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**IMPACT OF ALKALI PRETREATMENT AND TORREFACTION ON  
GLUCOSE PRODUCTION FROM WHEAT STRAW**

A Thesis in

Agricultural and Biological Engineering

by

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## ABSTRACT

Bioethanol has captured the attention of the world as a renewable fuel alternative to petroleum-based liquid fuels that can maintain environmental quality and energy security for countries. Lignocellulosic biomass has been researched for its potential as an ethanol feedstock due largely to the fact that it does not necessarily compete with food crops. In this study, wheat straw (an agricultural residue) was chosen as the potential feedstock. This study evaluated the possibility of using torrefaction, alkaline pretreatment, and their combination as a pretreatment process. The effect of torrefaction time, torrefaction temperature, and alkaline concentration was investigated in terms of glucose yields. Pretreatment with torrefaction alone resulted in 66-74% lower glucose yields when compared to. Pretreatment with NaOH resulted in 82-89% increase in glucose yield relative to un-pretreated wheat straw. Pretreatment with both NaOH and torrefaction resulted in 8-68% increase in glucose yield. The highest glucose yield of  $359.1 \pm 1.8$  mg glucose  $\text{g}^{-1}$  raw wheat straw is achieved for samples that were not torrefied but were alkaline pretreated with 1% NaOH solution. Increasing the torrefaction temperature decreased the glucose yield. Alkaline pretreatment dramatically improved glucose yield for samples torrefied at 220°C. This study demonstrated the potential of torrefaction as a pretreatment when combined with an alkaline solution.

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## Chapter 1

### **Introduction**

World energy use is steadily rising in response to growing human population and rising standards of living. According to the International Energy Outlook (IEA, 2017), energy intake in the world is expected to increase by 28% from 2015 to 2040. Fossil fuels such as oil, coal, and natural gas are the main sources that are being used to meet this increasing demand. Concerns about the sustainability of fossil fuel based energy have led to development of alternatives to replace fossil fuels. Biofuels, which are produced from biomass (organic material that comes from plants, animals, and their byproducts) are a promising option that already occupies a sizeable niche in the liquid fuel sector, providing a potentially renewable and sustainable replacement for petroleum-based liquid fuel. Bioethanol is the leading biofuel in the world. (Alvira et al. 2010; Talebnia et al. 2010). According to Talebnia (2010), Licht (2006) states that bioethanol production was approximately 45 and 49 billion liters per year in 2005 and 2006, and has more than doubled in 10 years (Renewable Fuels Association, accessed on: 02/2018 ). Countries like the United States, Canada, Brazil, Japan, India, and Europe are establishing internal biofuel markets and plans to use biofuels (Mussatto et al., 2010). The USA and Brazil are the main bioethanol producers in the world, producing about 85% of global production together.

Ethanol is called bioethanol if the substrate used for the production is organic based. In general, sources for bioethanol can be classified as sugars, starch, or lignocellulose. Bioethanol is categorized as first generation (sugars and starch), or second generation

(lignocellulose) depending on the substrate. Also, the conversion processes differ, primarily according to the way sugar is obtained for fermentation. Extraction is the only process needed for sugar crops, whereas starch crops need an additional step called hydrolysis to convert starch to glucose for fermentation. On the other hand, lignocellulosic biomass has to be pretreated prior to hydrolysis to increase enzyme accessibility (Zabed et al., 2017).

The USA and Brazil use corn and sugarcane, respectively as primary ethanol feedstocks. However, lignocellulosic biomass has captured attention as a future feedstock for ethanol production due to the fact that it does not necessarily compete with food crops since it can utilize agricultural residues, herbaceous crops from non-agricultural land, forestry wastes, wood, waste paper, and municipal waste (Saini et al., 2015). Wheat straw, an agricultural residue, and lignocellulosic feedstock is a byproduct of wheat grain production. According to The International Grains Council (2018) wheat is estimated to be the second major grain produced after corn in the upcoming year.

Like all lignocellulosic feedstocks, the main components of wheat straw are cellulose, hemicellulose, and lignin. In order for ethanol production from lignocellulosic feedstocks to be economical, the process must yield high amounts of ethanol per kg of feedstock. Pretreatment is a key step for achieving high yields of ethanol from lignocellulosic feedstocks.

The concept of this project is to study two pretreatment types and their combinations for glucose production, leading to ethanol production. Alkaline pretreatment and torrefaction are chosen for the pretreatment experiments.

## Chapter 2

### Literature Review

#### 2.1. Introduction

Energy demand and consumption in the world is growing every day (EIA 2017). Perhaps as a response to this, people are becoming more aware of the environmental issues related to using non-renewable energy. This awareness in the society has led to new and alternative sources for energy. Bioenergy that is derived from plant-based organic materials is a renewable alternative source for petroleum derived fuels (Balat et al. , 2008). Solid biomass, biogas, biodiesel, and bioethanol can be categorized as bioenergy types.

This literature review presents background information about bioenergy, biofuels, bioethanol, feedstocks used for bioethanol, pretreatment processes for lignocellulosic ethanol, and wheat straw as a potential source for glucose production.

#### 2.2. Bioenergy

Bioenergy can be defined as energy that is derived from organic materials such as; starch and sugar crops, oil-rich seeds, lignocellulosic feedstocks, and organic wastes. This energy can be in the form of heat, power, or biofuel for transportation (Khanal and Li, 2015). Biological materials that are used to produce energy either by directly burning or

by processing to produce another type of fuel are referred to as biomass (Lerner and Lerner, 2008a).

### **2.2.1. Bioenergy from Solid Biomass**

Wood, dedicated crops, logging residues, dried agricultural residues and animal waste have been used for heat and cooking for centuries if not millennia. Biomass is the most widely used form of renewable energy in the world, accounting for 14% of 18% of all renewables (World Energy Resources Bioenergy, 2016). The main use of solid biomass is for heating via combustion and producing electricity by using steam (Jones, et al. 2014). Deforestation and pollutants that are emitted to the air are problems associated with direct use of solid biomass (World Energy Resources Bioenergy, 2016).

### **2.2.2. Bioenergy from Biogas**

Biogas is a form of bioenergy, which can be produced from biomass via bacteria in an anaerobic digester or landfill. Biogas can be used to replace natural gas or propane in heat and power production or it can also be used as a transportation fuel. Biogas consists of several different gasses, methane and carbon dioxide being the main components (Weiland, 2010).

Biogas can be produced from organic wastes, food processing wastes, lignocellulosic biomass, animal manure and other substrates as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as their main components.

Producing methane with anaerobic digestion consists of four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In the hydrolysis step, organic material is broken down to monomers. Acidogenic microorganisms then ferment monomers into hydrogen, carbon dioxide, and low molecular weight volatile organic acids. In the third step, organic acids are converted to acetic acid via acetogenic microorganisms. Finally, methanogenic microorganisms convert acetic acid, hydrogen, and carbon dioxide to methane and carbon dioxide (Martins das Neves et al., 2009; Weiland, 2010).

### **2.2.3. Bioenergy from Liquid Biofuel**

Biofuel is a specific term used for liquid fuels produced from biological materials that can be used as transportation fuel. Common examples for biofuel are; biodiesel and bioethanol (Khanal and Li, 2015).

#### **2.2.3.1. Biodiesel**

The American Society for Testing and Materials (ASTM) defines biodiesel as “a fuel comprised of monoalkyl esters of long-chain fatty acids derived from vegetable oils or animal fats, designated B100” (Hoekman et al. 2012). Biodiesel can be produced by using vegetable oil feedstock, animal fats, and waste sources. While soybean oil is the main source in America for biodiesel production, European countries use rapeseed oil and palm oil is used in Asia (Hoekman et al., 2012; Roy et al. 2016).

Biodiesel is produced via transesterification, which is a series of chemical reactions where triglycerides from the substrate reacts with alcohol to produce fatty acid alkyl esters (biodiesel) and glycerol. Transesterification is affected by parameters such as; free fatty acid content and moisture content of the substrate, type of catalyst, reaction time and temperature, mixing intensity, organic solvent usage, alcohol to oil ratio and type of alcohol. Among these parameters alcohol to oil ratio affects the ester yield the most. Transesterification reaction is an equilibrium reaction and in order for the reaction to approach complete conversion, excess alcohol is often added. (Meher et al. 2006).

#### **2.2.3.2 Bioethanol**

Bioethanol is also known as ethyl alcohol, ethanol or chemically  $C_2H_5OH$  or EtOH. Bioethanol is a liquid fuel that is produced from different types of biomass (Balat et al., 2008). Its favorable properties as an alternative fuel for petroleum-based fuels include its low toxicity, low boiling point, high octane number and energy content (Kumar et al. 2016).

Bioethanol has been used in internal combustion engines since 1897 and has been considered to be a suitable alternative to fossil fuel due in part to the fact that it is renewable and oxygenated (Balat et al., 2008; Saini et al., 2015). Bioethanol production in the world was around 97.2 billion liters in 2015 (Renewable Fuels Association,2018) which makes it the most common liquid biofuel in the world (Biodiesel, the next most common accounted for only 4.8 billion liters in 2015). The United States and Brazil are currently the leading ethanol producers in the world right now as shown in Table 2.1 (Renewable Fuels

Association,2018). Around 60% of the bioethanol production in the world comes from maize and 30% from sugar based crops (OECD/FAO, 2017). Several feedstocks and their potential for ethanol production are given in Table 2.2.

Table 2.1 Ethanol production in the world \*

Region	Ethanol Production (million liters)	
	2015	2016
USA	56,051	58,027
Brazil	26,850	27,615
European Union	5,250	5,213
China	3,078	3,199
Canada	1,650	1,650
Thailand	1,264	1,219
Argentina	799	999
India	799	852
Rest of the world	1,480	1,855

\*Renewable Fuels Association (Accessed on: 02/2018)



Table 2.2 Representative of different biomass and their ethanol yield. \*

<b>Raw biomass</b>	<b>Bioethanol potential yield (L/tonne)</b>
Sugarcane	70-90
Sugar beet	95-107
Sorghum bagasse	250
Corn	370-470
Oat	264
Sweet potatoes	125-170
Potato	80-100
Wheat	376-435
Corn cob	510
Corn stover	450
Wheat straw	490

\*(Adapted from Zabed et al., 2017)

## **2.3. Production of Bioethanol**

Bioethanol can be obtained from feedstocks that contain fermentable sugar or materials that can be converted into fermentable sugar. The conversion process and the technology used for ethanol production depends on the raw material.

### **2.3.1. Sugar as a Feedstock for Ethanol Production**

Sugar producing feedstocks that are used for fermentation include; fruits, sugarcane, sugar beets, and sweet sorghum. Another commonly used feedstock is molasses, which is a byproduct of sugar production from sugarcane and sugar beets. Use of these

feedstocks varies by region and country. Brazil uses sugarcane as their primary bioethanol sugar feedstock whereas India uses molasses, and Europe and North America use sugar beets (Zabed et al., 2017).

Producing bioethanol from sugar crops is relatively easy when compared to starch or lignocellulosic crops. Sugars can be broken down to monosaccharides by microorganisms such as *Saccharomyces cerevisiae* without needing any type of pretreatment prior to fermentation (Cardona and Sánchez, 2007; Sánchez and Cardona, 2008). The general process of producing bioethanol from sugar crops is depicted in Figure 2.1. There are several byproducts of ethanol production from sugar crops. Bagasse can be used to produce electricity; whereas vinasse and filter cake can be used as fertilizer (Zabed et al., 2017).

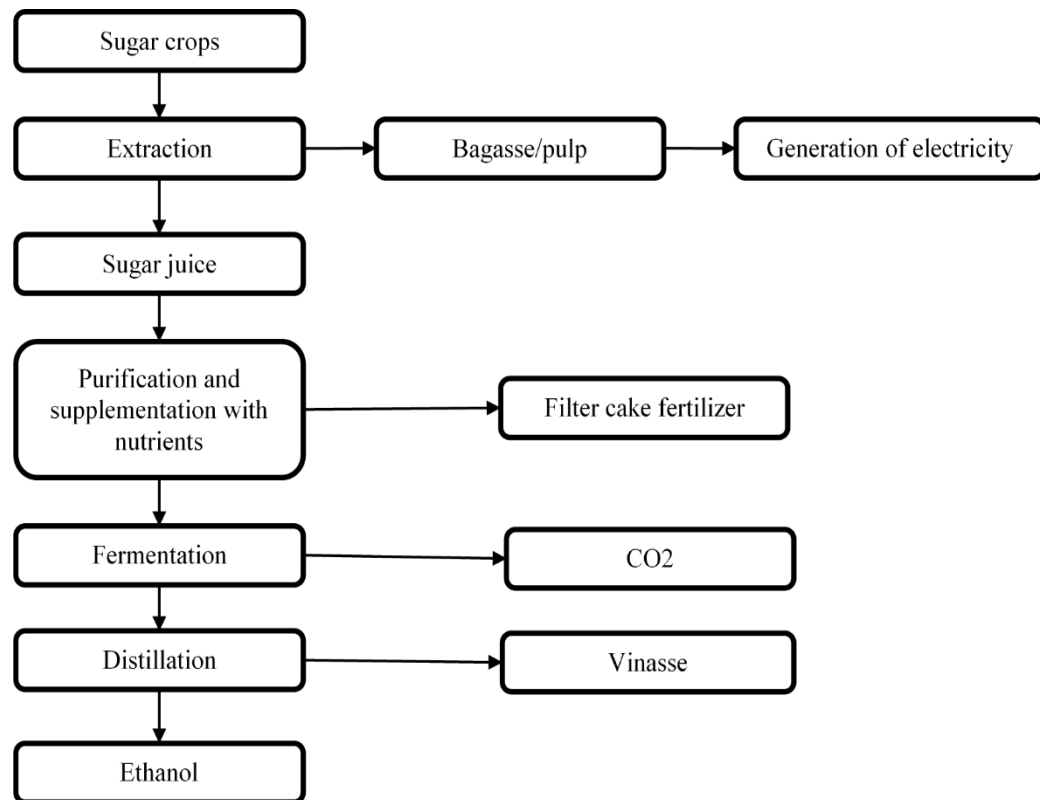


Figure 2.1 Flowchart for ethanol production from sugar crops (Adapted from Zabed et al., 2017).

### **2.3.2. Starch as a Feedstock for Ethanol Production**

Starch-based feedstocks are commonly used in North America and Europe for ethanol production (Balat et al., 2008; Cardona and Sánchez, 2007). Commonly used starch-based cereals include corn and wheat for ethanol production. In tropical countries, tubers like cassava are used as a starch material to produce ethanol (Cardona and Sánchez, 2007).

Starch is a polymeric carbohydrate consisting amylose, a linear polymer of glucose with  $\alpha$ -1, 4 bonds, and amylopectin, a branched chain of glucose that has  $\alpha$ -1, 6 bonds at the branch points. In order to produce bioethanol from starch-based materials, the chains of glucose molecules in the feedstocks need to be converted into fermentable sugar. This conversion process is called hydrolysis and consists of reacting water with a large molecule to form two or more small molecules (Lerner and Lerner, 2008b). In the case of starch hydrolysis, amylase enzymes catalyze the breaking down of starch into glucose. Figure 2.2. shows the general process of producing ethanol from starch-based materials. The first step of the process is milling to extract the starch. Milling can be done using either a dry or wet process. Once the starch is separated it is used to produce fermentable sugars by hydrolysis. The sugar is then fermented into ethanol. Depending on the milling process used, different byproducts will be produced. (Sánchez and Cardona, 2008).

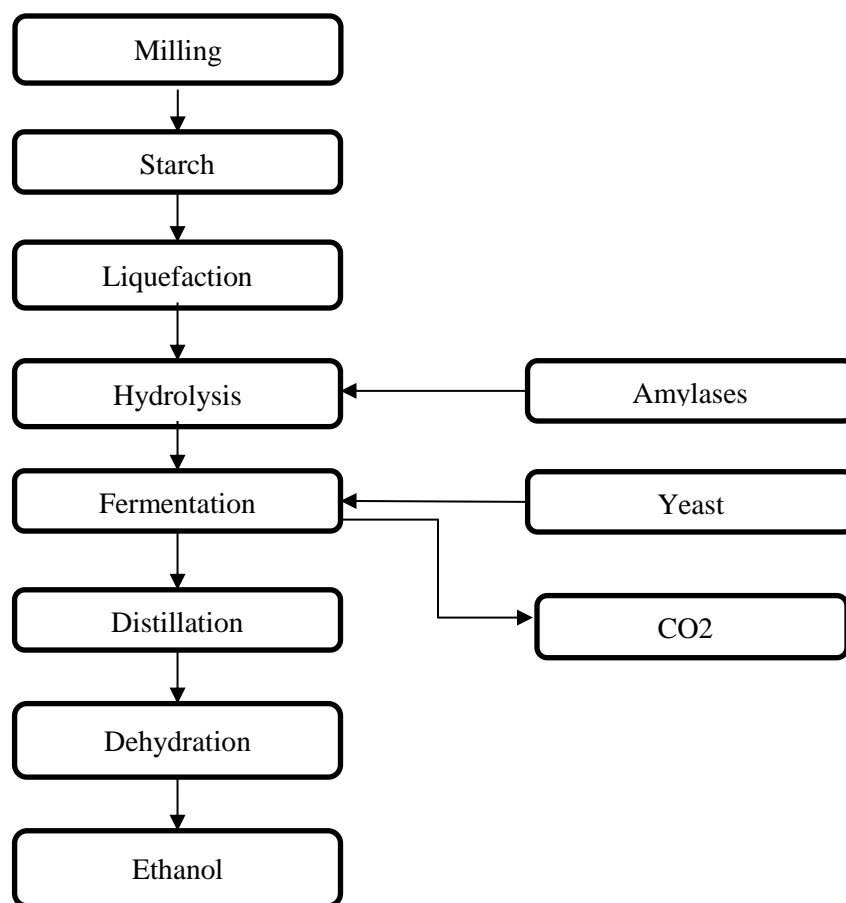


Figure 2.2 Flowchart of bioethanol production from starch (Adapted from Zabed et al., 2017).

