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EVALUATION OF DIFFERENT SOURCES OF HYDROXYL ON BIOMASS PRETREATMENT AND HYDROLYSIS

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EVALUATION OF DIFFERENT SOURCES OF HYDROXYL ON BIOMASS PRETREATMENT AND HYDROLYSIS

THESIS

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science in Biosystems and Agricultural Engineering in
the College of Engineering at the University of Kentucky

By

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Lexington, Kentucky

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2015

ABSTRACT OF THESIS

EVALUATION OF DIFFERENT SOURCES OF HYDROXYL ON BIOMASS PRETREATMENT AND HYDROLYSIS

Lignocellulosic biomass pretreatment is a fundamental step in the production of renewable fuels and chemicals. It is responsible for the disruption and removal of lignin and hemicellulose from the lignocellulosic matrix, improving the enzymatic hydrolysis of cellulose. Alkaline pretreatment has been shown to be successful on agricultural residues and dedicated energy crops. The objective of this study was to evaluate the pretreatment of switchgrass, wheat straw, corn stover, and miscanthus using calcium hydroxide, potassium hydroxide, and sodium hydroxide at the same hydroxyl concentration, 60% moisture content, and two temperatures for seven days. Enzymatic hydrolysis was also performed and the glucose produced measured. The composition of cellulose, hemicellulose, and lignin before and after pretreatment were quantified according to the standard procedures developed by the NREL for biomass. The hydrolysis was performed at 50°C and 150 rpm. The enzyme loading was 60 FPU/g cellulose. Overall, calcium hydroxide pretreatment resulted in the lowest delignification and structural carbohydrates after pretreatment, as well as lowest glucose yield; In addition to having a higher cost and carbon dioxide emission then sodium and potassium hydroxides. Sodium hydroxide and potassium hydroxide had similar performance in terms of composition changes due to pretreatment and glucose yield after enzymatic hydrolysis.

KEYWORDS: Alkaline Pretreatment, Enzymatic Hydrolysis, Agricultural Residues, Dedicated Energy Crops, Molar Basis, High Solids

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EVALUATION OF DIFFERENT SOURCES OF HYDROXYL ON BIOMASS PRETREATMENT AND HYDROLYSIS

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February 19, 2015
Date

Dedicated to the memory of
my loved twin brother Carlos
who shared my grown-up dreams

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Chapter One: INTRODUCTION

The amount of energy consumed has increased over the last century due to increases in the world population and industrialization. Crude oil is the largest primary energy source worldwide (IEA). However, its availability has been estimated to be declining, which could be crucial, in the view of the fact that many nations' economies depend on oil (Sun & Cheng, 2002).

Moreover, there are several local, regional, and global impacts regarding the utilization of energy from fossil fuels such as coal and oil. Local impacts include solid waste disposal sites for ash from coal burning, as well the production of carbon monoxide and smog as pollution. Regional impacts are generally the consequence of acid rain, which is generated from sulfur dioxide and nitrogen oxides released during combustion (Brown, 2003a). Nonetheless, the main environmental concern has to do with global climate change. Combustion of fossil fuels release carbon dioxide, which is believed to contribute to the greenhouse effect in the atmosphere, and hence global climate change (Kumar, Barrett, Delwich, & Stroeve, 2009). There are additional economic and political risks associated with fossil fuel use. In the US, a primary policy for utilizing biomass in the production of fuels is the Energy Independence and Security Act (EISA) of 2007, where the volume of renewable fuel blended into transportation fuels will increase from 34 billion liters in 2008 to 136 billion L by 2022. By 2022, 61 billion L is mandated to be from cellulosic biomass (corn cobs, switchgrass, etc.) and an additional 19 billion L classified as an advanced biofuel that meet targets for reduced greenhouse gas emissions.

With increased awareness of the finite nature of fossil fuels, environmental concerns such as pollution control, and changes in the social, economic, and cultural values the international scientific community has intensively searched for alternative sources of energy and material (Khuad & Singh, 1993). The development of technologies which allow the use of energetic resources in nature, such as lignocellulosic biomass, could be a simple solution to these issues (Wyman & Goodman, 1993).

