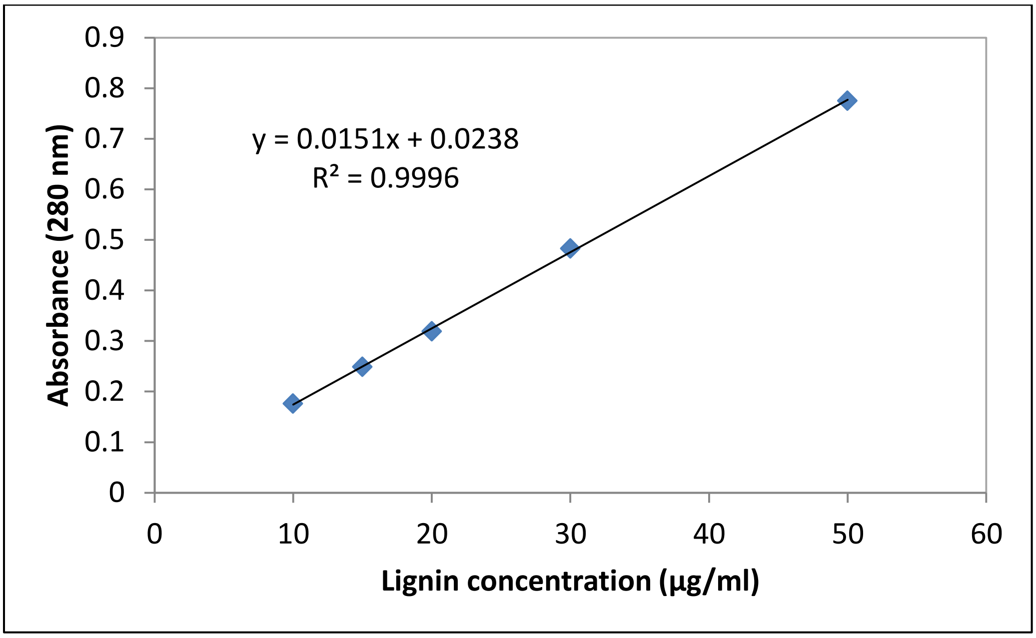

Figure B.3 Lignin calibration curve

APPENDIX C

STRESS-STRAIN CURVES

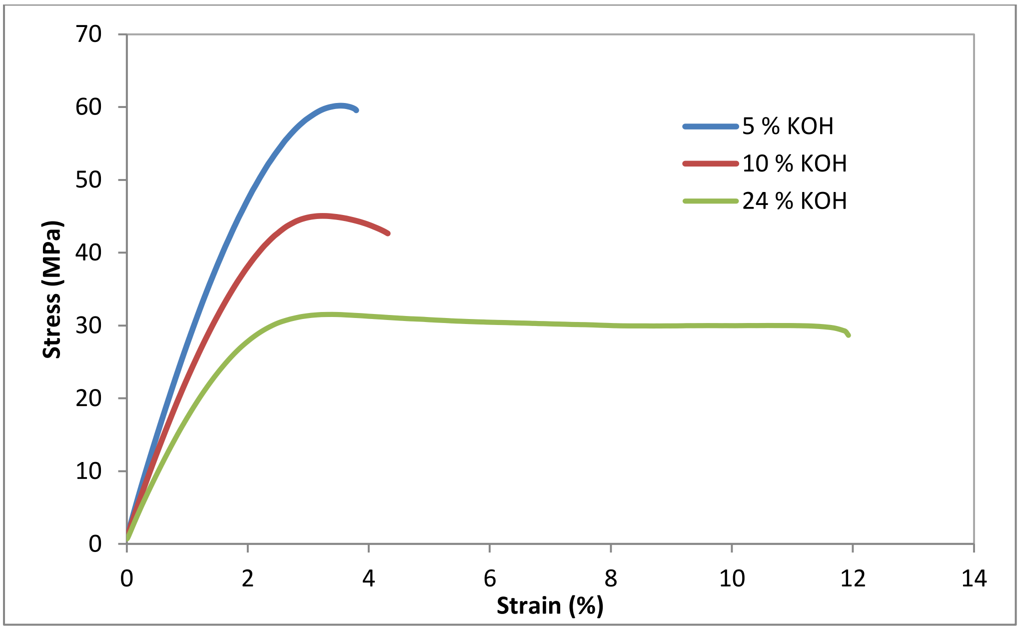

Figure C.1 Effect of KOH concentration on the stress-strain curves of the films. Each sample stress-strain curve was chosen among the other stress-strain curves on the basis of giving an overall perspective about the stress-strain behavior of the films.