### 2.3.3. Lignocellulosic Feedstocks for Ethanol Production

Lignocellulosic feedstocks can be categorized as; energy crops, aquatic plants, forest biomass and wastes, agricultural residues, and organic fractions of municipal solid waste (Zabed et al. 2016). Most of these feedstocks are readily available as byproducts of other operations without needing any additional land or affecting food and fiber crop production. Those feedstocks that would need additional land, such as vegetative or short rotation crops, can often be grown on marginal or degraded land without competing with food and fiber crops. According to Kim and Dale, (2004) lignocellulosic feedstocks can

produce up to 442 billion liters of ethanol per year and if all crop residues and wasted crops are added to the process this number can go up to 491 billion liters.

Lignocellulosic biomass consists mainly of three polymers; cellulose, hemicellulose and lignin, along with other compounds such as pectin, protein, extractives, and ash. The relative ratio among these compounds differs with species, age and stage of growth (Bajpai, 2016b). The compositions of different lignocellulosic feedstocks are shown in Table 2.3.

Table 2.3 Representative chemical composition of various lignocellulosic feedstocks\*

Name	Cellulose	Hemicellulose	Lignin
Energy crops, dedicated plants			
Switch grass	30-50	10-40	5-20
Miscanthus	38.2-40	18-24.3	24.1-25
<i>Municipal solid wastes (MSW) and Industrial wastes</i>			
General MSW	33-49	9-16	10-14
<i>Agricultural residues and agro-wastes</i>			
Corn stover	38-40	24-26	7-19
Corn cob	42-45	35-39	14-15
Rice straw	28-36	23-28	12-14
Wheat straw	33-38	26-32	17-19
<i>Woods</i>			
Softwood	35-40	25-30	27-30
Hardwood	45-50	20-25	20-25
Poplar	47.6-49.9	27.4-28.7	18.1-19.2

\*Adapted from Zabed et al., 2017

The most abundant component in the plant cell is cellulose, a linear homopolysaccharide with repeating anhydrous glucose molecules that are linked by  $\beta$ -(1,4) glycosidic bonds (Figure 2.3) (Saini et al., 2015). These glucose molecules that form cellulose are the main sugar components of lignocellulosic feedstocks used for fermentation. Chains of cellulose form microfibrils and bundled microfibrils which in turn form cellulose fibers. Cellulose microfibrils can contain up to 500-14,000 glucose molecules forming glucan chains that are bonded with hydrogen and van der Waals bonds. Cellulose microfibrils are insoluble in water and are the core component of the cell wall (Mohnen et al. 2009). Cellulose in lignocellulosic feedstocks consists of both crystalline and amorphous forms. When these two forms are compared, amorphous cellulose can be digested more easily by enzymes (Laureano-Perez et al. 2005).

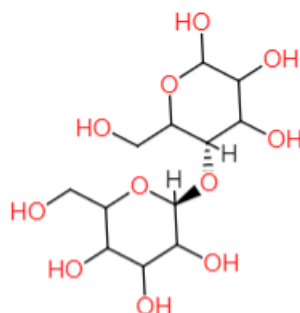


Figure 2.3 Structure of cellulose

Another type of polysaccharide in the lignocellulosic feedstock is hemicellulose, which consists of both five (arabinose, xylose, galactose) and six (D-mannose, D-galactose, and D-glucose) carbon sugars (Laureano-Perez et al., 2005; Saini et al., 2015). Hemicellulose composition is dependent on the type of plant, cell tissue, glycosidic

linkages, type of side chains and degree of polymerization (Chundawat et al., 2011). Hemicellulose can form a coat around cellulose microfibrils and also link microfibrils to one and other. Hemicellulose can also link with the phenolic portion of the plant known as lignin (Laureano-Perez et al., 2005). Hemicellulose is a more complex polysaccharide when compared to cellulose due to it containing different types of polymers. Because of the heterogeneous structure, a series of enzymes are needed to hydrolyze hemicellulose.

Lignin is a heterogeneous, amorphous, and cross-linked aromatic polymer. Lignocellulosic feedstocks can contain 2-40% lignin (Saini et al., 2015). The type of bonds lignin can form are; hydrogen bonding, ionic bonding, covalent ester linkages, ether linkages, and van der Waals bonds (Laureano-Perez et al., 2005). The composition of the lignin varies with plant species and cell structure, largely depending on the molecule's monomeric units. Basic monomeric units are; p-hydroxyphenyls (H), guaiacyls (G), and syringyls (S). Lignin in hardwood is mainly composed of G and S monomers, softwood lignin is mainly G monomers and herbaceous plants have a variation of all three with different ratios (Chundawat et al., 2011). These monomers are generally not fermentable, thus lignin is less valuable to the biofuel production process.

The cell wall of the plant is generally a multilayer structure (Figure 2.4.) with middle lamella, primary wall, and secondary wall (Zhao et al., 2012). Cellulose is surrounded by hemicellulose and lignin which makes it harder for enzymes to access. This makes pretreatment a necessity. Enzymatic digestibility of lignocellulosic feedstocks can be affected by structural and compositional factors such as; cellulose crystallinity, presence of lignin, hemicellulose coating around cellulose, porosity, feedstock particle size, surface

area, and degree of polymerization (Alvira, Tomas-Pejo, Ballesteros, and Negro, 2010; Laureano-Perez et al., 2005).

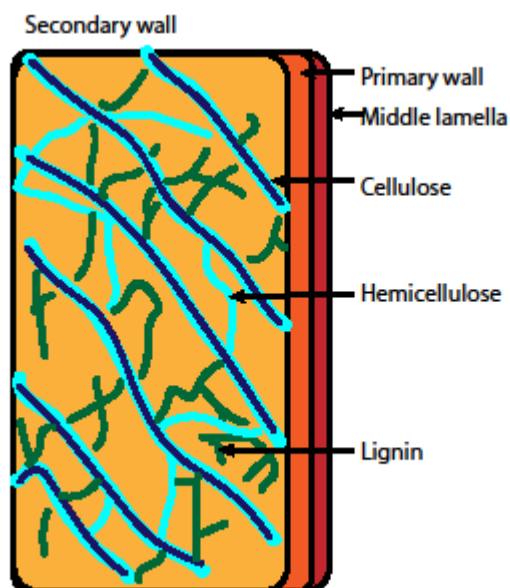


Figure 2.4 Schematic diagram of plant cell wall (Adapted from Zhao et al., 2012)

Studies show that cellulose crystallinity plays a role in initial hydrolysis rate but lignin content is the main effect on overall conversion (Laureano-Perez et al., 2005, Chang and Holtzapple 2000). Besides these two parameters, pore size, pore volume and surface area might have an effect on overall conversion as well.

To overcome or reduce the impact of limiting factors, microbial ethanol production from lignocellulosic biomass requires transforming the feedstock in a multi-step process (Figure 2.5.). The first step is pretreatment where the matrix of cellulose and lignin is broken down in order to provide access to the polymer chains of cellulose and hemicellulose. The second step is hydrolysis, which breaks down the polymers resulting in monomer sugar solutions. The third step is fermentation of the sugars to ethanol and the final step is purifying the produced ethanol (Taherzadeh and Karimi, 2007).



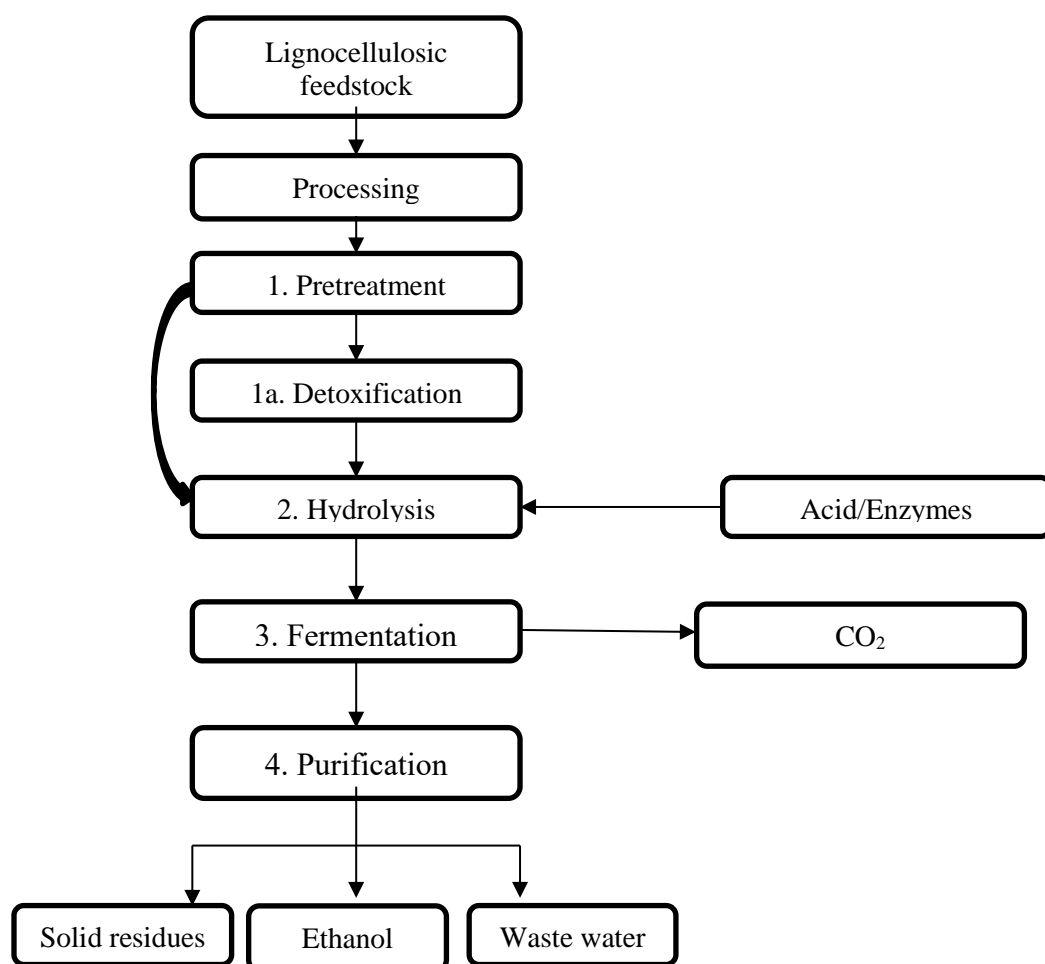


Figure 2.5 Ethanol production from lignocellulosic feedstocks (Adapted from Zabed et al., 2016)

### 2.3.4. Pretreatment of Lignocellulosic Feedstock

The objective of pretreatment is to break down the lignocellulosic matrix (Figure 2.6.), decrease the degree of cellulose crystallinity and increase the amount of amorphous cellulose portion (Sánchez and Cardona, 2008). An ideal pretreatment should have the following properties:

1. It should increase sugar yield from hydrolysis.
2. It should have little or no degradation or loss of carbohydrates.
3. It should form minimal amounts of byproducts that might have an inhibitory effect on hydrolysis and fermentation.
4. It should be cost-effective.

Pretreatment methods can be categorized as: physical, biological, thermochemical, chemical or a combination of these (Kumar et al. 2009; Sun and Cheng, 2002; Taherzadeh and Karimi, 2008).

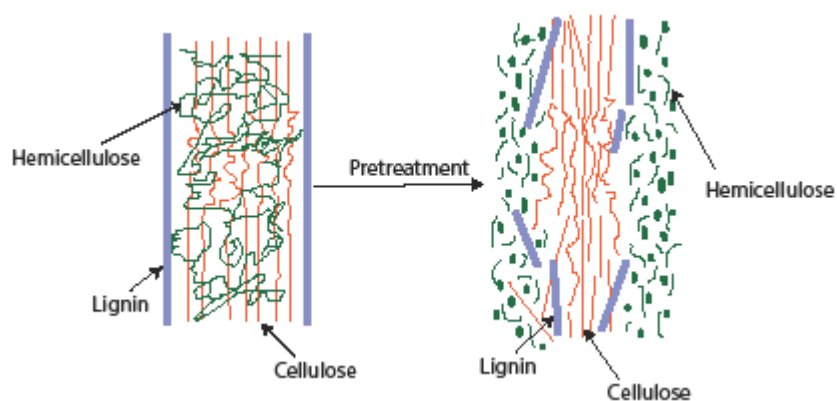


Figure 2.6 Schematic of the role of pretreatment in the conversion of biomass to fuel  
(Adapted from Kumar et al. 2009).

#### 2.3.4.1. Physical Pretreatment

Physical pretreatment impacts the structure of the feedstock without using extra chemicals and thus producing toxic residues. Mechanical comminution, irradiation, and extrusion can be categorized as physical pretreatment types for lignocellulosic materials (Taherzadeh and Karimi, 2008).

### *Mechanical comminution*

The main purpose of mechanical comminution is to reduce the particle size of the material and crystallinity of the cellulose. As a result of size reduction, an increase of available specific surface area and reduction of the degree of polymerization can be achieved (Alvira et al., 2010; Hendriks and Zeeman, 2009). Depending on the desired particle size, chipping, grinding or milling can be used as a comminution process. Particle size can be reduced to 10-30 mm after chipping and 0.2-2 mm after milling or grinding (Sun and Cheng, 2002). According to Hendriks and Zeeman (2009), comminution can increase the hydrolysis yield of lignocellulosic biomass by 5-25% while the enzymatic hydrolysis time is reduced by 23-59%. These results depend on the type of the biomass, type of milling being used and the milling time. This pretreatment is not considered to be economically feasible due to high energy requirements.

### *Extrusion*

Extrusion is a process in which the material is forced through a die, exposing it to heating, mixing, and shearing. During the process, both chemical and physical changes can occur in the material. It is believed that while passing through an extruder, lignocellulosic structure can be disrupted leading to defibrillation, fibrillation and shortening of the fibers. Due to these changes carbohydrates become more accessible for enzymatic hydrolysis. Enzyme application during extrusion is considered to be a potential pretreatment for cellulosic ethanol production (Alvira et al., 2010).

#### **2.3.4.2. Biological Pretreatment**

Biological pretreatment consists of using microorganisms to degrade lignin and hemicellulose. Brown-, white-, and soft-rot fungi are the commonly used microorganisms. Among these microorganisms, white-rot fungi are believed to be most effective for pretreatment of lignocellulosic materials (Sun and Cheng, 2002). Effectiveness of lignin removal during the biological pretreatment process relies on the lignolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase which are produced by basidiomycete (Sindhu et al. 2016). Corn stover, switchgrass, and hardwood pretreated with *Ceriporiopsis subvermispota* result in 2 to 3-fold increase on glucose yield when compared to untreated samples while wheat straw, and soybean straw glucose yields do not improve. Addition of glucose and malt extract as external carbon source to the process increases wheat straw cellulose digestibility by 10% (Wan and Li 2011).

Overall, biological pretreatment has low energy requirements, functions in mild environmental conditions, and does not need chemicals. Despite these advantages, biological pretreatment is not a favored approach because the rate of hydrolysis in most biological pretreatment processes is very low (Sun and Cheng, 2002). More research has to be conducted on basidiomycetes fungi and their capability to delignify the lignocellulosic material fast and efficiently in order to achieve a cost-competitive pretreatment process (Alvira et al., 2010).

### 2.3.4.3. Chemical Pretreatment

#### *Ozonolysis*

Ozone is a strong oxidizing agent that is soluble in water. Ozone is electron deficient in the terminal oxygen. Because of this it attacks lignin which is an electron rich substrate when compared to carbohydrates (Travaini et al., 2016). During the process, lignin is the main component that degrades. Although hemicellulose is somewhat affected by the process, cellulose is not. Ozone pretreatment can be applied to biomass such as, wheat straw, bagasse, green hay, cotton straw, and poplar sawdust (Kumar et al., 2009). Delignification of wheat straw with ozone indicates that ozone pretreated samples have 75% cellulose digestion at the end of enzymatic hydrolysis whereas untreated samples only have 20% (Binder et al. in 1980). The main factors to be considered during ozone treatment are; particle size, moisture content, ozone concentration in the gas flow, type of biomass and air/ozone flow rate. In a study of rye and wheat straw that is pretreated with ozone, results show enzymatic hydrolysis yields increase while acid soluble lignin is partially removed. The same study also states moisture content (below 30%) and type of the biomass to be most significant parameters of ozone pretreatment process.