Conversion of lignocellulosic biomass has numerous advantages because the carbon within the biomass does not result in releasing additional carbon dioxide into the atmosphere. Bioenergy is different from the energy that arises from burning fossil fuels, since it is produced from plants grown today and with short life cycle, which contributes to the improvement of energy security (Kumar, Barrett, Delwich, & Stroeve, 2009). One potential pathway to renewable liquid fuels from lignocellulosic biomass comprises the following three steps: pretreatment of the lignocellulosic material; hydrolysis of carbohydrate polymers into fermentable sugars; and sugar fermentation into a biofuel.

The goal of pretreatment is to increase access to the cellulose and hemicellulose molecules by causing cell wall disruption, and reducing crystallinity of the lignocellulosic material, thus improving the efficiency of the hydrolysis process (Bragatto, 2010). Pretreatment has been identified as one of the major economic costs in biomass conversion (Tomás-Pejó, Alvira, Ballesteros, & Negro, 2011) and, therefore, different types of pretreatment have been intensively studied in order to optimize the biological conversion to biofuels.

Laureano-Perez et al., 2005 reported that some alkaline pretreatments yield highly digestible cellulose and produce liquid streams with significant quantities of extracted lignin and polymeric hemicellulose. A number of alkaline pretreatments have been investigated and include lime (Kaar & Holtzapple, 2000; Xu J. , Cheng, Sharma-Shivappa, & Burns, 2010a; Sirohi & Rai, 1998), sodium hydroxide (Varga, Szengyel, & Réczey, 2002; Silverstein, Chen, Sharma-Shivappa, Boyette, & Osborne, 2007; Kingsley & Thrash Jr., 2010) , and potassium hydroxide (Bales, Kellogg, & Miller, 1979; Sharma, Palled, Sharma-Shivappa, & Osborne, 2013; Ong, Chuah, & Chew, 2010) that have resulted in an increase in digestible cellulose.

After pretreatment, hydrolysis of the biomass can be performed through acid or enzymatic catalysts. Acid hydrolysis is predominantly carried out with sulfuric or hydrochloric acid, which can be applied dilute or concentrated. For concentrated-acid hydrolysis, sulfuric acid is attractive due to its low price and high sugar yields, however the large volume of the acid required, and its high boiling point make recovery and reuse difficult. On the other hand, hydrochloric acid has higher volatility, but is significantly more expensive and corrosive (Brown, 2003b). With dilute-acid hydrolysis, a reduced quantity of acid is needed, but high temperatures are required, which result in lower yields of simple sugars and greater quantities of fermentation inhibitors such as acetic acid and furfural (Brown, 2003b).

Enzymatic hydrolysis utilizing cellulases, which are highly specific, are applied to the pretreated biomass to perform hydrolysis where cellulose is the substrate (Béguin & Aubert, 1994). Cellulases are usually a mixture of several enzymes, including xylanases, that allow for hydrolysis of hemicellulose as well, thus increasing the yield of reducing sugars produced (Duff & Murray, 1996). Enzymatic hydrolysis is more advantageous than acid hydrolysis because it is normally conduced at mild temperatures and have limited corrosion issues (Duff & Murray, 1996), thus reducing the environmental impact and the costs associated with equipment resistant to corrosion.

Following the hydrolysis step, fermentation results in the production of a biofuel and CO2 by applying yeasts or bacteria to the sugar rich hydrolysate. Numerous yeast species, including *Saccharomyces cerevisiae* which is the most commonly employed, have been shown to be efficient at fermenting six-carbon sugars (Brown, 2003b). Nonetheless, the efficient conversion of lignocelullosic biomass to fuels would require fermentation of five-carbon sugars derived primarily from the hemicellulose fraction. Finding microorganisms able to ferment both pentoses and hexoses with high yields is still a challenge. Maximum yields obtained from organisms capable of fermenting both pentose and hexoses are on the order of 50g/L compared to 150g/L for hexose-fermenting *Saccharomyces cerevisiae* (Brown, 2003b).