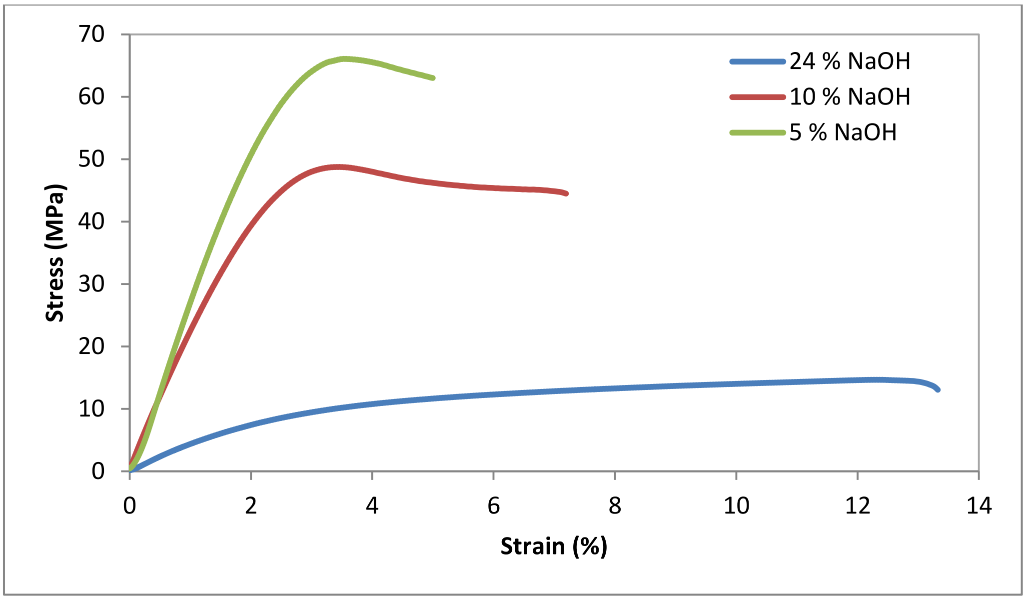

Figure C.2 Effect of NaOH concentration on the stress-strain curves of the films. Each sample stress-strain curve was chosen among the other stress-strain curves on the basis of giving an overall perspective about the stress-strain behavior of the films.

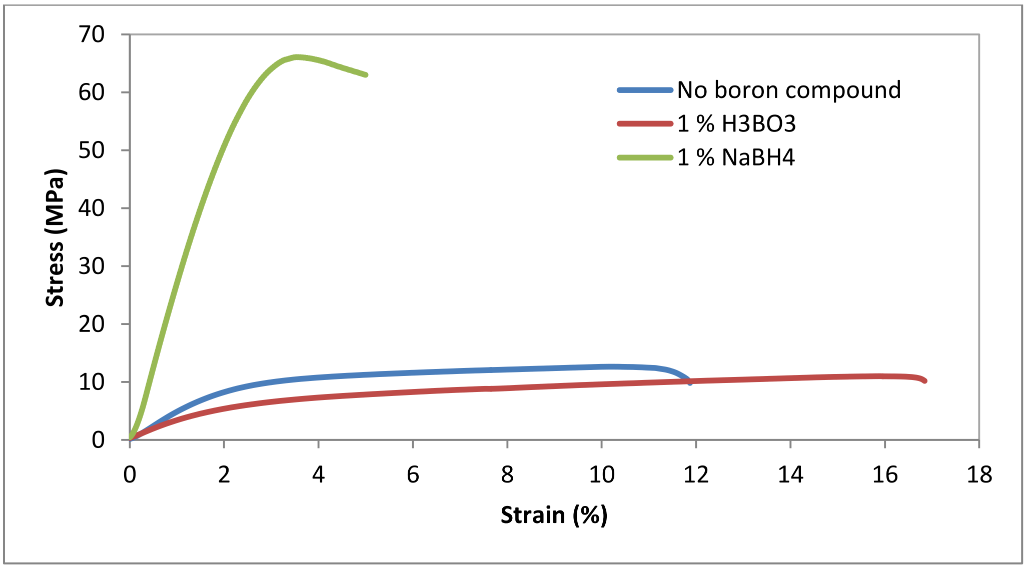

Figure C.3 Effect of boron compound addition and type on the stress-strain curves of the films. Each sample stress-strain curve was chosen among the other stress-strain curves on the basis of giving an overall perspective about the stress-strain behavior of the films.