Ozone pretreatment of sugarcane bagasse yields more than 92% carbohydrate recovery, increasing glucose yield 6.64% to 41.79% without producing furfural or HMF (Travaini et al., 2013). Despite the process being able to be conducted at room temperature and without producing inhibitory products, this process utilizes a significant amount of ozone which makes the process costly (Sun and Cheng, 2002).

### *Acid Pretreatment*

The purpose of using acid in the pretreatment process is to solubilize the hemicellulose in the biomass to make cellulose more accessible for enzymatic hydrolysis. Commonly used acids for this pretreatment are sulfuric and hydrochloric acid. This process can be performed at high temperatures by using dilute acid or at low temperatures by using concentrated acid. When the operating conditions are compared, concentrated acid has an advantage with lower pretreatment temperature (e.g. 40 °C). Despite this advantage, using acid concentrations up to 30-70% makes the process very corrosive and dangerous. Concentrated acid pretreatment needs to be conducted in corrosion resistant vessels. Concentrated acid pretreatment has high investment process and maintenance costs making the process undesirable for commercial scale operations (Taherzadeh and Karimi, 2008).

Dilute acid (usually below 4 %) can be used as a pretreatment before enzymatic hydrolysis or as a hydrolysis process to produce fermentable sugars (See section 2.3.5). This pretreatment process can be executed at high (e.g. 180 °C) temperatures and low retention time (e.g. 5 min) or lower temperatures (e.g. 120 °C) with higher retention time (e.g. 30-90 min) (Alvira et al., 2010; Taherzadeh and Karimi, 2008). Rice straw pretreated with dilute H<sub>2</sub>SO<sub>4</sub> (1% w/w) for 1-5 min at 160°C or 180°C followed by an enzymatic hydrolysis achieves a maximum sugar yield of 83% (Hsu et al., 2010). Saha et al. (2005) observed that pretreating wheat straw with 0.75% v/v of H<sub>2</sub>SO<sub>4</sub> for 1 h at 121°C could obtain up to 74% hydrolysis yield. Corn cob pretreated and hydrolyzed with phosphoric acid shows that glucose yields increase as the pretreatment temperature (100-160°C) and time increases (5-60 min). Although the highest glucose yield is achieved at 160° C for 60 min, optimum conditions are also dependent on furfural production (Satimanont et al., 2012).

Combination of sulfuric acid and hydrochloric acid was used to pretreat sweet sorghum bagasse (Heredia-Olea et al.,2012). Results show all three pretreatments resulted in similar total fermentable sugar yields.

Dilute acid pretreatment is more effective for increasing the accessible area of populus spp than hot water and alkaline pretreatment due to high amount of hemicellulose removal, but it is not effective on removing lignin (Meng et al., 2015).

One drawback of dilute acid pretreatment is, depending on the temperature of the process, inhibitory products such as furfural and HFM can be produced. Dilute H<sub>2</sub>SO<sub>4</sub> pretreated wheat straw hydrolysate shows detectable amounts of furfural and HMF as the pretreatment temperature rises to 180-190°C but when pretreatment conditions are kept at mild acid concentrations and moderate temperatures the hydrolysate does not contain any detectable inhibitory compounds (Saha et al., 2005). HMF and furfural are also a function of pressure; during dilute acid pretreatment as the pressure increases the amount of inhibitory products produced increases. Maximum xylose conversion and minimum furfural production are achieved when pretreatment is conducted at 15 psi (Karimi et al.,2006).

#### *Alkaline Pretreatment*

Alkaline pretreatment is based on the effect bases have on lignocellulosic biomass. Lignin content of the biomass is the main factor influencing pretreatment effectiveness (Sun and Cheng, 2002). Commonly used bases for this process are; sodium hydroxide, ammonia, calcium hydroxide and potassium hydroxide. Alkaline pretreatment is more

effective for lignin degradation, with little cellulose and hemicellulose degradation compared to acid or hydrothermal processes (Han et al., 2012).

Alkaline pretreatment is more suitable for feedstocks with low lignin content, like hardwood, agricultural residues, and herbaceous crops rather than softwood which has relatively higher lignin content (Chen et al., 2013). NaOH pretreated hardwood birch and softwood spruce shows an increase of 11 and 2.5 fold on their enzymatic hydrolysis efficiency (Mirahmadi et al., 2010).

An increase in alkaline concentration mainly increases the delignification and subsequently cellulose conversion yield (Silverstein et al., 2006). Sodium hydroxide pretreated cotton stalks show highest delignification (65%) with 2% NaOH in 90 min at 121°C and cellulose conversion (60.8%) when compared with sulfuric acid, ozone, and hydrogen peroxide pretreatments. Aqueous sodium hydroxide (0.5-2.0%) pretreated barley straw leads to maximum of 84.8% lignin and 79.5 % hemicellulose removal with a maximum sugar yield of 86.5% after enzymatic hydrolysis when pretreatment conditions are 105°C for 10 min (Azizul; et al., 2012). Effects of SO<sub>2</sub>, Na<sub>2</sub>CO<sub>3</sub>, and NaOH on enzymatic digestibility of grape marc has been investigated by Vaccarino et al., (1987). Among all three chemicals NaOH has the highest degrading result when conditions were 1 % NaOH and at 120 °C.

Alkaline loading is a dominant parameter for NaOH pretreated corn stover (Chen et al., 2013). Results shows an increase in alkaline loading and reaction temperature are two parameters that increase the hydrolysis yield. Furthermore, their results show a better correlation between corn stover digestibility with alkaline loading rather than alkaline concentration.



Alkaline pretreatment can be done at low temperatures with a longer reaction time. Lignin degradation and sugar conversion of KOH pretreated switchgrass was observed to be 28.5% and 91.8% respectively when the pretreatment conditions were 21°C, 2% KOH and reaction time of 48h (Sharma et al., 2013). Aqueous ammonia pretreated oil palm empty fruit bunches (Jung et al.,2011) and corn stover (Li and Kim, 2011) reach highest digestibility in 12h when pretreatment temperature is 60 °C.

Lime is another alkali that gives high hydrolysis conversion rates for feedstocks such as switchgrass (Garlock et al., 2011), sugarcane bagasse (Rabelo et al., 2008), and corn stover (Kaar and Holtzaple, 2000). The main drawback of lime pretreatment is that, although dependent on temperature, it has a relatively long reaction period. Optimum conditions for lime pretreatment of corn stover are found to be 4 weeks when reaction temperature is 55 °C (Kim and Holtzaple, 2005).

#### **2.3.4.4. Thermo-chemical Pretreatment**

##### *Liquid hot water*

Liquid hot water pretreatment is done by exposing biomass to water under pressure at elevated temperatures without any additional catalyst or chemicals. This process increases cellulose accessibility mainly by solubilizing the hemicellulose (Alvira et al., 2010). Liquid hot water pretreatment can improve the enzymatic hydrolysis of agricultural feedstocks like brewers spent grain, corn cob, corn husk and wheat straw (Michelin and Teixeira, 2016). Hemicelluloses in bamboo samples were highly removed when pretreated with hot water while small amounts of lignin and cellulose were degraded. Hot water

pretreatment (200°C for 120 min) increases the “cellulose to glucose” conversion from 15.7% to 75.7% when compared with untreated samples (Xiao et al., 2014). Although liquid hot water pretreatment has advantages like not needing catalysts, and low levels of inhibitory compound formation, the amount of energy and water needed makes the process unfavorable for commercial scale (Alvira et al., 2010).

### *Steam explosion*

This pretreatment process consists of exposing biomass to high temperature and pressure for a period of time then suddenly depressurizing. When the pressure is suddenly released, fibers in the biomass explode as the water in the cells flashes to steam. In addition, high temperature hydrolyzes the acetyl groups in hemicellulose causing cellulose to be more accessible for enzymes. Hemicellulose degradation during the pretreatment makes the process more favorable for materials with high hemicellulose content (Alvira et al., 2010). Besides the composition of the biomass, also particle size, moisture, residence time of the pretreatment, and temperature are parameters that have an effect on steam explosion pretreatment. During steam explosion pretreatment, compounds that are inhibitory to further processes for ethanol production can be formed (Alvira et al., 2010; Sun and Cheng, 2002). Wheat straw pretreated with steam explosion (170°C-220°C) was used to investigate enzymatic hydrolysis performance (Horn et al., 2011). A maximum “cellulose to glucose” yield of 90% could be achieved at a process temperature of 210°C. Although more severe conditions also have similar glucose yield, these conditions also lead to xylose loss and inhibitory product formation.

### *Ammonia fiber explosion (AFEX)*

In this process, pressure and moderate temperature (60–100°C) is used to pretreat the biomass in the presence of liquid ammonia. Once the pressure is suddenly released, ammonia inside the biomass flashes to a gaseous state, causing the structure of the biomass to be disrupted. This process induces cellulose de-crystallization, hemicellulose hydrolysis, lignin depolymerization, and also an increase in surface area. The overall effect is an increase in enzyme accessibility and cellulose digestibility (Kim et al. 2016; Laureano-Perez et al., 2005). AFEX pretreatment can improve hydrolysis yields of herbaceous crops and agricultural residues. AFEX pretreated (100°C for 30 min) Bermuda grass had 94.8% cellulose to glucose yield while chemical composition almost stayed the same (Lee et al., 2010). The effect of AFEX pretreatment on switchgrass increases up to a point with increasing ammonia loading (max 1:1), increasing reactor temperature, and increasing moisture content (max 80%) (Alizadeh et al., 2005). In another study, AFEX pretreatment at 160°C for 5 min resulted in 96% glucan and 81% xylan conversion at the end of enzymatic hydrolysis of pretreated *Miscanthus* (Murnen et al., 2007).

AFEX pretreatment is effective at increasing the hydrolysis yield by removing lignin and hemicellulose. Ammonia can be recovered at the end of the process but liquid ammonia being expensive, the system is unfavorable for commercial application (Bajpai, 2016a).

### *Torrefaction*

Torrefaction is another thermo- chemical pretreatment process. It takes place under atmospheric pressure and in an inert environment at temperatures between 200-300°C.

During the process, biomass decomposes, giving off water and volatiles (Kongkeaw and Patumsawad, 2011; Wang et al., 2011).

Torrefaction has been researched for several decades. As stated by Bergman et al., (2005) although torrefaction was first reported in 1930's it wasn't until 1980-1990's it was commercialized and received attention as a pretreatment. In 1990, Pentananunt et al. studied combustion characteristics of torrefied wood. Their study showed that torrefied wood had a high combustion rate and less smoke than raw wood.

The torrefaction process can be divided into five regions based on the pretreatment temperature (Bergman et al., 2005). In the first region (50-150 °C) biomass loses moisture and low volatile components. Second, (120-160°C) lignin starts to soften. Third (150-200°C) is the region where depolymerization of the biomass begins. At higher temperatures the fourth region occurs. In this region polymers that are still intact and solid structures from the previous stage start to devolatilize and carbonize. With increasing the temperature even higher, extensive carbonization and devolatilization of the polymers and solids occur. Hemicellulose is the most reactive component followed by cellulose and lignin. Due to hemicellulose being the most reactive component, most mass loss of biomass during torrefaction can be related to hemicellulose degradation (Bergman et al., 2005; Chen and Kuo, 2011).

Parameters that affect the torrefaction process are; reaction temperature and time, heating rate, pressure, feedstock, moisture of the feedstock, and feedstock particle size (Tumuluru et al., 2011). Research on torrefaction often focuses on physical and chemical changes like; compositional changes, mass and energy yields, grindability, and pelletization (Bridgeman et al., 2008).

As mentioned previously, mass loss occurs during torrefaction due to the loss of moisture, volatile matter, and decomposition of biomass compounds. Biomass with a higher composition of hemicellulose shows a greater mass loss when compared to biomass that has a lower level of hemicellulose (Bridgeman et al., 2008; Deng et al., 2009; Prins et al., 2006a; Sadaka and Negi, 2009; Shang et al., 2012). In a study that looked at energy and mass loss during torrefaction of willow, red canary grass, and wheat straw shows that torrefied biomass can contain up to 96% of its original energy content (Bridgeman et al., 2008). The same study also reports increased calorimetric values and heating value yield when compared to raw samples, consistent with an increase in carbon composition and decrease in oxygen and hydrogen composition. The change in the calorific value is related to chemical compositional changes in structural carbohydrates during the process. In another study (Arias et al., 2008), as the O/C ratio decreases the calorimetric value increases with respect to torrefaction temperature and time.

The increase in torrefaction temperature and time also increases the energy density due to more mass being lost than energy. Results from Prins et al. (2006b) shows that the energy density of willow (torrefied at 270° C) and beech (torrefied at 280°C) can increase by 17% and 20% when torrefied. Results from the same study also shows torrefied deciduous wood types and straw produce more volatiles than coniferous wood due to differences in hemicellulose side chains.

Grindability is another aspect that is improved by torrefaction. For example, the percentage of fine particles increases with the increase in torrefaction temperature (Deng et al., 2009). Torrefied woody biomass also shows improved grindability characteristics with the percentage of particles in the smallest sieve increasing after torrefaction (Arias et

al., 2008). Degradation of hemicellulose can be associated with grindability characteristics. In a study by Shang et al., (2012) neither the grindability properties or the chemical composition of the biomass change until torrefaction temperature is increased to 200°C. Grindability value increases when the torrefaction temperature is between 250-300°C.

Thermogravimetric Analysis (TGA) has been used to study the effect of torrefaction on hemicellulose, cellulose, and lignin of bamboo, willow, coconut shell and wood (Chen and Kuo, 2010). Results show decomposition temperatures are subjected to change with type of biomass. Torrefaction at mild temperatures (240°C) leads to significant hemicellulose depletion without significant impact on cellulose and lignin. In this concept, mild torrefaction can be suitable pretreatment for fuel production.

Biomass torrefaction studies are mostly associated with supply chain benefits and combustion studies; few studies have been reported that investigate the effect of torrefaction as a pretreatment process for hydrolysis and fermentation. Using torrefaction as pretreatment for biomass has been reported for olive prunings (Chiaramonti et al. 2011), rice straw (Sheikh et al. 2013), waste money bills (Sheikh et al., 2013a), and Norway spruce (Normark et al., 2016). Chiaramonti et al (2011) reports torrefied (180-220°C) and ground olive prunings have a glucose yield of 35% which is slightly lower (39%) than enzymatically hydrolyzed raw olive prunings. Increasing the torrefaction temperature results in reduced sugar yields for Norway Spruce (Normark et al., 2016). Torrefied rice straw (220°C for 40 min) and waste money bills (180°C for 40 min) are reported to have 60.68% and 44.89% increases in sugar yield when compared to untreated samples respectively (Sheikh et al. 2013, Sheikh et al., 2013a).

### 2.3.5. Hydrolysis of Biomass

Hydrolysis in ethanol production from lignocellulosic feedstock consists of the separation of carbohydrate chains in cellulose and/or hemicellulose. Hydrolysis is an important step for starch-based and lignocellulosic feedstocks (Aditiya et al., 2016). Hydrolysis of lignocellulosic biomass can be done either using acid or enzyme treatment.

Acid hydrolysis can be carried out either with dilute or concentrated acids. Sulfuric acid, hydrochloric acid, nitric acid, trifluoroacetic acid, and phosphoric acid can be used for hydrolyzation of carbohydrates. Drawbacks of using acid hydrolysis include the need for high temperatures, acid recovery or neutralization, and the possibility of production of inhibitory products (Zabed et al., 2017).

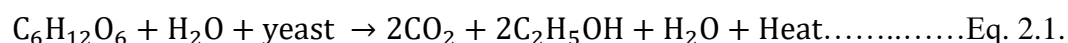
Hydrolyzing cellulose and hemicellulose via enzymes is a slower process when compared to acid hydrolysis but produces lower levels of inhibitory compounds, thus making the process favorable (Sánchez and Cardona, 2008). Enzymatic hydrolysis usually takes place at low temperatures around 45-50 °C and would not cause any corrosion problems (Zabed et al., 2017).

In enzymatic hydrolysis, cellulose (glucose linked by  $\beta$ -(1,4) glycosidic bonds) is depolymerized by a group of enzymes known as cellulases. Cellulase follows a multi-step process using endoglucanase, exoglucanase, and cellobiase to break down the glycosidic bonds in cellulose. Endoglucanase randomly breaks down glycosidic bonds in amorphous regions of cellulose or at the surface microfibrils producing free chain ends. Exoglucanase releases cellobiose from non-reducing ends of glucan chains. The outcome of both

processes, cellobiose, is a disaccharide which is further hydrolyzed to glucose by cellobiase (J. Lee, 1997).