1.1 Goal

The main aim of this study was to evaluate the alkaline pretreatment chemicals (calcium hydroxide, potassium hydroxide, and sodium hydroxide) on biomass (switchgrass, wheat straw, corn stover, and miscanthus) based on compositional changes after pretreatment, sugar yield after enzymatic hydrolysis, cost, and potential environmental impacts.

1.2 Specific Objectives

In order to achieve the overall goal of this study, the following steps were accomplished:

1. Measure the change in composition of biomass (switchgrass, wheat straw, corn stover, and miscanthus) before and after pretreatment with calcium hydroxide, potassium hydroxide, and sodium hydroxide;
2. To quantify the yields of reducing sugars after pretreatment with the three chemicals and the four biomass types as a result of enzymatic hydrolysis; and
3. To estimate the economic and environmental viability of the three pretreatment chemicals and four biomass types investigated.

Chapter Two: BACKGROUND

2.1 Feedstock

Wastes and dedicated energy crops have been considered a significant feedstock for the production of biobased products. Waste products are defined as agricultural residues and animal manures that have no apparent value, while dedicated energy crops refer to herbaceous crops grown for purposes other than food or feed (Brown, 2003c). Some of the common biorenewable resources studied are switchgrass, miscanthus, wheat straw and corn stover, shown in Figure 2.1. It has been estimated that there is over a billion tons of biorenewable resources available in the US (US Department of Energy, 2011).

Figure 2.1: Biomass feedstock: (A) switchgrass (B) miscanthus (C) corn stover (D) wheat straw. Pictures from Michael Montross.

Switchgrass is a perennial grass native to North America, where its range extends from Quebec to Central America (McLaughlin, et al., 1999). It is resistant to pest and plant diseases (Nlewem & Thrash Jr., 2010), and is capable of growing in several soil and climate conditions, ranging from arid sites in the shortgrass prairie to brackish marshes and open woods (Xu J. , Cheng, Sharma-Shivappa, & Burns, 2010b; McLaughlin, et al., 1999). Switchgrass has a relatively low production cost since it requires few fertilizers and pesticides and has a high yield potential (Mann, et al., 2009). Lee (2006) reported annual biomass yields up to 34 dry Mg/ha (15 dry tons/acre). Moreover, it presents a high net energy gain, producing 540 % more energy than what was consumed to grow and process it into ethanol (Schmer, Vogel, Mitchell, & Perrin, 2008). Due to its physiological characteristics, it is also beneficial to the soil since it is capable of fixing carbon by multiple metabolic pathways with a higher water use efficiency than other grass species (McLaughlin, et al., 1999).

Miscanthus is a genus that comprises a group of more than ten grass species, most of which are native to Asia. Giant miscanthus is a perennial warm-season C4 plant with high yield potential (Clifton-Brown, Stampfl, & Jones, 2004; Heaton, Voigt, & Long, 2004), efficient conversion of radiation to biomass, efficient use of nitrogen (N) and water, and good pest and disease resistance (Beale & Long, 1995). Small trials in the Midwestern United States indicated that giant miscanthus can yield more than double the biomass of traditional switchgrass varieties (Heaton, Dohleman, & Long, 2008).

Wheat straw is a residue after the harvest of the grain. Since wheat is the world's largest cultivated crop, grown in over 115 nations and under a wide range of environmental conditions, wheat straw is abundantly available (Talebnia, Karakashev, & Angelidaki, 2010). Nelson (2005) evaluated the potential wheat straw removal in the Eastern and Midwestern United States based on rainfall and wind erosion. It was estimated that over 8 million metric tons of wheat straw spring and winter wheat were available based on erosion being the limiting factor. Therefore, wheat straw is a potential biofuel feedstock based on the planted wheat acreage.

Corn stover is the residue of a corn plant after grain harvest, including the leaves, stalks, husks, and cobs. It is widely available in the United States, with an estimated quantity of 80-110 million dry tons that could be sustainably collected (Kadam &

McMillan, 2003). The majority of the available corn stover could be used as a biofuel feedstock since only a small portion is currently used for other applications, such as animal bedding, animal feed, and soil erosion protection (Schell, Farmer, Newman, & McMillan, 2003; Kadam & McMillan, 2003). In addition, the low costs associated with nutrient replacement and harvesting activities contribute to the attractiveness of corn stover as a feedstock for the production of ethanol and other bioenergy and biobased products. It has been estimated that corn stover could supply as much as 25 percent of the feedstocks for biofuel production by 2030 (Koundinya, 2009).