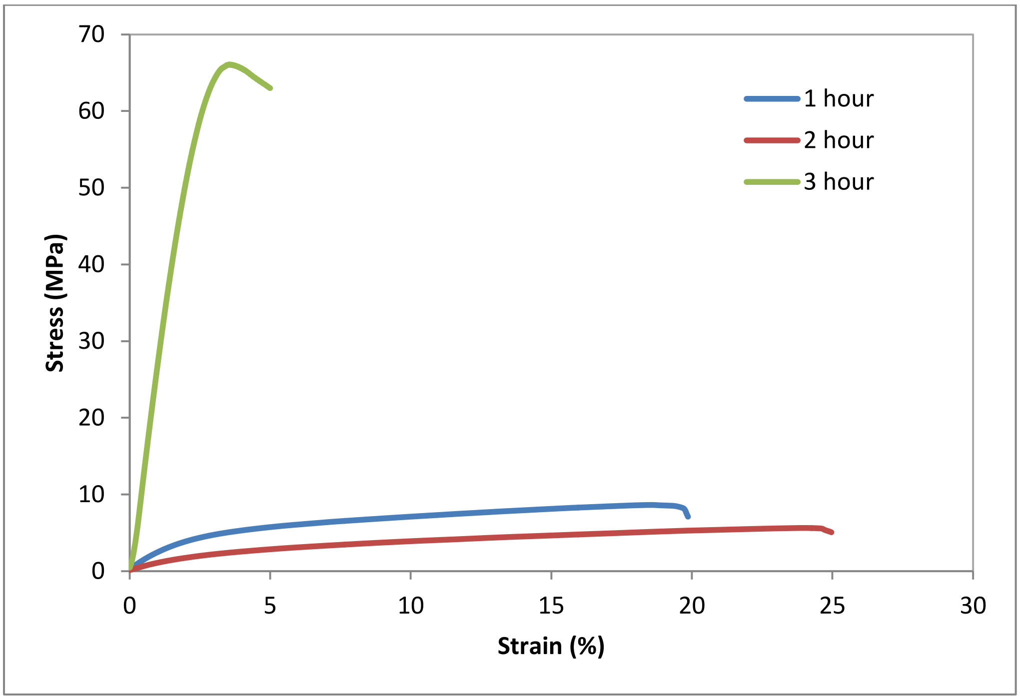

Figure C.4 Effect of pretreatment time on the stress-strain curves of the films. Each sample stress-strain curve was chosen among the other stress-strain curves on the basis of giving an overall perspective about the stress-strain behavior of the films.

APPENDIX D

THE RESULTS OF ONE-WAY ANALYSIS OF VARIANCE (ANOVA) TESTS

Table D.1 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 24 % NaOH and 10 % NaOH.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	6.891	6.891	21.9	0.000
Within groups	14	4.406	0.315		
Total	15	11.296			

Table D.2 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 24 % NaOH and 5 % NaOH.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	0.005	0.005	0.03	0.877
Within groups	9	1.599	0.178		
Total	10	1.603			

Table D.3 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 10 % NaOH and 5 % NaOH.

Source	**Degrees of freedom**	**Sum of squares**[*]	**Mean Square**[**]	**F**[***]	**p**[****]
Between groups	1	7.342	7.342	19.83	0.000
Within groups	15	5.554	0.370		
Total	16	12.895			

Table D.4 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 24 % KOH and 10 % KOH.

Source	**Degrees of freedom**	**Sum of squares**[*]	**Mean Square**[**]	**F**[***]	**p**[****]
Between groups	1	1.501	1.501	2.05	0.174
Within groups	14	10.249	0.732		
Total	15	11.749			

Table D.5 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 24 % KOH and 5 % KOH.