### 2.3.6. Fermentation

Fermentation is a metabolic process by which microorganisms produce stored energy from organic compounds. In ethanol production, bacteria or yeasts convert soluble sugar available in the hydrolysate into ethanol and carbon dioxide. Carbon sources that can be used for ethanol production include glucose, disaccharides, xylose, and glycerol. Among these carbon sources, glucose can be converted to ethanol via baker's yeast *S. cerevisiae*, bacterium *Z. mobilis* and xylose can be directly fermented by yeasts such as *Pachysolen tannophilus*, *Candida shehatae* and *Pichia stipis*. Xylose can also be fermented when the microorganism used is baker's yeast (Sarris and Papanikolaou, 2016). Taking the chemical reaction (Eq. 2.1.) into consideration theoretically, 1 kg of glucose can produce 0.51 kg of ethanol and 0.48 kg of carbon dioxide (Aditiya et al., 2016; Zabed et al., 2017).



Depending on the kinetic properties of the microorganism and substrate, fermentation can be carried out in different modes; batch, fed batch, and continuous. Batch fermentation is a closed system in which all the required substances for microorganism growth and product formation are added to the system at the beginning. Fed-batch is a similar system to batch but the difference is it is not a closed system. During fed-batch fermentation, substrates, nutrients, and inducers can be added to the system at a given time. Continuous mode, on the other hand, is a system where the fresh medium is fed to the



bioreactor and the spent medium with cells is removed from the bioreactor at the same rate (Macauley-Patrick and Finn, 2008).

Fermentation processes can also be categorized depending on the configuration of the process as Separate Hydrolysis and Fermentation (SHF), Simultaneous Saccharification and Fermentation (SSF), or Consolidated Bioprocessing (CBP) (Zhao et al., 2012). In Separate Hydrolysis and Fermentation (SHF), cellulose is hydrolyzed to glucose and when the hydrolysis is finalized the hydrolysate is fermented and converted into ethanol. By using this process, each step can be carried out under optimal conditions. The main concern about this process is microbial contamination during hydrolysis which may affect ethanol yield (Balat et al., 2008; Sánchez and Cardona, 2008)

In the case of SSF, hydrolysis and fermentation are carried out in the same unit at the same time. Higher ethanol yields can be achieved with SSF than SHF. But due to the fact that enzymatic hydrolysis and ethanol fermentation are carried out at different optimum temperatures it is difficult to optimize the system (Sánchez and Cardona, 2008).

The third configuration, consolidated bioprocessing (CBP), is an emerging technology. In this process, cellulase production, hydrolysis, and fermentation are combined into a single step. When compared with other configurations, CBP can lower the production cost due to fewer process steps and no additional enzyme purchase (Aditiya et al., 2016).

## **2.4. Ethanol Production from Wheat Straw via Alkaline Pretreatment**

Wheat is one of the most widely grown crops in the world. According to USDA's "World Agricultural Production Report" (2018), wheat production in the world was around 735.3 and 750.4 million metric tons in the years of 2015/16 and 2016/2017. Wheat straw is an agricultural residue of wheat production, making it one of the most abundant forms of agricultural biomass in the world. A small amount of wheat straw is used as livestock feed, or as bedding for livestock, but a large part of the wheat straw is discarded on the field or burned directly (Talebnia, Karakashev, and Angelidaki, 2010) Using wheat straw for ethanol production can be an alternative use instead of burning.

Removing all the wheat residue from the field might lead to problems such as lower soil quality, needs for additional fertilizer, decreasing soil organic carbon stock, and soil erosion (Wang et al., 2013). The amount of residue that can be removed from the site depends on the weather, crop rotation, soil fertility, slope of the land, and tillage practices (Kerstetter and Kim Lyons, 2001).

The exact composition of wheat straw depends on wheat species, soil, and climate conditions. Cellulose, hemicellulose and lignin content of wheat straw typically are in the range of 33-40, 20-25, and 15-20 (%w/w) (Prasad et al., 2007).

Wheat straw can be a low-cost feedstock for ethanol production due to its availability, low lignin content, and renewability. About a kg of wheat straw can be obtained for every 1.3-1.4 kg of wheat grain harvested. Wheat straw as a potential feedstock has been researched in terms of compositional changes from pretreatment, hydrolysis, and fermentation (Govumoni et al., 2013).

Pretreatment of wheat straw with various alkaline solutions and their effect on cell wall composition has been investigated (Sun et al., 1995). When wheat straw is pretreated with 1.5% NaOH for 144h at 20° C, 80% of hemicellulose and 60% of lignin is degraded. As alkaline concentration increases, the rigid and organized structure of wheat straw becomes distorted. Also, the surface becomes more homogeneous which is related to lignin removal (Barman et al., 2012). As the pH of the solution (pH 10-13) increases an increase of enzymatic monosaccharide release from wheat straw solid fraction has been observed (Pedersen et al., 2011).

Degradation of hemicellulose and lignin during alkaline pretreatment enhances the glucose and, for that, ethanol yield as well. Similar to alkaline strength, increasing pretreatment temperature also maximizes hemicellulose and lignin solubilization and enhances enzymatic hydrolysis when temperature is increased to 121 °C from 60 °C (McIntosh and Vancov, 2011). However when the temperature increases to 140°C from 100 °C no significant change in hydrolysis yield is observed (Pedersen et al., 2011).

Alkaline peroxide pretreated wheat straw can achieve a maximum of 96.7% total conversion (hemicellulose and cellulose) by enzymatic hydrolysis without any production of inhibitory products (Saha and Cotta, 2006). When *E. coli* is used as microorganism for fermentation in SHF and SSF configurations, yields of 0.29 g g<sup>-1</sup> straw and 0.23g g<sup>-1</sup> straw can be achieved.

Potassium hydroxide and recycled black liquor as a pretreatment has also been investigated (Liu et al., 2015). Recycling black liquor results in 180-206% higher sugar yields from raw wheat straw. But when compared to fresh KOH, sugar yields are about 40-

45% lower. The system also reduces the chemical requirement by 25% and water use by 75%.

The effect of pretreatment conditions on biomass properties was modeled by Jaisamut (2013) using Expert Designer software. Their results show that sodium hydroxide concentration and pretreatment temperature are the factors most affecting conversion efficiency. At optimum conditions (80°C, 39 min, 0.18 g NaOH and 0.06 g lime per g of raw biomass),  $93.1 \pm 1.0\%$  conversion of cellulose to glucose after enzymatic hydrolysis and  $80.3 \pm 1.2\%$  yield of monosaccharides (glucose plus xylose and arabinose) from cellulose and hemicellulose of wheat straw are theoretically achievable.

Zhang et al., (2013) reports an optimization of SSF parameters after alkaline pretreatment of wheat straw. Variables include; temperature, enzyme loading, yeast concentration and pH. Optimum conditions for SSF of NaOH pretreated wheat straw are reported to be 37.5 °C, enzyme loading at 35 FPU g<sup>-1</sup> substrate, a yeast concentration of 10 g L<sup>-1</sup> and a pH of 4.6.

Combination of other pretreatment methods or agents with alkaline pretreatment can give positive results. Combining mechanical pretreatment (grinding) with NaOH pretreatment has been studied (Han et al., 2012). Results shows reducing particle size to 120 meshes from 40 meshes increases the enzymatic hydrolysis yield, thus further reduction does not lead to significant changes. Alkaline pretreatment followed by screw press mechanical pretreatment enhances the enzymatic hydrolysis whereas only screw press pretreatment does not improve enzymatic hydrolysis (Yan et al., 2017).

## **2.5. State of the Art**

Harvesting and transportation are important aspects of lignocellulosic ethanol production. Torrefaction and densification of biomass can reduce logistics cost. Torrefaction process reduces the moisture content of the biomass while increasing the energy density, hydrophobicity, and grinding properties. When these advantages are taken into consideration it will be beneficial to know the possibility of using torrefaction as a pretreatment step. While studies have investigated the biochemical conversion of wheat straw to ethanol, a need exists to study how torrefaction affects the yield of glucose and ethanol produced from wheat straw. Also, information on the combined impact of alkaline pretreatment and torrefaction on wheat straw is not currently available in the literature. Understanding how torrefaction alone and in combination with alkaline pretreatment affects glucose yields will be a significant contribution towards the effective use of wheat straw as a bioenergy source.

## Chapter 3

### Goal and Objectives and Hypotheses

#### 3.1. Goal

The main goal of this research is to produce glucose from wheat straw by evaluating different pretreatments such as alkaline pretreatment, torrefaction and their combination.

#### 3.2. Objectives

Four specific objectives will be pursued in the course of this research project:

1. Determine the effect of different degrees of torrefaction pretreatment on sugar yield of wheat straw.
2. Determine the effect of different degrees of alkaline pretreatment on sugar of wheat straw.
3. Determine the effect of using both torrefaction and alkaline pretreatment on sugar of wheat straw.
4. Determine if an optimal combination of torrefaction and alkaline pretreatment for sugar yield can be identified.

### 3.3. Hypotheses

The hypotheses related to the four research objectives are as follows:

1. Torrefaction will increase the sugar yield (g glucose produced per g raw wheat straw).

*Null Hypothesis:* Sugar yield of torrefied biomass is not greater than raw wheat straw.

*Alternate Hypothesis:* Sugar yield of torrefied biomass is greater than raw wheat straw.

2. An increase in alkaline concentration will increase the enzymatic hydrolysis efficiency (mg glucose produced per g raw wheat straw).

*Null Hypothesis:* Hydrolysis efficiency is equal for all alkaline concentrations.

*Alternate Hypothesis:* Hydrolysis efficiency differs with alkaline concentration.

3. The combination of torrefaction and alkaline pretreatment will increase sugar yield, relative to using only one process.

*Null Hypothesis:* Sugar yield of combined torrefied and alkaline treated biomass is not greater than only torrefied or only alkaline treated biomass and both torrefied and alkaline pretreated biomass are the same.

*Alternate Hypothesis:* Sugar yield of combined torrefied and alkaline pretreated biomass is greater than only torrefied or alkaline pretreated biomass.

## Chapter 4

### **Materials and Methods**

The purpose of this section is to describe the materials used, experimental designs and the procedure to analyze the results for enzymatic hydrolysis yield from wheat straw via alkaline pretreatment, torrefaction, and the combination of those two pretreatment processes.

#### **4.1. Wheat Straw Preparation**

Post grain harvested and field dried wheat straw was obtained from The Penn State Agronomy Research Farm. Wheat straw was ground using a knife mill (Munson SCC-10S, State College, PA) with a 1.5 mm screen. Ground wheat straw was stored in plastic bags (Great value zipper bags) at room temperature.

#### **4.2. Torrefaction**

Ground wheat straw was torrefied using a Lindeberg/Blue Lab oven (Model BF51828C-1, State College, PA). Batch torrefaction was used to pretreat the raw wheat straw samples. About 40 g of raw biomass was weighed in a tray and placed inside the oven. Torrefaction was conducted under nitrogen atmosphere. In order to remove the oxygen from the reactor, nitrogen gas was directed into the oven at the rate of 3.5 L/min for 15 min before turning on the oven and continued to flow at that rate until the experiment was concluded. Three different torrefaction temperatures (180, 220, 260°C) and three



torrefaction process residence times (45, 60, 90 min) were selected as torrefaction pretreatment conditions. Residence time is measured from the time the reactor reaches the desired temperature until the sample is removed from the oven. Torrefied samples were cooled down and left at room temperature in open air for 24 h before storage. Samples were then stored in plastic bags at room temperature until further use.

### **4.3. Thermogravimetric Analysis (TGA)**

Degradation characteristics of the raw and torrefied samples (one measurement per treatment) were analyzed via thermogravimetric analysis in order to assess the compositional changes during torrefaction. This analysis was conducted in the Material Characterization Lab at Penn State's Materials Research Institute. Equipment that was used is Discovery Series TGA Q5500 coupled with Discovery MS (TA Instruments, State College, PA). Analysis was done under an inert atmosphere of nitrogen gas at a heating rate of 20° C per minute.

### **4.4. Alkaline Pretreatment**

Dilute sodium hydroxide (NaOH) at concentrations of 0.75%, 1%, and 2% (w/v) were used to pretreat raw and torrefied wheat straw samples. The pretreatment of wheat straw was performed in Erlenmeyer flasks with 4 g of dry biomass and alkaline solution at a solid loading of 10% (w/v). All pretreatments were carried out in an autoclave (Beta Star Laboratory Sterilizer, State College, PA) at 121°C (15 psi) for one hour. After taking the samples out of

the autoclave they were cooled at room temperature. The solid fraction of the biomass was recovered by filtering through a cloth filter in a vacuum filtration system (Thermo Fisher Nalgene, 300-4000 Reusable Vacuum Filter, State College, PA). Samples were washed with DI water until pH of the filtrate was neutralized to remove any excess alkali or inhibitory products. Pretreated and washed samples were stored in a plastic bag at 4°C until enzymatic hydrolysis. Alkaline pretreatment of raw and torrefied samples was replicated four times. Replications were conducted by selecting subsample from the raw and torrefied samples and subjecting it to alkaline pretreatment. This was repeated four times. Subsamples were also collected and dried prior to hydrolysis to determine moisture content.

#### **4.5. Enzyme Hydrolysis**

All treatments were subjected to enzymatic hydrolysis. Cellulase enzyme blend Cellic CTec2 (Novozyme Corporation, distributed by Sigma-Aldrich, St. Louis, MO) was used for this process. Activity of the enzyme was measured to be 109.6 FPU/ml. Enzymatic hydrolysis was performed by adding 0.15 g of dry biomass to total of 10 mL suspension solution inside a 15 mL centrifuge tube and placing it in a rotary shaker in an incubator at 50°C for 72 h. The suspension used for enzymatic hydrolysis consisted of 500 µL 1M sodium citrate buffer (pH 4.8), 40µL tetracycline solution and 30µL cycloheximide as antimicrobial agents to prevent microbial growth, and enzyme. An excessive dosage of enzyme was used to avoid enzyme limitation (60 FPU / g of dry biomass). In order to make the final solid loading 1.5%, DI water was added. Enzyme blanks and substrate blanks were also prepared and subjected to enzymatic hydrolysis. After this 72-h hydrolysis step, the

tubes were subjected to heating (99 °C) in a water bath for 10 min to inactive the enzymes. The hydrolysate was centrifuged at 4000 rpm for 20 min and the supernatant was collected in 2 ml microcentrifuge tubes and stored at -20°C for sugar analysis.

#### **4.6. Design of Experiments and Analysis**

For most measurements, a full factorial design was used, in which every combination of variables was tested. However, in order to minimize the number of pretreatment experiments, a Box-Behnken response surface method (RSM) was used to investigate the effect of combining torrefaction with alkaline pretreatment on mass yield and glucose yield. Torrefaction temperature, torrefaction time, and alkaline concentration were the independent parameters assessed. In a full factorial design, the number of treatments would be 27 ( $3^3$ ) and 4 replications would add up to 108 data points. The Box-Behnken RSM model allows the number of treatments to be 15; 12 edge and 3 center points. With 4 replications, a total number of data points added up to 60. RSM design tables and evaluation of the data was done using Minitab Software (Minitab Inc., State College, PA). Replications were blocked and experimental combinations were run in random order to minimize the effects of unexpected variability from performing experiments at different times. Levels were coded as -1, 0, and 1 as shown in table 4.1.

Table 4.1 Various factors considered with their different levels.

Factors	Coded	Coded variable levels		
		-1	0	1
Torrefaction temperature (°C)	X1	180	220	260
Torrefaction time (min)	X2	45	60	75
NaOH (w/v)	X3	0.75	1	2

A diagram of the overall process is shown in Figure 4.1., including grinding, torrefaction, alkaline pretreatment, hydrolysis and glucose measurement.

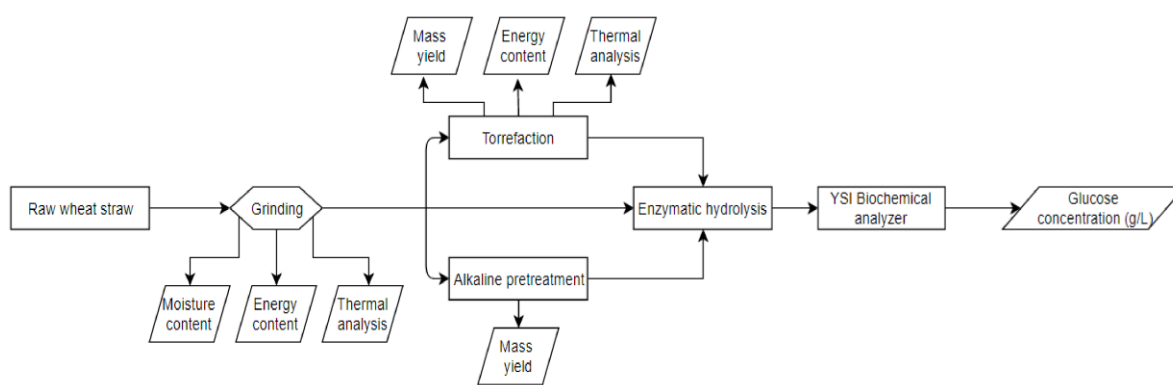


Figure 4.1 Overall diagram of the research process.