2.2 Lignocellulosic Biomass

Lignocellulosic biomass, such as agricultural residues and herbaceous crops, are complex substrates widely available in the world, which have a great potential as a feedstock for bioenergy and biobased product production. The potential use of these residues is strongly influenced by their chemical composition. Regardless of its origin, approximately 90% of lignocellulosic plant matter is composed of cellulose, hemicellulose, and lignin (Wyman & Goodman, 1993). Typically cellulose will make up 40% to 50% of the plant dry matter; hemicellulose 25% to 35%; and lignin 15% to 25% of the dry matter (Wyman & Goodman, 1993). Besides these components, the lignocellulosic matrix also contains a small quantity of pectin, protein, extractives, and ash (Kumar, Barrett, Delwich, & Stroeve, 2009). The chemical composition of switchgrass, miscanthus, corn stover, and wheat straw is summarized in Table 2.1

Table 2.1: Chemical composition of lignocellulosic materials.

Components (%wt)	**Lignocellulosic material**			
	Swtichgrass	**Miscanthus**	**Corn stover**	**Wheat straw**
Cellulose	43	38	31	33
Hemicellulose	23	24	29	19
Lignin	24	15	13	17
Ash	4	2	7	10
Other	4	6	20	17

Source: (Brown, 2003c; Sun & Cheng, 2002; Kumar, Barrett, Delwich, & Stroeve, 2009; McDonald, Bakhshi, Mathews, & Roychowdhury, 1983; Zhu & Pan, 2010a; Vrije, Haas, Tan, Keijsers, & Claassen, 2002).

Cellulose is a linear chain polymer, with the formula $(C_6H_{10}O_5)_n$, formed by only anhydrous glucose molecules (six carbon sugar units) linked by β-(1,4) D-glycosidic bonds (Aguiar & Ferraz, 2011; Jorgensen, Kristensen, & Fekby, 2007). This linkage forms cellobiose, the repeat unit that constitutes cellulose chains (Kumar, Barrett, Delwich, & Stroeve, 2009). Cellulose chains in lignocellulose aggregate in crystalline and amorphous portions, which are defined as organized and less organized regions, respectively. The different characteristics of both forms can dictate the selectiveness of the enzymatic degradation. The amorphous portions are more flexible than the crystalline ones, which are very resistant to tensile, stretch, and solvolysis (Laureano-Perez, Teymouri, Alizadeh, & Dale, 2005; Fengel & Wegener, 1989).

Cellulose fibers swell when in contact with water or other solvents. Swelling can occur in two different ways: intercrystalline and intracrystalline. Intercrystalline swelling happens when the solvent, usually water, penetrates in the amorphous components of the midrofibrils, as well in the space among amorphous regions. Intracrystalline swelling, in contrast, is characterized by infiltration of a solvent, such as acid, alkaline and salt solutions, in the crystalline components of cellulose (Rabelo, 2010).

Hemicelluloses, on the other hand, are branched polysaccharides composed of a variety of sugars including pentoses (xylose and arabinose), hexoses (glucose, mannose, galactose); and a small amount of uronic acid and deoxy-hexoses (Fengel & Wegener, 1989). Xylan is the main polysaccharide in hemicellulose, and it is formed by β-(1,4) D-xylose bonds with acetyl group and/or arabinose unit ramifications, varying from plant to plant (Rabelo, 2010; Bragatto, 2010). Different from cellulose, hemicelluloses chains do not aggregate (Kumar, Barrett, Delwich, & Stroeve, 2009).