Source	**Degrees of freedom**	**Sum of squares**[*]	**Mean Square**[**]	**F**[***]	**p**[****]
Between groups	1	3.610	3.610	14.95	0.002
Within groups	14	3.380	0.241		
Total	15	6.990			

Table D.6 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 10 % KOH and 5 % KOH.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	0.456	0.456	0.85	0.381
Within groups	14	7.789	0.556		
Total	15	8.244			

Table D.7 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 24 % NaOH and 24 % KOH.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	0.721	0.721	2.52	0.141
Within groups	11	3.145	0.286		
Total	12	3.866			

Table D.8 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 24 % NaOH and 10 % KOH.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	0.051	0.051	0.07	0.791
Within groups	11	7.554	0.687		
Total	12	7.605			

Table D.9 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 24 % NaOH and 5 % KOH.

Source	**Degrees of freedom**	**Sum of squares***	**Mean Square****	**F*****	**p******
Between groups	1	0.6682	0.6682	10.72	0.007
Within groups	11	0.6853	0.0623		
Total	12	1.3535			

Table D.10 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 10 % NaOH and 24 % KOH.

Source	**Degrees of freedom**	**Sum of squares***	**Mean Square****	**F*****	**p******
Between groups	1	4.022	4.022	9.63	0.006
Within groups	17	7.100	0.418		
Total	18	11.122			

Table D.11 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 10 % NaOH and 10 % KOH.

Source	**Degrees of freedom**	**Sum of squares***	**Mean Square****	**F*****	**p******
Between groups	1	11.046	11.046	16.32	0.001
Within groups	17	11.509	0.677		
Total	18	22.555			

Table D.12 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 10 % NaOH and 5 % KOH.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	16.402	16.402	60.09	0.000
Within groups	17	4.640	0.273		
Total	18	21.042			

Table D.13 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 5 % NaOH and 24 % KOH.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	0.674	0.674	1.88	0.195
Within groups	12	4.293	0.358		
Total	13	4.967			

Table D.14 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 50 % NaOH and 10 % KOH.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	0.098	0.098	0.14	0.719
Within groups	12	8.702	0.725		
Total	13	8.800			

Table D.15 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 5 % NaOH and 5 % KOH.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	0.880	0.880	5.76	0.034
Within groups	12	1.833	0.153		
Total	13	2.713			

Table D.16 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 1 % H_3BO_3 and 1 % $NaBH_4$.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	1.680	1.680	7.48	0.012
Within groups	24	5.390	0.225		
Total	25	7.070			

Table D.17 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 1 % $NaBH_4$ and no boron compound.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	2.534	2.534	9.05	0.006
Within groups	27	7.561	0.280		
Total	28	10.096			

Table D.18 One way Anova test results which were conducted to compare the tensile energy to break values of the films with 1 % H_3BO_3 and no boron compound.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	0.029	0.029	0.17	0.685
Within groups	21	3.635	0.173		
Total	22	3.664			

Table D.19 One way Anova test results which were conducted to compare the tensile energy to break values of the films with pretreatment times of 1 hour and 2 hours.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	0.154	0.154	0.61	0.446
Within groups	15	3.761	0.251		
Total	16	3.915			

Table D.20 One way Anova test results which were conducted to compare the tensile energy to break values of the films with pretreatment times of 1 hour and 3 hours.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	1.469	1.469	4.89	0.037
Within groups	24	7.202	0.300		
Total	25	8.671			

Table D.21 One way Anova test results which were conducted to compare the tensile energy to break values of the films with pretreatment times of 2 hours and 3 hours.

Source	Degrees of freedom	Sum of squares*	Mean Square**	F***	p****
Between groups	1	2.264	2.264	8.09	0.010
Within groups	21	5.875	0.280		
Total	22	8.138			

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* Sum of squares is the mean sum value of squared deviations of each subgroup mean from the overall mean.

** Mean square is the mean sum value of squared deviations of each item from its subgroup mean.

*** $F = \frac{Mean\ square\ for\ between\ groups}{Mean\ square\ for\ within\ groups}$ and F value compares the actual variation of the mean values of groups with the expected variation of the mean values of groups.

**** p value is the indicator of significance level.