Several types of data were collected to allow for characterization of the performance of the system. This included sample mass, moisture content, heat of combustion (energy content), and glucose concentration. These data were in turn used to carry out a mass balance analysis and to calculate mg glucose per g pretreated wheat straw, and mg glucose per g raw wheat straw.

#### 4.6.1. Moisture Analysis

To determine the moisture content of raw, torrefied, and alkaline pretreated wheat straw, samples were weighed before and after drying at 105 °C in an oven (VWR, Signature) for 48 h. Moisture content was calculated on a wet basis, as follows:

$$MC_{WB} = \frac{M_W - M_D}{M_W} \times 100 \dots \dots \dots \text{Eq. 4.1}$$

Where:

$MC_{WB}$  = moisture content on a percent basis,

$M_W$  = total weight (wet basis)

$M_D$  = dry weight.

#### 4.6.2. Mass Balance/ Mass Yield

The amount of solids recovered after pretreatment was measured for use in the mass balance of the process. For torrefaction pretreatment, tray and samples were weighed before and after the torrefaction process. This information was combined with sample moisture content data to determine dry mass loss during the pretreatment. For alkaline pretreatment, pre-dried and weighed cloth filters were used for filtration process. Alkaline pretreated solid was filtered on the cloth and was dried in an oven at 105°C. Solid residue from alkaline pretreatment and cloth filter were then weighed. Mass balance of the process was then determined as follows:

$$\% \text{ Mass yield} = \frac{\text{amount of dry biomass recovered after pretreatment (g)}}{\text{amount of dry biomass added to pretreatment process(g)}} \times 100 \dots \dots \dots \text{Eq. 4.2.}$$

#### **4.6.3. Thermogravimetric Analysis (TGA)**

Raw and torrefied samples weight changes were measured as a function of temperature at a constant heating rate of 20°C per min. Change in the weight (%) of the samples were plotted against temperature to visualize regions corresponding to decomposition of the biomass compounds. Rate of the weight change was obtained by taking the first derivative of TGA data. Differential thermal gravimetric (DTG) curve was obtained by plotting first derivative of TGA against temperature. Area of the peaks obtained in DTG curves are proportional to the weight loss at corresponding temperature range.

#### **4.6.4. Heat of Combustion**

The heat of combustion of raw and torrefied wheat straw was analyzed using Parr Oxygen Bomb Calorimeter (Model 1341, Moline, IL). Approximately 0.7 g of sample was weighed and 10 cm of fuse was attached to the fuel capsule. The sample vessel containing the fuel capsule was sealed, filled with oxygen and placed in an insulated water bath. A stirrer was then turned on for five min to reach equilibrium while recording temperature every minute. The calorimeter sample was ignited at the 6<sup>th</sup> minute and temperatures were recorded every 30 sec. The resulting temperature rise, which is proportional to the sample's energy content, is measured and the heat of combustion then calculated. This was carried out on the raw biomass and each torrefaction treatment (one measurement per treatment).

#### 4.6.5. Glucose Concentration

Subsamples of the hydrolysate liquid from all treatments were analyzed for glucose using a YSI 2700 Analyzer (YSI, Yellow Springs, OH). The YSI 2700 is a biochemical analyzer that uses specific membranes, buffers and calibration solutions to characterize sample composition. Substrate concentration is proportional to the current conducted. Glucose concentrations (g/L) in the samples were analyzed by first diluting the samples with DI water to get the samples in the range that was provided by the manufacturer, then injecting the sample into the device for analysis. The dilution ratio used in the experiments was 1:4, based on a preliminary test of the procedure. Glucose yields are calculated as shown;

$$Y_{GR} = \frac{\frac{mg \text{ glucose}}{mL} \times 10 \text{ mL}}{M_R (g)} \dots\dots\dots \text{Eq. 4.3}$$

$$Y_{GP} = \frac{\frac{mg \text{ glucose}}{mL} \times 10 \text{ mL}}{M_P (g)} \dots\dots\dots \text{Eq. 4.4}$$

Where:

$\frac{mg \text{ glucose}}{mL}$  is from YSI analysis

10 mL= total volume of assay

$Y_{GR}$ = *Glucose yield per gram of raw sample*

$Y_{GP}$ = *Glucose yield per gram of pretreated sample*

$M_R$ = *inital raw biomass added*

$M_P$ = *inital pretreated biomass added*

#### **4.6.6. Statistical Analysis**

Collected data were used to test the project's hypotheses (Section 3.3) and analyze the effect of different pretreatment types and levels. ANOVA analysis was performed using statistical software Minitab for comparing mean glucose levels for different experimental conditions. The level of significance was set at 0.05. Tukey HSD test was performed to statistically compare groups. Student t-test was used for hypotheses testing. Design of the experiments and analysis of combined effect of torrefaction and alkaline pretreatment on mass yield and glucose yield was conducted using Response Surface Methodology (Box-Behnken) via Minitab.



## Chapter 5

### Results and Discussion

Glucose production from wheat straw and effect of two different pretreatments were investigated. General overview of the results are shown in Table 5.1.

Table 5.1 General overview of the results

	Min	Mean	Max
Torrefaction mass yield (%)	35	66	92
Torrefaction energy content (MJ/KgDM)	17.01	18.94	20.72
Alkaline pretreatment mass yield (%)	53	57	60
Alkaline and torrefaction mass yield (%)	34	54	73
Glucose concentration in hydrolysate (g/L) (Torrefied and alkaline pretreated)	0.03	5.8	9.6

### 5.1. Mass Yield

#### 5.1.1. Mass Yield for Torrefaction

Dry mass yield after torrefaction ranged from 35% to 92% with respect to torrefaction temperature and time as shown in Table 5.2. Increasing the torrefaction

temperature and torrefaction time decreases the mass yield. Wheat straw torrefied at 180°C for 45 min has the highest dry mass yield with 92%. Increasing the torrefaction time to 75 min decreases dry mass yield to 90%. The lowest mass yields were observed at a process temperature of 260°C. At this temperature and 45min torrefaction time, mass yield is about 37%, and at 75 min the mass yield decreases to 35%. Statistical analysis indicates that torrefaction temperature has a statistically significant effect on mass yield ( $p \leq 0.05$ ) (Table 5.3) whereas torrefaction time does not. Effect of torrefaction time and temperature on mass yield of torrefied wheat straw is shown in Figure 5.1.

Table 5.2 Mass yield results for torrefaction

Torrefaction temperature (°C)	Torrefaction time (min)	Mass yield (%)
		Mean of 2 samples (CV%)
180	45	92 (0.02)
180	60	91 (0.02)
180	75	90 (0.5)
220	45	78 (0.4)
220	60	73 (0.2)
220	75	68 (0.6)
260	45	37 (1.0)
260	60	36 (0.01)
260	75	35 (0.8)

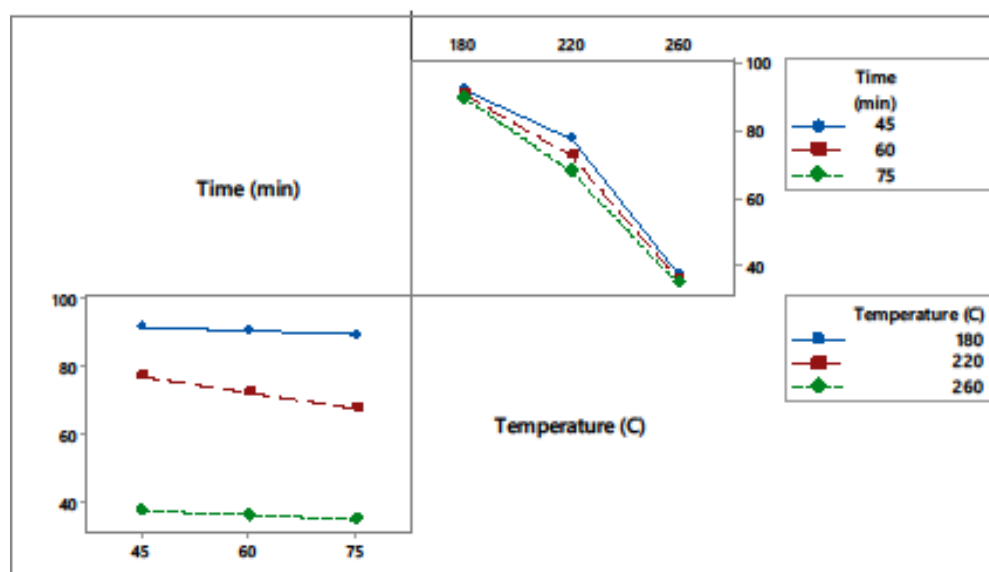


Figure 5.1 Effect of torrefaction time and temperature on mass yield of torrefied wheat straw.

Table 5.3 ANOVA for mass yield of torrefaction

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Torrefaction time (min)	1	0.88	0.88	0.03	0.857
Torrefaction temperature (°C)	1	352.38	352.38	13.49	0.000
Torrefaction time (min)* Torrefaction temperature (°C)	1	0.09	0.09	0.00	0.955
Error	14	365.58	26.11		
Total	17	9516.37			

Wheat straw samples torrefied at 180°C had noticeably higher mass yield than samples torrefied at 220°C or 260°C. At every torrefaction temperature, 45 min torrefaction had the highest mass yield. Mass loss during torrefaction can be associated with loss of moisture and low volatile components, as well as decomposition, devolatilization and carbonization of other polymers and solids as the temperature increases. According to Shang et al., (2012) hemicellulose starts to decompose around 200°C and temperatures below this do not cause any structural change for wheat straw. Results from the present study also indicate that there might not be major structural changes when wheat straw is torrefied at 180°C because of the high mass yield which could be related to dehydration reactions and volatilization of extractives (Tumuluru et al., 2011). The greater mass loss during torrefaction temperatures at 220°C and 260°C is possibly due to depolymerization reactions of hemicellulose and other components. Mass loss of samples torrefied at 260°C is greater than the expected hemicellulose content (20-32%); this suggests that thermal decomposition of cellulose and/or lignin is likely to be occurring at this temperature.

### **5.1.2. Mass Yield for Alkaline Pretreatment**

Alkaline pretreated raw wheat straw mass yield for alkaline concentrations (w/v %) 0.75%, 1%, and 2 % are 60%, 58%, and 53% respectively. Results are given as averages of two replications. As the alkaline concentration increases, mass yield decreases (Table 5.4). Statistical analysis shows alkaline concentration has a significant effect on mass yield for alkaline pretreated (not torrefied) wheat straw (Table 5.5).

Table 5.4 Mass yield results for alkaline pretreated wheat straw

Alkaline concentration (% w/v)	Solid recovery (%) Mean of 2 samples (CV%)
0.75	60 (2.7)
1	58 (3.5)
2	53 (2.0)

Table 5.5 ANOVA results for alkaline pretreated samples mass yield

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Alkaline concentration (w/v %)	1	60.940	60.940	26.34	0.007
Error	4	9.253	2.313		
Total	5	70.193			

Solid losses during only alkaline pretreatment range from 40-47% depending on the alkaline concentration that is being used. Similar solid loss data and pretreatment conditions have been reported for wheat straw in the literature (McIntosh and Vancov, 2011; Sharma et al., 2013). Assuming that the lignin content in wheat straw ranges between 17-19% this magnitude of mass loss could be an indication of hemicellulose degradation or even cellulose degradation during pretreatment.

### 5.1.3. Mass Yield for Torrefied and Alkaline Pretreatment

Alkaline pretreatment's effect on mass yield for torrefied wheat straw samples was also investigated. The highest mass yield is observed for samples torrefied at 260°C for 60 min and pretreated with 0.75 % alkaline solution. The lowest mass yield was observed for samples torrefied at 220°C for 75 min and pretreated with 2 % alkaline solution. Table 5.6 shows average mass yield for torrefied and alkaline pretreated samples.

Table 5.6 Mass yield results for torrefied and alkaline pretreated samples according to RSM model

Torrefaction temperature (°C)	Torrefaction time (min)	Alkaline concentration (% w/v)	Mass yield (%) of torrefied biomass Mean of 2 samples (CV %)	Mass yield (%) of raw dry biomass Mean of 2 samples (CV %)
180	45	1	54 (9.7)	53 (9.7)
180	60	0.75	58 (6.9)	56 (6.9)
180	60	2	51(2.1)	49(2.1)
180	75	1	58 (2.4)	55 (2.4)
220	45	0.75	58 (3.4)	48 (3.4)
220	45	2	45 (0.1)	37 (0.1)
220	60	1	49 (4.3)	37 (4.3)
220	60	1	49 (4.6)	38 (4.6)
220	60	1	48 (4.0)	38(4.0)
220	75	0.75	53 (3.7)	38(3.7)
220	75	2	34 (11.7)	24 (11.7)
260	45	1	69 (4.2)	27(4.2)
260	60	0.75	73 (1.1)	28 (1.1)
260	60	2	53 (0.3)	20 (0.3)
260	75	1	66 (1.7)	24 (1.7)

All three parameters (temperature, time, alkaline concentration) have a significant effect on % mass yield of the process ( $p \leq 0.05$ ) (Table 5.7). Among all three parameters, alkaline concentration has the most dramatic effect on mass yield with a negative relationship. For all samples that were torrefied at 260°C, relatively high mass yields were achieved relative to the mass of the torrefied sample, suggesting that the alkaline solution is less effective on severely torrefied biomass. Both temperature and square of temperature have a significant effect. This suggests that torrefaction temperature and mass yield have a nonlinear relationship. Torrefaction time is statistically significant, which is different than the samples that were torrefied but not alkaline treated.

Moderate solid loss during alkaline pretreatment is likely caused by lignin and hemicellulose solubilization. However, extensive solid loss can be an indication of cellulose breakdown and glucose loss during pretreatment. Because of this, it may be preferable to for the process not to have extensive solid loss. However, final glucose yield per gram of dry biomass could be better indication for determining this possibility.

Table 5.7 ANOVA results for torrefied and alkaline pretreated samples mass yield from RSM model

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	10	2706.99	270.70	57.61	0.000
Blocks	1	44.75	44.75	9.52	0.006
Linear	3	1202.96	400.99	85.34	0.000
Alkaline concentration	1	874.46	874.46	186.11	0.000
Torrefaction time	1	95.26	95.26	20.27	0.000
Torrefaction temperature	1	233.25	233.25	49.64	0.000
Square	3	1088.97	362.99	77.25	0.000
alkali*alkali	1	31.11	31.11	6.62	0.019
time*time	1	5.69	5.69	1.21	0.285
temp*temp	1	1078.30	1078.30	229.49	0.000
2-Way Interaction	3	164.79	54.93	11.69	0.000
time*alkali	1	44.64	44.64	9.50	0.006
temp*alkali	1	95.93	95.93	20.42	0.000
temp*time	1	24.22	24.22	5.15	0.035
Error	19	89.28	4.70		
Lack-of-Fit	15	88.81	5.92	51.34	0.001
Pure Error	4	0.46	0.12		
Error	29	2796.27			

The RSM generated equation for mass yield for alkaline pretreated torrefied wheat straw is shown in equation 5.1 and associated plots are shown in Figure 5.2.

$$Y_{MR} = 43.05 - 7.393 X_3 - 2.620 X_2 + 4.099 X_1 + 3.46 X_3^2 + 0.878 X_2^2 + 12.084 X_1^2 -$$

$$2.175 X_3 * X_2 - 3.188 X_3 * X_1$$

$$- 1.740 X_2 * X_1 \dots\dots\dots \text{Eq 5.1}$$



where  $X_1$ ,  $X_2$ ,  $X_3$  are torrefaction temperature ( $^{\circ}\text{C}$ ), torrefaction time (min) and alkaline concentration (w/v%) respectively.

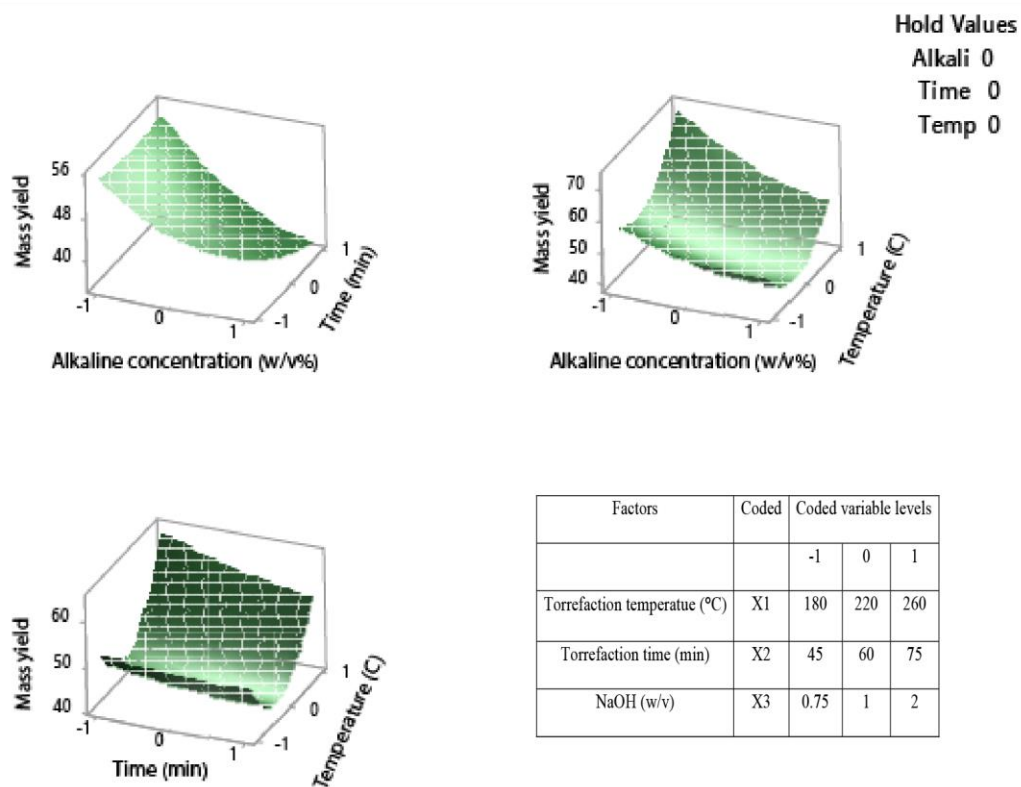


Figure 5.2 Surface plots of model regression equation for mass yield % (g solid recovered  $\text{g}^{-1}$  pretreated biomass) in response to the interactions of torrefaction temperature, torrefaction time, and alkaline concentration.