Lignin is very different in that it is a naturally-occurring aromatic macromolecule made of phenolic monomers derived from p-coumaryl, coniferyl, and sinapyl alcohols cross-linked by alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds (Kumar, Barrett, Delwich, & Stroeve, 2009; Jorgensen, Kristensen, & Fekby, 2007; Santos, 2012). Lignin can be divided into three groups based on their structural components: (i) Guaiacyl lignin – typical of softwoods; (ii) Guaiacyl-syringyl lignin – commonly present in hardwoods; and (iii) monocot lignin – co-polymer of all three lignin precursors. The solubility and reactivity of lignin under either acid or alkaline conditions depend on the monolignois forming the polymer (Bragatto, 2010; Santos, 2012).

The three lignin precursors contribute significantly to the recalcitrance of the biomass, being responsible for structural support, resistance against microbial attack, water and nutrient transport, and stress responses (Kumar, Barrett, Delwich, & Stroeve, 2009; Simons, Loqué, & John, 2010).

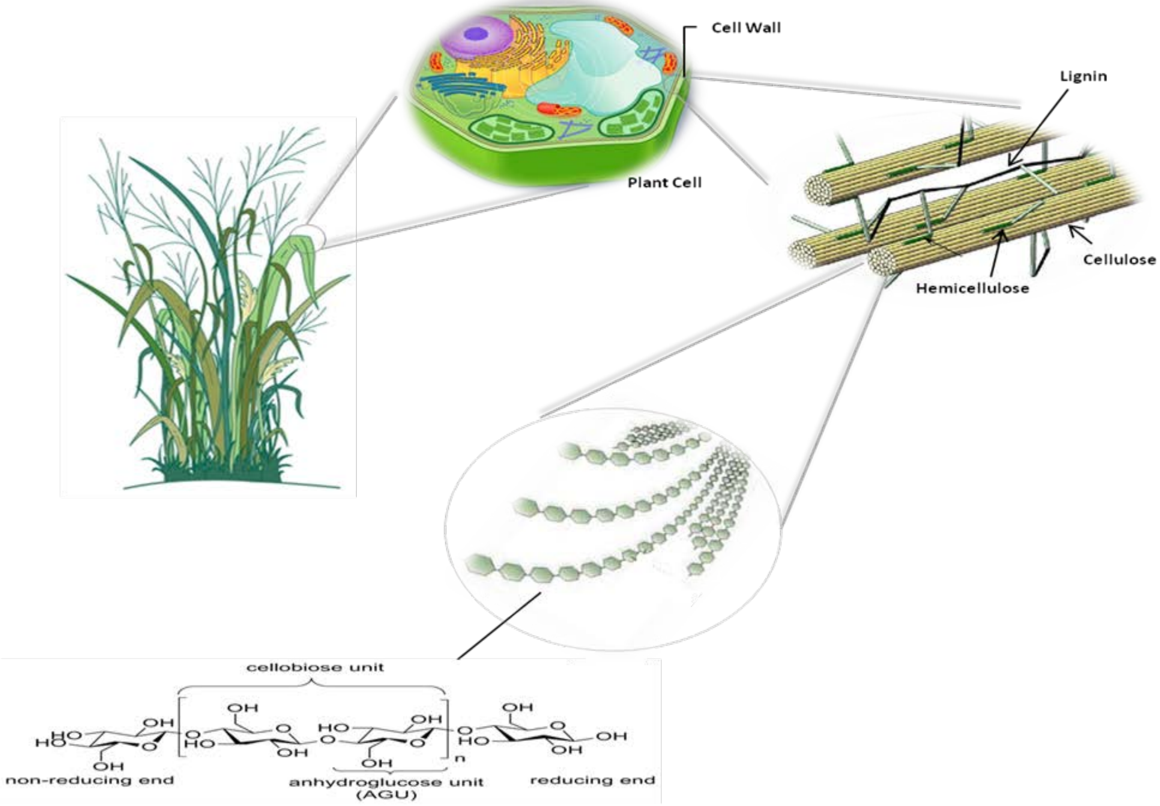


Figure 2.2: Plant cell wall structure of lignocellulosic materials.

Figure 2.2 shows the two polysaccharides in lignocellulosic matter, cellulose and hemicellulose, bounded together by lignin that comprise the cell wall. The glucose chains are tightly linked by Van der walls and hydrogen bonds, both side-to-side and top-to-bottom, compounding the elementary fibrils and, consequently, the microfibrils of the cellulose, which promote the rigidity of the plant (Kumar, Barrett, Delwich, & Stroeve, 2009; Laureano-Perez, Teymouri, Alizadeh, & Dale, 2005). Among the cells, there is also a thin layer of lignin called the middle lamella, which give cohesion to the plant structure. Due to this cohesion and to the fact that the cell wall has low porosity, the biodegrading process is difficult and slow in nature (Aguiar & Ferraz, 2011). Thus, to successfully convert lignocellulosic biomass into liquid transportation fuels or other chemicals, the lignin that provides the cohesion needs to be broken down prior to utilizing the sugars.

2.3 Biofuels from lignocellulosic materials

The production of biobased products from lignocellulosic based on enzymatic hydrolysis comprises three main steps: pretreatment, hydrolysis, and fermentation. The bioconversion process is summarized in Figure 2.3.

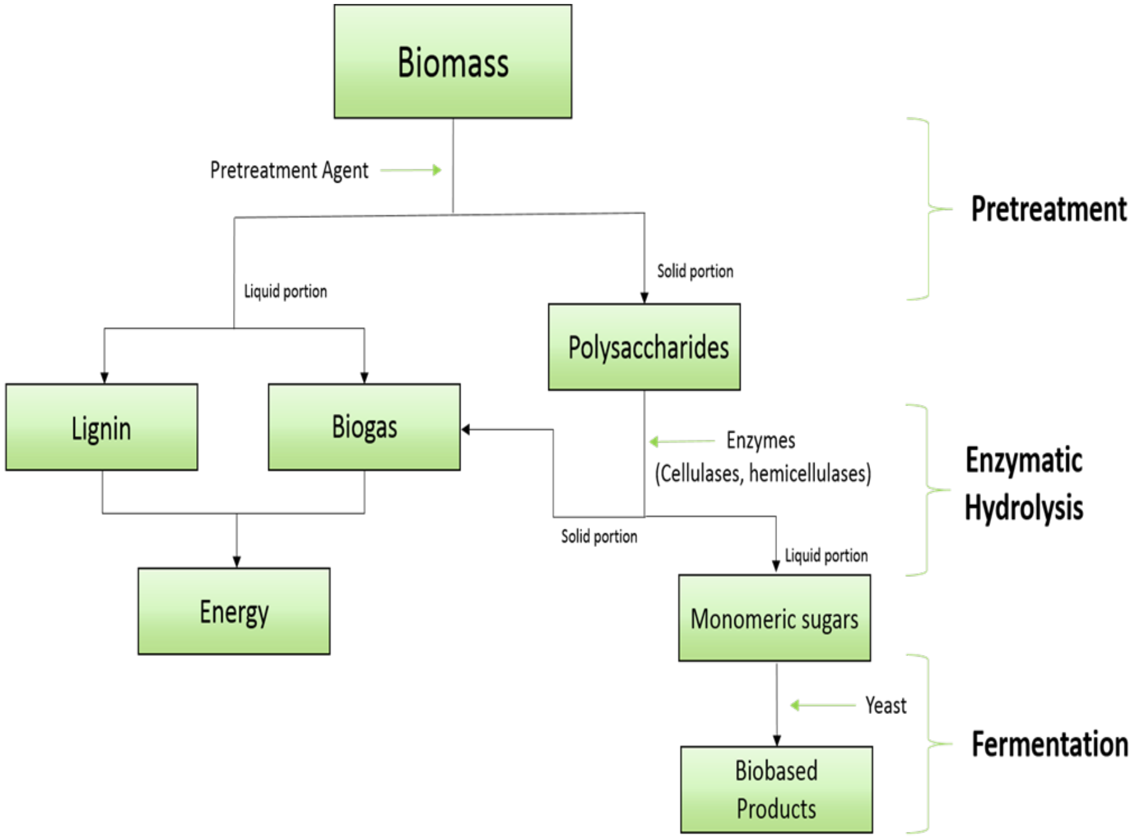


Figure 2.3: Typical bioconversion process utilizing pretreatment, enzymatic hydrolysis and fermentation.