#### 5.1.4 Mass Balance

Overall, mass yield data were used to form a mass balance for the whole process to further use for glucose yield per gram of raw wheat straw. A schematic flow diagram (Figure 5.3) shows the steps and mass flows for every step in the experiment. In order to

form a connection between produced glucose and amount of raw biomass used, a mass balance was formed for the process inside the dotted lines using the mass yield percentages calculated for both pretreatment types. Pretreatment conditions and mass balance are given in Table 5.8. For every enzymatic hydrolysis experiment, 0.15 g of dry pretreated or raw biomass was used. For each pretreatment condition, the amount of raw biomass needed in the beginning in order to obtain the 0.15 g of dry material needed for enzymatic hydrolysis was calculated. Calculations were done using mean values of mass yields calculated in the previous sections (Eq. 4.2).

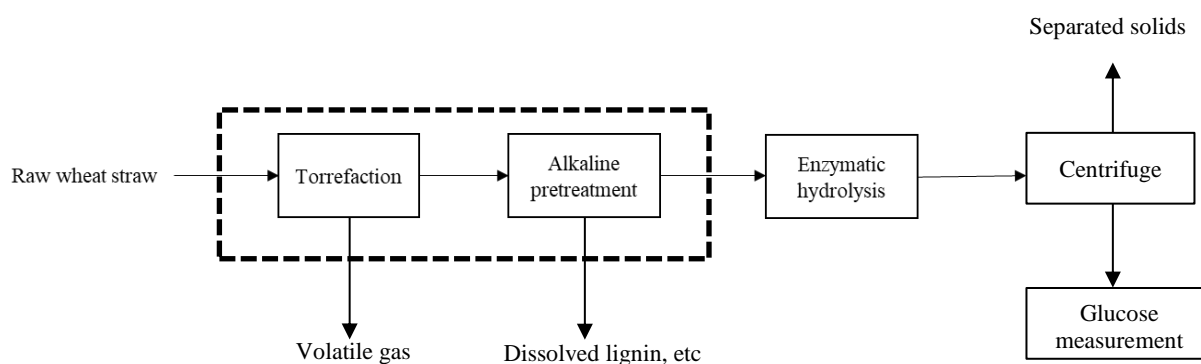


Figure 5.3 Flow diagram of the pretreatment process

Table 5.8 Mass balance for all pretreated samples

Pretreatment conditions	Raw biomass required for 0.15g pretreated biomass (g DM)	g DM torrefied biomass	g DM pretreated sample used for enzymatic hydrolysis
Raw wheat	0.15	N/A	0.15
Alkaline pretreatment			
0.75%	0.25	N/A	0.15
1%	0.26	N/A	0.15
2%	0.28	N/A	0.15
Torrefaction			
180 °C,45 min	0.16	0.15	0.15
180 °C,60 min	0.16	0.15	0.15
180 °C,75 min	0.17	0.15	0.15
220 °C,45 min	0.19	0.15	0.15
220 °C,60 min	0.21	0.15	0.15
220 °C,75 min	0.22	0.15	0.15
260 °C,45 min	0.40	0.15	0.15
260 °C,60 min	0.42	0.15	0.15
260 °C,75 min	0.43	0.15	0.15
Combined pretreatment (Temp, Time, Alkaline concentration)			
180 °C,45 min,	0.29	0.28	0.15
180 °C,60	0.27	0.26	0.15
180 °C,60 min,	0.30	0.29	0.15
180 °C,75 min,	0.27	0.26	0.15
220 °C,45	0.31	0.26	0.15
220 °C,45 min,	0.41	0.34	0.15
220 °C,60 min,	0.40	0.31	0.15
220 °C,75	0.39	0.31	0.15
220 °C,75 min,	0.61	0.31	0.15
260 °C,45 min,	0.55	0.28	0.15
260 °C,60	0.54	0.44	0.15
260 °C,60 min,	0.74	0.22	0.15
260 °C,75 min,	0.62	0.21	0.15

## 5.2. Thermogravimetric Analysis

Thermogravimetric analysis mass measurements of raw and torrefied wheat straw samples are shown in Figure 5.4. Three stages can be hypothesized during the heating process; drying, primary devolatilization, and secondary devolatilization. The first stage likely corresponds to loss of moisture and release of some low volatiles and occurs in the test at temperatures of 30°C to 130°C. The second stage likely corresponds to thermal decomposition of hemicellulose, cellulose, and possibly lignin and would correspond to the steep portion of the graph. As can be seen from the graph, mass loss during this stage is significant. The third stage consists of a slower rate of mass loss and may correspond to decomposition of lignin and other “low volatility” components.

The DTG distributions of torrefied and raw samples are given in Figure 5.5. The main peak observed in the curve is between 250-350°C and the maximum rate of mass loss falls between 310°C and 342°C for all the samples. Cellulose’s decomposition temperature falls in this range (Chen et al., 2010). As the torrefaction temperature increases the area under the peak is decreasing. Since the main peak observed on DTG graph is assumed to be mainly correlated with cellulose, the decrease in the intensity of the peak could be attributed to cellulose degradation during torrefaction. Wheat straw torrefied at 260°C has the smallest peak at this region indicating cellulose content is lower for these samples. When thermal analysis is taken into account samples we might be losing most of the available cellulose during torrefaction at 260°C which would make these samples unfavorable for enzymatic hydrolysis.





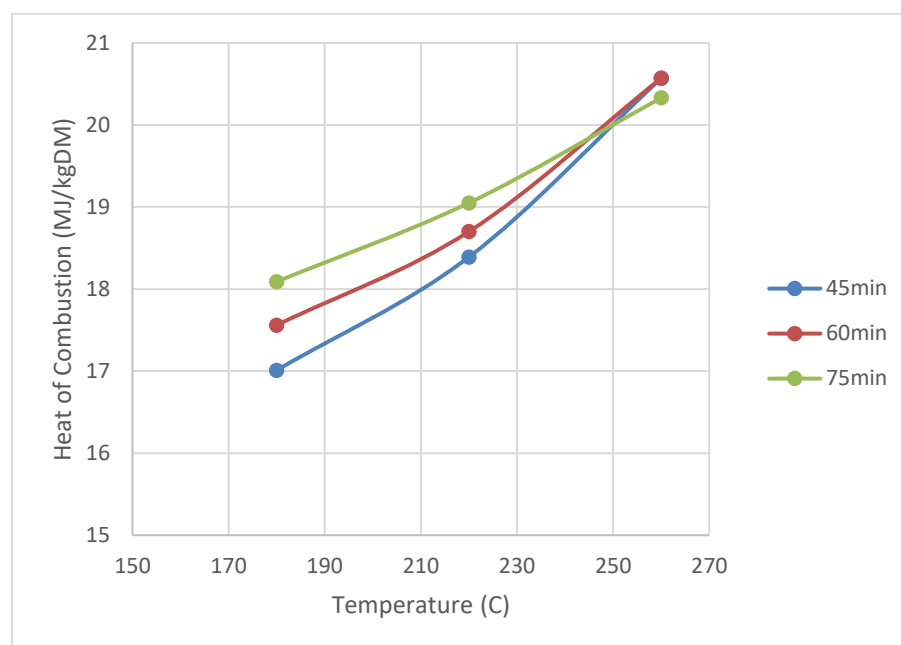


Figure 5.6 Effect of torrefaction temperature on heat of combustion value of torrefied wheat straw

Torrefaction time has a positive correlation with heat of combustion for 180°C and 220°C but when the temperature is increased to 260°C a decrease is observed with the increase of torrefaction time (Figure 5.7.). Statistical analysis shows torrefaction temperature and torrefaction time have a significant effect ( $p \leq 0.05$ ) on heat of combustion (Table 5.9.). All of the samples had higher heat of combustion values than raw biomass except for the sample that was torrefied at 180 ° C for 45 min (Figure 5.7.). Raw wheat straw has a heat of combustion value of 17.28 MJ/kgDM whereas wheat straw torrefied at 180 ° C for 45 min had a heat of combustion value of 17.07 MJ/kgDM. This could be due to energy loss being greater than mass loss at this torrefaction temperature and time perhaps due to volatilization of high energy extractives during this very mild torrefaction treatment. The maximum increase (19.9%) in heat of combustion was observed for samples torrefied

at 260°C for 45 min. Results show that, as the torrefaction temperature increases, the energy content of the wheat straw increases. Low energy density of biomass is a drawback for handling and logistics purposes.

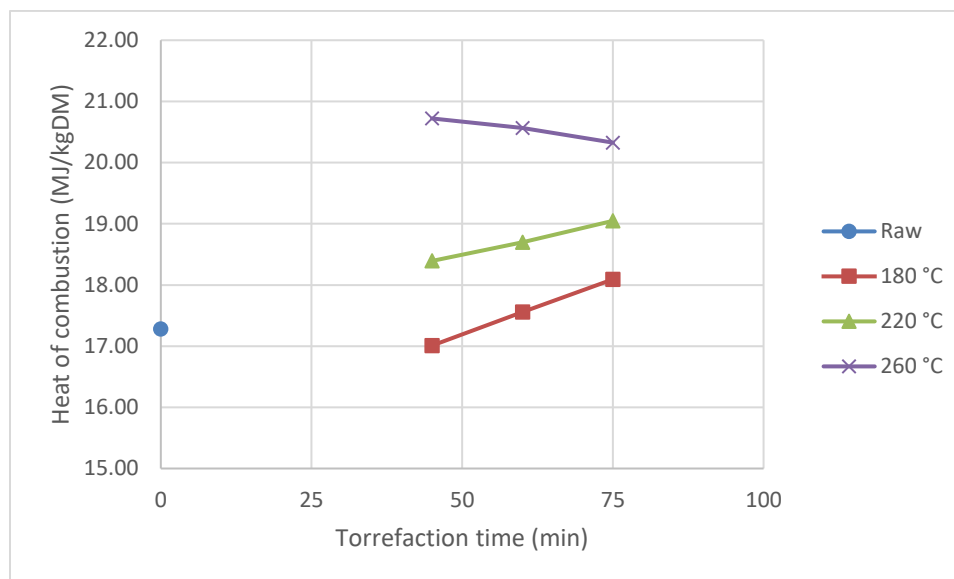


Figure 5.7 Effect of torrefaction time on heat of combustion value of torrefied wheat straw.

Table 5.9 ANOVA table for effect of torrefaction temperature and time on heat of combustion

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperaure	1	2.1169	2.11691	41.26	0.001
Time	1	0.6580	0.65799	12.82	0.016
Temperaure*Time	1	0.5457	0.54570	10.64	0.022
Error	5	0.2565	0.05131		
Total	8	14.4678			



## 5.4. Enzymatic Hydrolysis

### 5.4.1. Glucose Yield from Enzymatic Hydrolysis of Torrefied Wheat Straw

Nine different torrefaction conditions (three torrefaction times and three torrefaction temperatures) and their effect on glucose yield were evaluated. Glucose yields from torrefied samples, after hydrolysis, are shown in Table 5.10. Among all the torrefied samples, the highest and only glucose yields were for samples torrefied at 180°C. Once the torrefaction temperature was increased to 220°C or 260 °C no glucose was detected. The maximum mg glucose yield per gram torrefied biomass is at 180°C for 60 min; whereas maximum glucose yield per gram raw biomass occurs when samples are torrefied at 180°C for 45 min. Statistical analysis indicates that torrefaction time for samples torrefied at 180°C has significant impact on glucose production ( $p \leq 0.05$ ) (Table 5.11). Statistical analysis (Tukey's test) also shows there is no significant difference in mg glucose g<sup>-1</sup> raw biomass between samples that were torrefied at 180°C for 45 min and 60 min. This result aligns with mass yield of torrefied samples since no significant mass yield difference was observed between samples torrefied at 180°C for 45 min and 60 min. This could suggest that, at 180°C, increasing the torrefaction time to 60 min from 45 min does not result in a difference in the composition of the wheat straw. Once the torrefaction time is increased to 75 min there is a decrease observed in terms of mg produced g<sup>-1</sup> raw wheat straw and mg glucose per gram of torrefied biomass.

None of the torrefied samples have glucose yield that is as high as raw wheat straw. In fact, samples torrefied at 180°C have 66-74% lower glucose yields when compared to enzymatically hydrolyzed raw wheat straw.

Table 5.10 Effect of torrefaction time and temperature on glucose concentration

Torrefaction temperature (°C)	Torrefaction time (min)	mg glucose g <sup>-1</sup> torrefied biomass Mean of 3 samples (%CV)	mg glucose g <sup>-1</sup> dm raw biomass Mean of 3 samples
Raw		-	190.1 (3.6)
180	45	65.2 (1.8)	63.8 (1.8)
180	60	65.7 (0.9)	63.7 (0.9)
180	75	51.6 (52.6)	50.2 (3.5)
220	45	ND	ND
220	60	ND	ND
220	75	ND	ND
260	45	ND	ND
260	60	ND	ND
260	75	ND	ND

Table 5.11 ANOVA table for effect of torrefaction time on glucose yield (mg glucose/ g raw biomass)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Torrefaction time (min)	1	277.825	277.825	19.7	0.003
Error	7	98.701	14.100		
Total	8	376.526			

Results show that torrefaction temperature and torrefaction time have a negative impact on glucose yield via enzymatic hydrolysis. Moderate and severe torrefaction temperatures result in no glucose production. Previous studies using torrefaction as a pretreatment also show that increasing the torrefaction temperature causes lower yields of glucose production. Testing of torrefied and enzymatically hydrolyzed olive prunings (Chiamonti et al. 2011) shows that as the torrefaction temperature increases from mild (180-220°C) to severe (240-280°C) conditions, glucose yield decreases. Torrefaction of Norway spruce at torrefied at temperatures between 260-310°C at different durations results in lower yields of glucose when compared to raw wood (Normark et al., 2016). Sheikh et al. (2013) reports that torrefied rice straw at 220°C for 40 min shows sugar yield can be increased by 60.68% when compared with the untreated sample but further increasing the torrefaction temperature leads to a decrease in glucose yield. The reason for differences between the above studies and this study could be due to the difference in torrefaction process, biomass properties, or experimental technique. For example, Sheikh et al. (2013) report samples to be torrefied first then ground.

Cellulose decomposition is a slow process which starts around 250°C. A great amount of cellulose might be degrading at severe torrefaction temperatures resulting in low or even no glucose production. However, it seems unlikely that cellulose is breaking down when torrefied at 220°C, but cellulose depolymerization may be an issue for the 260°C torrefied biomass. As to the 220°C treatments, it is likely that other mechanisms are causing the dramatic reduction in yield. It is reported in the literature that torrefaction temperatures above 200°C increase the pH of the biomass (Sadaka and Negi, 2009). The increase in the pH of the biomass could be inhibitory for enzymes used in the hydrolysis step where the optimum conditions of the enzymes are around pH 4.8. Production of inhibitory products, pH and structural changes during torrefaction should be further investigated in order to better characterize the relationship between torrefaction and glucose yield.

#### **5.4.2. Glucose Yield from Enzymatic Hydrolysis of Alkaline Pretreated Wheat Straw**

Measured glucose concentrations from alkaline treated (not torrefied) samples are shown in Table 5.12. The highest glucose yield per gram of pretreated wheat straw was observed for samples that were alkaline pretreated with 2% NaOH solution. Increasing the alkaline concentration increases the amount of glucose produced from alkaline pretreated wheat straw. This result suggests that, after pretreatment, the amount of available cellulose for enzymatic hydrolysis in the pretreated samples increases with respect to NaOH concentration. On the other hand, when the overall process is taken into account, the highest amount of glucose produced per g dm raw wheat straw is achieved when the NaOH concentration is 0.75% or 1%. ANOVA results (Table 5.13) indicate that NaOH

concentration has a statistically significant impact on glucose yield. Tukey's pairwise comparison (Table 5.14) suggests that, although samples pretreated with 2% NaOH produced higher glucose per gram of pretreated sample, this is not the case for overall yield. Samples pretreated with 2% NaOH solution have the lowest overall glucose yield. This result shows that increasing the alkaline concentration does not increase the amount of polysaccharides available for enzymatic hydrolysis. The decrease on glucose yield when mass loss during the pretreatment is taken into account suggests that any increase in cellulose availability due to the pretreatment process is counteracted by another mechanism or mechanisms, such as biomass loss during the processing step.

Results show that pretreating wheat straw with 0.75% or 1% (w/v) NaOH increases the released glucose yield per gram of raw wheat straw by nearly 1.9 fold when compared to enzymatically hydrolyzed raw wheat straw. The maximum yield of 359.1 mg glucose per gram of raw biomass was achieved with 1% NaOH pretreated enzymatically hydrolyzed wheat straw.

Table 5.12 Effect of alkaline concentration on glucose yield

NaOH concentration (% w/v)	mg glucose g <sup>-1</sup> pretreated biomass Mean of 8 samples (CV %)	mg glucose g <sup>-1</sup> dm raw biomass Mean of 8 samples (CV %)
0.75	594.4 (3.0)	358.9 (3.0)
1	621.6 (1.8)	359.1 (1.8)
2	658.9 (1.8)	346.8 (1.8)

Table 5.13 ANOVA table for effect of alkaline concentration on glucose yield (mg glucose g<sup>-1</sup> dm raw biomass)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
NaOH concentration (% w/v)	1	767.02	767.02	11.65	0.002
Error	21	1416.7	67.46		
Total	23	2215.7			

Table 5.14 Tukey's pairwise comparison of glucose yield (mg glucose g<sup>-1</sup> dm raw biomass)

Alkaline concentration	N	Mean	Grouping	
1.00	8	359.11	A	
0.75	8	358.95	A	
2.00	8	346.79		B

#### 5.4.3. Glucose Yield from Enzymatic Hydrolysis of Torrefied And Alkali Pretreated Wheat Straw

Table 5.15 shows the overall results for experiments that were designed by RSM. For this analysis, three parameters; torrefaction temperature, torrefaction time, and alkali concentration and their effects on glucose yield was investigated. Maximum mg glucose produced per gram of pretreated wheat straw was observed for samples torrefied at 180°C for 60 min and 2% NaOH pretreated followed by 220°C for 75 min and 2% NaOH pretreated samples. All the samples except for those torrefied at 260°C produce a higher amount of glucose per gram of pretreated sample when compared to enzymatically

hydrolyzed raw wheat straw. This increase ranged between 1.7-3.3 fold. Torrefaction temperature and alkaline pretreatment have a statistically significant ( $p \leq 0.05$ ) impact on mg glucose produced per g of pretreated sample (Table 5.16). Although torrefaction time is not statistically significant, ( $p > 0.05$ ) the interaction term of alkaline concentration and torrefaction time is significant. The RSM generated equation for mg glucose  $g^{-1}$  pretreated wheat straw is shown in Equation 5.2 and associated plots are in Figure 5.8.

Table 5.15 Glucose yield of torrefied and alkali pretreated wheat straw.

Torrefaction temperature (°C)	Torrefaction time (min)	Alkaline concentration (%w/v)	mg glucose/ g pretreated biomass Mean of 8 samples (CV%)	mg glucose/ g raw biomass Mean of 8 samples (CV%)
180	45	1	583.1 (2.4)	306.5 (2.4)
180	60	0.75	554.4 (2.7)	311.4 (2.7)
180	60	2	635.2 (3.6)	313.8 (3.6)
180	75	1	579.9 (4.2)	318.2 (4.2)
220	45	0.75	431.1 (3.6)	206.0 (3.6)
220	45	2	606.1 (3.7)	222.4 (3.7)
220	60	1	473.7 (6.5)	178.0 (6.5)
220	60	1	445.0 (17.0)	167.2 (17.0)
220	60	1	499.5 (4.5)	187.7 (4.5)
220	75	0.75	315.7 (6.8)	120.3 (6.8)
220	75	2	625.4 (3.5)	153.1 (3.5)
260	45	1	5.0 (71.2)	1.4 (71.2)
260	60	0.75	2.1 (150.2)	0.6 (150.2)
260	60	2	11.2 (46.3)	2.3 (46.3)
260	75	1	5.1 (81.7)	1.2 (81.7)



Table 5.16 ANOVA table for torrefied and alkali pretreated wheat straw (mg glucose g<sup>-1</sup> pretreated wheat straw)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	16	6808493	425531	162.65	0.000
Blocks	7	5964	852	0.33	0.941
Linear	3	5672589	1890863	722.74	0.000
Temperature (C)	1	5350906	5350906	2045.27	0.000
Time (min)	1	9841	9841	3.76	0.055
NaOH(% w/v)	1	311841	311841	119.19	0.000
Square	3	1087441	362480	138.55	0.000
Temperature (C)*Temperature	1	1051350	1051350	401.86	0.000
Time (min)*Time (min)	1	2517	2517	0.96	0.329
NaOH(% w/v)*NaOH(% w/v)	1	4712	4712	1.80	0.183
2-Way Interaction	3	42499	14166	5.41	0.002
Temperature (C)*Time (min)	1	22	22	0.01	0.928
Temperature	1	6171	6171	2.36	0.128
Time (min)*NaOH(% w/v)	1	36306	36306	13.88	0.000
Error	103	269473	2616		
Lack-of-Fit	87	220032	2529	0.82	0.732
Pure Error	16	49440	3090		
Total	119	7077965			

$Y_{GP} =$

$$472.7 - 289.15 X_1 - 12.40 X_2 + 69.80 X_3 - 188.66 X_1^2 + 9.23 X_2^2 + 12.63 X_3^2 + 0.82 X_1 * X_2 - 13.89 X_1 * X_3 + 33.68 X_2 * X_3 \dots\dots\dots \text{Eq. 5.2.}$$

Where

$$Y_{GP} = \text{mg glucose } g^{-1} \text{ pretreated biomass}$$

$X_1$ ,  $X_2$ ,  $X_3$ , are torrefaction temperature ( $^{\circ}\text{C}$ ), torrefaction time (min) and alkaline concentration (w/v%) respectively.

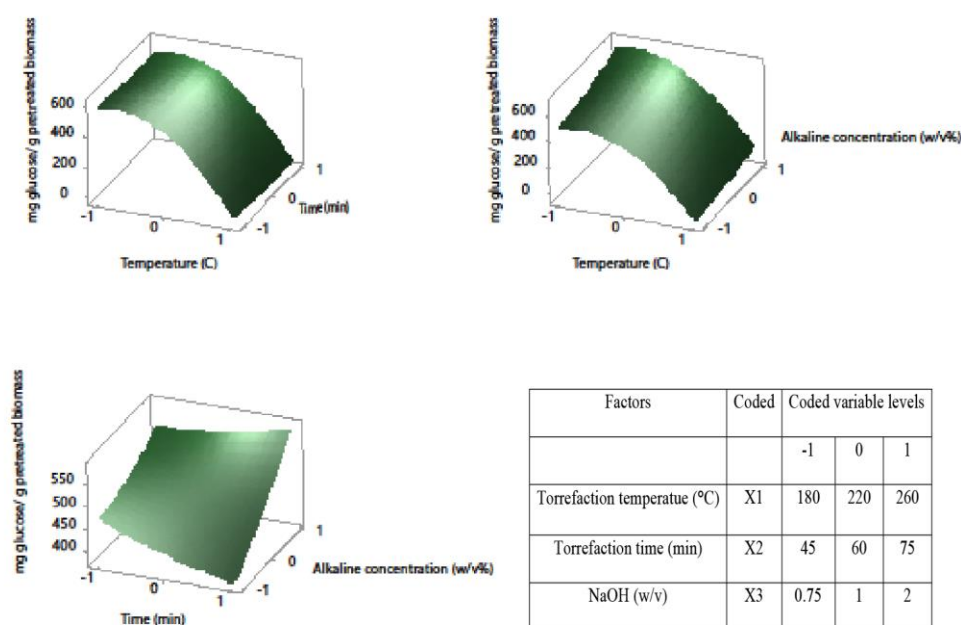


Figure 5.8 Surface plots of model regression equation for glucose yield ( $\text{mg glucose g}^{-1}$  pretreated biomass) in response to the interactions of torrefaction temperature, torrefaction time, and alkaline concentration.

Glucose yield per g raw biomass is calculated using mass yields from torrefaction and alkaline pretreatment to determine how much raw wheat straw would be needed in order to enzymatically hydrolyze 0.15g of pretreated wheat straw (Table 5.8). ANOVA results (Table 5.17) indicate that all three parameters have a significant effect ( $p \leq 0.05$ ) on mg glucose released per g of raw biomass. Torrefaction temperature and torrefaction time have a negative impact and alkaline concentration has a positive impact. Among all three parameters, torrefaction temperature has the most pronounced effect. When glucose yield

per gram of raw biomass is calculated, the maximum glucose yield occurs for samples torrefied at 180°C for 75 min and 1% NaOH pretreated. Alkaline pretreatment has a dramatic positive impact on glucose yield of torrefied biomass. Samples torrefied at 180°C from increase ~60 to ~300 mg glucose per gram of feedstock. Samples torrefied at 220°C increase from zero to ~150-220 mg glucose per gram of feedstock. Samples torrefied at 260 °C increase less notably, but still shifted from zero to ~2 mg per g of feedstock.

Samples torrefied at 180°C and alkaline pretreated have higher glucose yield (mg glucose g<sup>-1</sup> raw biomass) when compared to enzymatically hydrolyzed raw wheat straw. Samples torrefied at 220 °C for 45 min have comparable results to enzymatically hydrolyzed raw wheat straw. Further increase in the torrefaction temperature and time decreases the glucose yield lower than raw wheat straw yield. The maximum glucose yield per g raw biomass is nearly 1.7 times that of enzymatically hydrolyzed raw wheat straw. The RSM generated equation for mg glucose g<sup>-1</sup> raw wheat straw is shown in Equation 5.3 and associated plots are in Figure 5.9.

$Y_{GR} =$

$$177.65 - 155.54 X_1 - 18.31 X_2 + 7.05 X_3 - 20.03 X_1^2 - 0.81 X_2^2 - 0.63 X_3^2 - 2.97 X_1 * X_2 - 0.19 X_1 * X_3 + 3.33 X_2 * X_3 \dots \dots \dots \text{Eq. 5.3.}$$

Where

$Y_{GR} =$  mg glucose g<sup>-1</sup> raw biomass

$X_1, X_2, X_3$  are torrefaction temperature (°C), torrefaction time (min) and alkaline concentration (w/v%) respectively.

Table 5.17 ANOVA table for torrefied and alkali pretreated wheat straw (mg glucose g<sup>-1</sup> raw wheat straw)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	16	1586308	99144	227.84	0.000
Blocks	7	714	102	0.23	0.976
Linear	3	1573070	524357	1205.00	0.000
Temperature (C)	1	1548422	1548422	3558.37	0.000
Time (min)	1	21463	21463	49.32	0.000
NaOH(% w/v)	1	3185	3185	7.32	0.008
Square	3	11887	3962	9.11	0.000
Temperature (C)*Temperature	1	11849	11849	27.23	0.000
Time (min)*Time (min)	1	19	19	0.04	0.833
NaOH(% w/v)*NaOH(% w/v)	1	12	12	0.03	0.870
2-Way Interaction	3	637	212	0.49	0.691
Temperature (C)*Time (min)	1	282	282	0.65	0.422
Temperature	1	1	1	0.00	0.960
Time (min)*NaOH(% w/v)	1	354	354	0.81	0.369
Error	103	44820	435		
Lack-of-Fit	87	38667	444	1.16	0.390
Pure Error	16	6153	385		
Total	119	1631128			

It should be noted that when overall mass is taken into account alkaline pretreated (but not torrefied) wheat straw has higher glucose yields when it is compared with torrefied alkaline pretreated and enzymatically hydrolyzed wheat straw (student t-test with  $p \leq 0.05$ ). This suggests that cellulose loss might be accruing during the torrefaction process due to thermal decomposition.

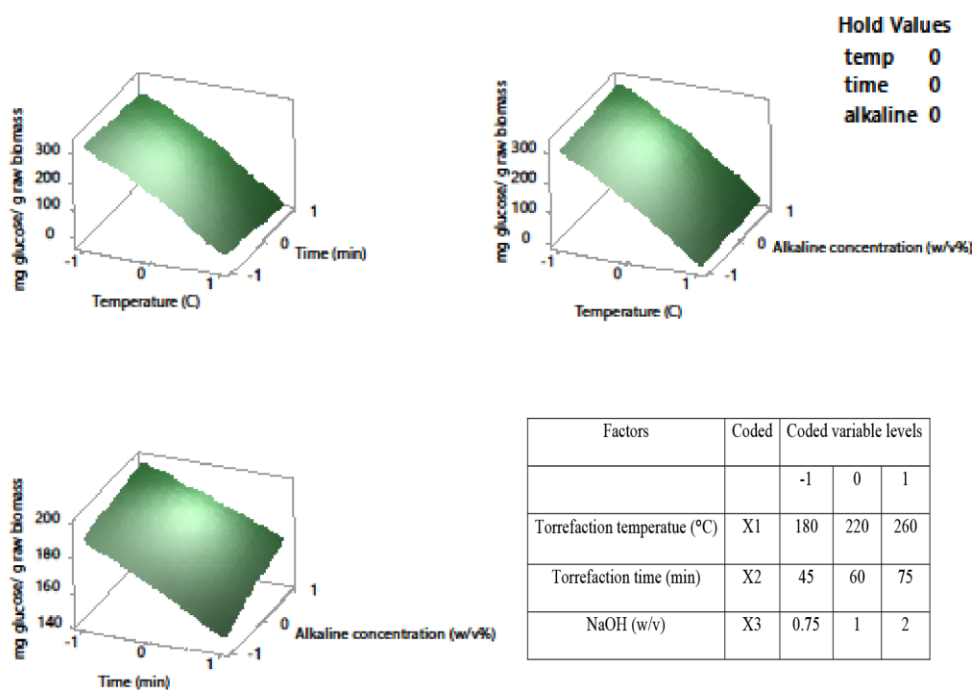


Figure 5.9 Surface plots of model regression equation for glucose yield ( $\text{mg glucose g}^{-1}$  raw biomass) in response to the interactions of torrefaction temperature, torrefaction time, and alkaline concentration.

These results indicate that torrefied and alkaline pretreated samples (except for samples torrefied at  $260^{\circ}\text{C}$ ) are more amenable to enzymatic hydrolysis than either raw or “torrefied only” samples with respect to glucose yield per gram of feedstock. This suggests that combined pretreatment is effective in terms of increasing the amount of available cellulose in the substrate. Samples torrefied at  $220^{\circ}\text{C}$  and  $260^{\circ}\text{C}$  do not produce detectable glucose but when they are combined with alkaline pretreatment, glucose is then obtainable. Still, samples torrefied at  $260^{\circ}\text{C}$  then alkaline pretreated have very low glucose yields. This result, as well as TGA analysis, strengthens the supposition about decomposition of most

of the available cellulose during torrefaction at 260°C. Samples torrefied at 220°C, when combined with alkaline pretreatment, produce glucose yields higher than raw wheat straw. This suggests that there might be an enzyme inhibitory product occurrence during torrefaction which is removed when subjected to an alkaline solution.

Some of the samples result in lower glucose yield ( $\text{mg glucose g}^{-1}$  raw biomass) when compared to enzymatically hydrolyzed wheat straw. The reason behind this might be the solid loss during the process being higher than effectiveness of the pretreatment process. Normark et al., (2015) reports that glucose yield from Norway spruce torrefied and pretreated with ionic liquid results in comparable glucose yields when compared to samples only pretreated with ionic liquid. Their results also show severe torrefaction (310°C), even with ionic liquid pretreatment, decreases the glucose yield. Their results align with results observed during this research.

If we assume a cellulose content of 36%, enzymatically hydrolyzed non-pretreated raw sample nearly digests 47% of the available cellulose. Alkaline pretreatment increases this number to 87-90%. For samples torrefied and alkaline pretreated, lower digestion yields suggest that either there is an excessive solid loss with part of it being cellulose, or the alkaline pretreatment process is less effective on torrefied samples.

Torrefaction severity, or mass loss %, is one way of combining torrefaction time and temperature into a single measure of the torrefaction process. As such, it allows for simplified visualization of the impact of torrefaction and alkaline pretreatment on glucose yield. Change in glucose yield ( $\text{g glucose g}^{-1}$  raw biomass) with respect to torrefaction severity (mass loss) is shown in Figure 5.4. The impact of combining torrefaction with

alkaline pretreatment can be clearly observed for samples with moderate and mild torrefaction severity.

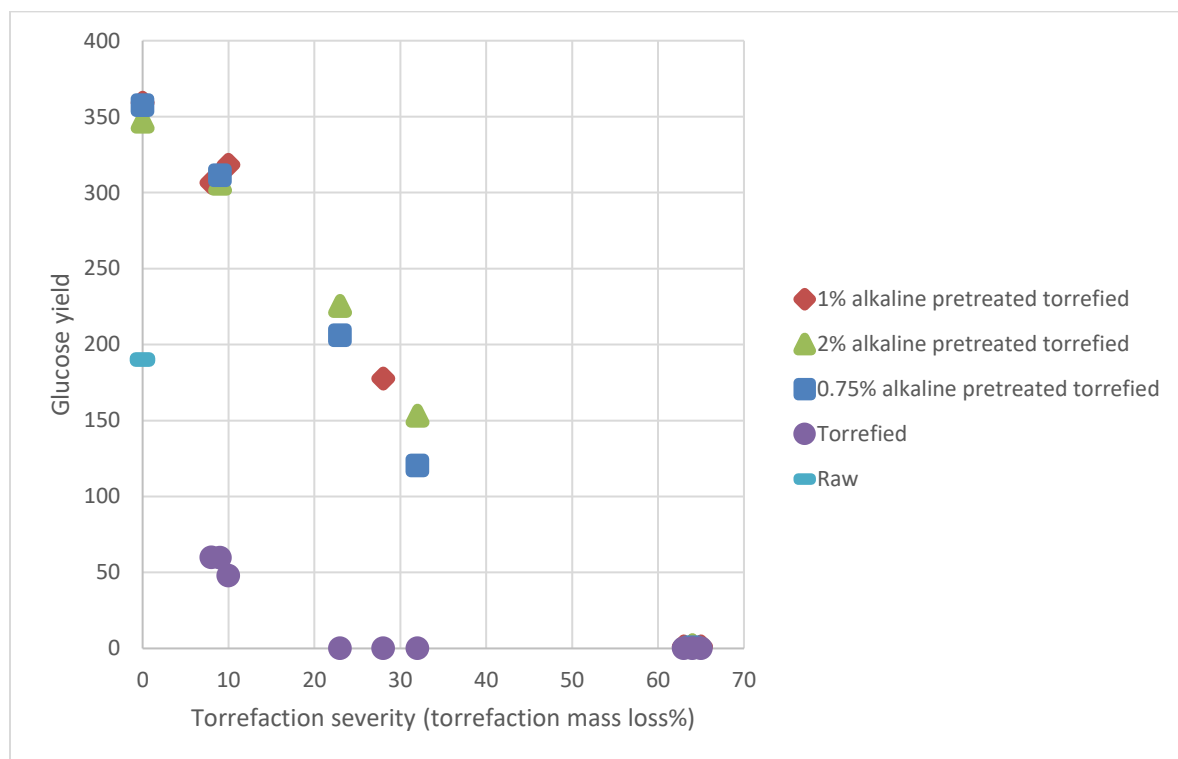


Figure 5.10 Change in glucose yield per g raw biomass with respect to torrefaction severity

If torrefaction is to be a practical component of a biofuel supply chain that utilizes microbial hydrolysis, the supply chain benefits will have to compensate for reduced yield. Alkaline pretreatment lessens the negative impact of torrefaction on glucose yield, but even so, only very mild torrefaction conditions appear to be able to achieve higher glucose yield than raw wheat straw.

## Chapter 6

### Conclusions

Torrefaction, alkaline pretreatment and the combination of these two process and their impact on glucose yield has been investigated. Without any additional pretreatment torrefaction as pretreatment resulted in decreased glucose yield. Moderate (220°C) and severe (260°C) torrefaction temperatures do not produce any glucose. Pretreating wheat straw with 1% alkaline solution increases glucose yields from 190.1 mg glucose g<sup>-1</sup> raw biomass to 359.11 mg glucose g<sup>-1</sup> raw biomass. This indicates that alkaline pretreatment is an effective pretreatment process for increasing glucose yield from wheat straw. Combining torrefaction with alkaline pretreatment shows potential as long as torrefaction conditions are not severe.

This research shows there is potential for mild torrefaction to be used as pretreatment when combined with alkaline chemical pretreatment. Pretreatment of torrefied biomass prior to enzymatic hydrolysis could be a promising process to allow for benefits of torrefied biomass to be realized while still obtaining high conversion yield. In order to optimize the process, further investigations on how torrefaction changes the chemical structure of wheat straw would be beneficial in order to better guide the selection and optimization of pretreatments. In order to overcome excessive mass loss during pretreatment, different torrefaction temperatures and torrefaction time combinations should be assessed.



The reduction of glucose yield due to torrefaction and alkaline pretreatment could be due to a number of factors, including thermal decomposition of cellulose, solubilization of cellulose during the washing step after alkaline pretreatment, production of inhibitory compounds, and shifts in pH. It is likely that more than one of these mechanisms is active in this system.

For further research, investigation of these factors could shed light on the degree to which glucose yield from torrefied samples can be enhanced.

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## Appendix A

### Mass Yield for Torrefied Wheat Straw

Temperature (°C)	Time (min)	Empty tray (g)	Full tray (g)	Out of oven mass (g)	% Mass yield
180	45	411.08	454.33	450.85	91.95
180	45	411.08	452.61	449.28	91.98
180	60	409.4	449.97	446.35	91.08
180	60	411.43	452.56	448.9	91.10
180	75	409.49	450.67	446.3	89.39
180	75	409.52	451.2	447.03	90.00
220	45	410.93	452.55	443.19	77.51
220	45	409.41	449.47	440.28	77.06
220	60	409.47	460.21	446.2	72.39
220	60	409.34	450.54	439.25	72.60
220	75	411.07	452.17	438.8	67.47
220	75	411.01	451.15	438.33	68.06
260	45	414.37	455.27	429.71	37.51
260	45	411.4	451.64	426.26	36.93
260	60	409.44	439.76	420.34	35.95
260	60	411.4	451.59	425.85	35.95
260	75	411.41	453.41	425.99	34.71
260	75	411.36	452.1	425.35	34.34

**Appendix B****Mass Yield for Alkaline Pretreated Wheat Straw**

Alkaline concentration (w/v%)	Sample used for pretreatment (g dm)	Amount solid recovered after pretreatment (g dm)	Mass yield (%)
0.75	3.9997	2.3690	59.23
0.75	3.9999	2.4620	61.55
1	4.0025	2.2550	56.34
1	4.0002	2.3680	59.20
2	4.0029	2.0770	51.89
2	3.9999	2.1350	53.38

### Appendix C

#### Mass Yield for Alkaline Pretreated Torrefied Wheat Straw

Temperature (°C)	Time (min)	Alkaline concentration (w/v%)	Sample used for pretreatment (g dm)	Amount solid recovered after pretreatment (g dm)	Mass yield (%)
180	45	1	4.0009	2.0170	50.4131
180	45	1	4.0007	2.2960	57.3906
180	60	0.75	4.0007	2.2050	55.1160
180	60	0.75	4.0007	2.4320	60.7901
180	60	2	4.0005	2.0090	50.2192
180	60	2	4.0008	2.0700	51.7403
180	75	1	4.0010	2.3405	58.4972
180	75	1	4.0011	2.2610	56.5088
220	45	0.75	4.0009	2.2690	56.7116
220	45	0.75	4.0006	2.3810	59.5167
220	45	2	4.0008	1.7847	44.6091
220	45	2	4.0009	1.7870	44.6655
220	60	1	4.0008	1.8830	47.0662
220	60	1	4.0009	1.9930	49.8144
220	60	1	4.0009	1.9000	47.4899
220	60	1	4.0008	2.0270	50.6655
220	60	1	4.0010	1.8880	47.1877
220	60	1	4.0009	2.0070	50.1631
220	75	0.75	4.0009	2.059	51.4628
220	75	0.75	4.0009	2.17	54.2385
220	75	2	4.0010	1.246	31.1419



220	75	2	4.0006	1.471	36.7699
260	45	1	4.0009	2.693	67.3090
260	45	1	4.0006	2.857	71.4151
260	60	0.75	4.0010	2.928	73.1809
260	60	0.75	4.0005	2.883	72.0667
260	60	2	4.0010	2.118	52.9362
260	60	2	4.0007	2.109	52.7164
260	75	1	4.0009	2.609	65.2095
260	75	1	4.0008	2.671	66.7624

### Appendix D

#### Glucose Yield from Enzymatic Hydrolysis of Torrefied Wheat Straw

Temperature (°C)	Time (min)	YSI reading (Glucose-EB-SB) (g/L)	mg glucose g <sup>-1</sup> torrefied DM	mg glucose g <sup>-1</sup> raw DM
180	45	0.9955	66.2633	64.83069
180	45	0.9655	63.9743	62.59117
180	45	0.9855	65.2995	63.88773
180	60	1.0075	66.0283	63.98376
180	60	0.9975	64.9973	62.98461
180	60	1.0075	66.0283	63.98376
180	75	0.7885	52.2462	49.85158
180	75	0.8285	54.5425	52.04258
180	75	0.7685	50.9210	48.58712
220	45	N/A	N/A	N/A
220	45	N/A	N/A	N/A
220	45	N/A	N/A	N/A
220	60	N/A	N/A	N/A
220	60	N/A	N/A	N/A
220	60	N/A	N/A	N/A
220	75	N/A	N/A	N/A
220	75	N/A	N/A	N/A
220	75	N/A	N/A	N/A
260	45	N/A	N/A	N/A
260	45	N/A	N/A	N/A
260	45	N/A	N/A	N/A
260	60	N/A	N/A	N/A

260	60	N/A	N/A	N/A
260	60	N/A	N/A	N/A
260	75	N/A	N/A	N/A
260	75	N/A	N/A	N/A
260	75	N/A	N/A	N/A

### Appendix E

#### Glucose Yield from Enzymatic Hydrolysis of Alkaline Pretreated Wheat Straw

Alkaline concentration (w/v%)	YSI reading (Glucose-EB- SB) (g/L)	mg glucose g <sup>-1</sup> alkaline pretreated DM	mg glucose g <sup>-1</sup> raw DM
0.75	9.4628	623.8010	376.7171
0.75	8.6128	574.1833	350.7787
0.75	8.7133	563.1625	344.0003
0.75	8.9383	594.2985	358.9004
0.75	8.6788	572.5373	349.7426
0.75	9.0988	599.1065	361.8039
0.75	8.9473	594.6676	359.1232
0.75	9.2773	613.5747	370.5414
1	9.3248	619.6053	357.9367
1	9.6548	625.8143	361.5235
1	9.6098	640.6500	370.0939
1	9.3998	626.6500	362.0063
1	9.5648	627.0528	362.239
1	9.2798	618.6500	357.3848
1	9.1883	612.5500	353.8609
1	9.1433	602.1436	347.8493
2	10.1900	669.6898	352.4709
2	10.1800	678.6667	357.1956
2	9.7473	649.8167	342.0113
2	9.8973	659.8167	347.2745
2	9.6428	642.8500	338.3446
2	9.8078	653.8500	344.1341

2	10.1518	666.5189	350.802
2	10.1068	649.9936	342.1044

**Appendix F**  
**Glucose Yield from Enzymatic Hydrolysis of Torrefied and Alkaline Pretreated**  
**Wheat Straw**

Temperature (°C)	Time (min)	Alkaline concentration (w/v%)	YSI reading (Glucose- EB-SB)	mg glucose g <sup>-1</sup> torrefied DM	mg glucose g <sup>-1</sup> raw DM
180	45	1	9.074	603.967	317.4032
180	45	1	8.754	582.0479	305.8841
180	45	1	9.028	580.773	305.2141
180	45	1	9.068	600.2118	315.4298
180	45	1	8.62	573.8403	301.5708
180	45	1	8.66	576.9679	303.2144
180	45	1	8.412	560.3219	294.4664
180	45	1	8.812	586.9228	308.446
180	60	0.75	8.446	559.3192	314.1053
180	60	0.75	8.366	554.4069	311.3466
180	60	0.75	8.628	571.9286	321.1865
180	60	0.75	8.468	562.329	315.7955
180	60	0.75	8.372	557.3901	313.0219
180	60	0.75	7.852	522.9089	293.6578
180	60	0.75	8.448	562.7873	316.0529
180	60	0.75	8.168	544.497	305.7813
180	60	2	9.6740	643.4919	317.8925
180	60	2	9.0340	601.1725	296.9862
180	60	2	8.4880	542.0317	267.77
180	60	2	8.6080	561.0449	277.1627
180	60	2	9.7560	649.707	320.9628

180	60	2	9.6360	641.7155	317.0149
180	60	2	10.0520	669.7136	330.8463
180	60	2	9.6520	643.4238	317.8588
180	75	1	8.9620	595.5609	326.769
180	75	1	8.6820	576.1574	316.1228
180	75	1	8.6280	549.0645	301.2577
180	75	1	8.8680	579.6988	318.0659
180	75	1	9.0680	604.4527	331.6478
180	75	1	9.2680	617.5373	338.8269
180	75	1	8.3640	557.1877	305.7146
180	75	1	8.4040	559.9009	307.2033
220	45	0.75	6.8180	453.1919	216.5369
220	45	0.75	6.4180	427.6785	204.3465
220	45	0.75	6.7720	442.5218	211.4387
220	45	0.75	6.8120	444.1778	212.23
220	45	0.75	6.3200	420.6939	201.0092
220	45	0.75	6.5200	434.5276	207.6191
220	45	0.75	6.3000	419.6727	200.5213
220	45	0.75	6.1000	406.3199	194.1413
220	45	2	9.622	633.1096	232.3512
220	45	2	9.502	631.3621	231.7099
220	45	2	9.032	569.9322	209.1651
220	45	2	9.192	589.7411	216.435
220	45	2	9.172	611.3688	224.3723
220	45	2	9.372	624.6667	229.2527
220	45	2	8.916	594.2495	218.0895
220	45	2	8.916	594.5823	218.2117
220	60	1	7.574	503.4833	189.2165

220	60	1	6.854	456.6411	171.6125
220	60	1	7.18	460.0795	172.9046
220	60	1	7.06	466.8849	175.4622
220	60	1	7.328	488.1428	183.4513
220	60	1	6.208	413.7839	155.5061
220	60	1	7.424	493.1449	185.3311
220	60	1	7.624	507.4007	190.6887
220	60	1	4.89	325.0184	122.1467
220	60	1	7.65	509.5923	191.5123
220	60	1	7.48	491.5232	184.7217
220	60	1	5.24	343.6021	129.1307
220	60	1	7.452	495.9404	186.3817
220	60	1	7.652	509.661	191.5381
220	60	1	7.256	483.6366	181.7578
220	60	1	6.016	401.0132	150.7067
220	60	1	7.466	496.2446	186.496
220	60	1	7.666	499.8207	187.84
220	60	1	7.06	470.4785	176.8128
220	60	1	7.34	476.8401	179.2036
220	60	1	7.412	494.0938	185.6877
220	60	1	7.452	496.7901	186.701
220	60	1	8.124	541.4556	203.487
220	60	1	7.804	519.9893	195.4197
220	75	0.75	4.726	312.6406	119.118
220	75	0.75	4.846	322.2824	122.7917
220	75	0.75	4.528	301.1439	114.7377
220	75	0.75	4.688	304.7718	116.12
220	75	0.75	5.032	335.0914	127.6719



220	75	0.75	4.952	328.8967	125.3117
220	75	0.75	5.156	343.5982	130.9131
220	75	0.75	4.156	276.9098	105.5044
220	75	2	9.158	609.2053	149.1285
220	75	2	9.438	628.3204	153.8077
220	75	2	10.028	665.1279	162.8179
220	75	2	9.588	623.2449	152.5653
220	75	2	9.456	630.2319	154.2756
220	75	2	8.816	587.5375	143.8244
220	75	2	9.532	635.3396	155.5259
220	75	2	9.372	624.4253	152.8542
260	45	1	0.138	9.056901	2.487243
260	45	1	0.058	3.865507	1.061561
260	45	1	0.124	7.906146	2.171218
260	45	1	0.072	4.665176	1.28117
260	45	1	0.124	8.260554	2.268547
260	45	1	0	0	0
260	45	1	0.092	6.129247	1.683239
260	45	1	0	0	0
260	60	0.75	0	0	0
260	60	0.75	0.074	4.927027	1.368547
260	60	0.75	0	0	0
260	60	0.75	0	0	0
260	60	0.75	0	0	0
260	60	0.75	0.056	3.732388	1.03672
260	60	0.75	0	0	0
260	60	0.75	0.124	8.259068	2.294065
260	60	2	0.234	15.58255	3.148358

260	60	2	0.278	18.46244	3.730222
260	60	2	0.096	6.252076	1.263194
260	60	2	0.104	6.883542	1.390777
260	60	2	0.136	9.054112	1.829328
260	60	2	0.18	11.98338	2.421169
260	60	2	0.244	16.26233	3.285704
260	60	2	0.072	4.797314	0.969268
260	75	1	0.038	2.529017	0.612963
260	75	1	0.066	4.393439	1.064847
260	75	1	0.116	7.540008	1.827488
260	75	1	0.14	9.004142	2.182354
260	75	1	0.088	5.854178	1.41889
260	75	1	0.172	11.45933	2.777424
260	75	1	0	0	0
260	75	1	0	0	0