2.4 Pretreatment

Lignocellulosic biomass pretreatment is an important first step in the bioconversion process since its main function is to remove lignin and hemicellulose from the plant material (Kumar, Barrett, Delwich, & Stroeve, 2009). Lignin and hemicellulose, when in high concentrations, obstruct the use of the cellulose since they hamper the access of the degrading enzymes. As a result, pretreatment causes cell wall disruption with significant increases in the surface area of cellulose (Bragatto, 2010). Besides that, pretreatment is responsible for a reduction in the cellulose microfibrils crystallinity, thus improving the efficiency of further chemical or enzymatic processes on the cellulose (McMillan, 1994).

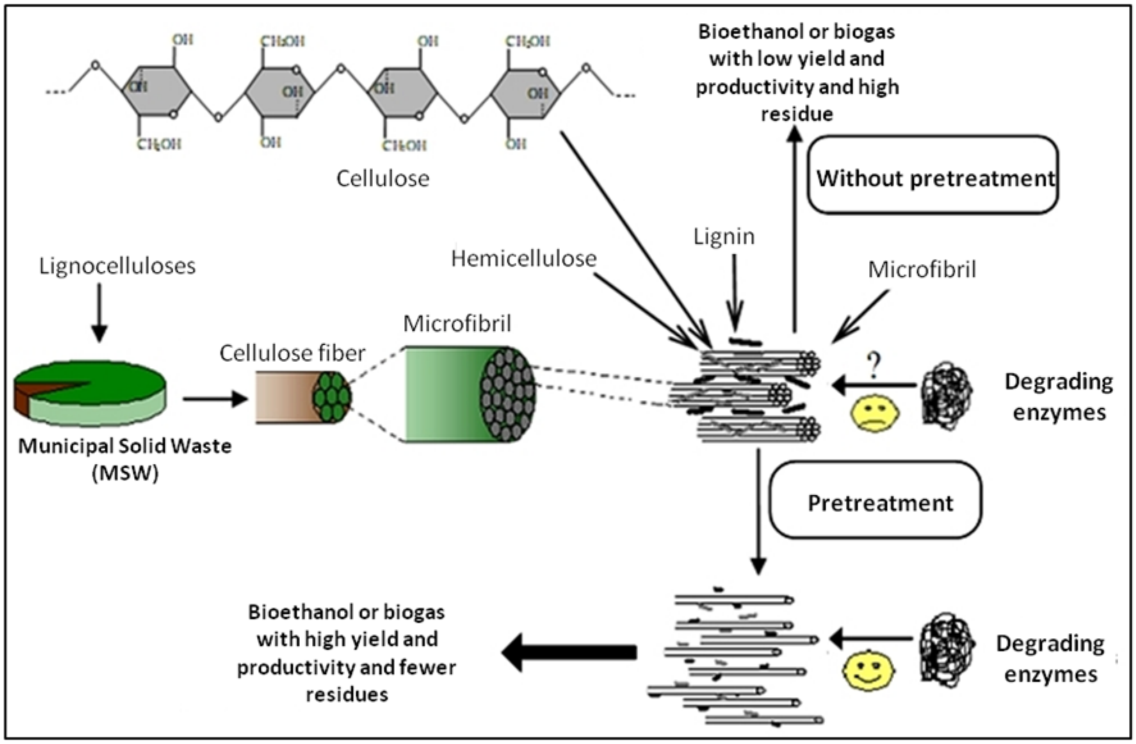


Figure 2.4: Pretreatment effect on lignocellulosic material. Source: (Taherzadeh & Karimi, 2008).

There are a wide variety of pretreatments with the aim of removing lignin and/or hemicellulose and increasing the cellulose availability. Pretreatment can be classified in three main categories: (i) physical, (ii) chemical, and (iii) biological. Physical pretreatment processes have proven to be ineffective due to the high energy requirements and biological pretreatment methods can be expensive and time consuming (Sharma, Palled, Sharma-Shivappa, & Osborne, 2013). Chemical pretreatments on the other hand have been intensively studied and alkaline pretreatment, in particular, has been considered effective on agricultural residues and herbaceous crops (Hsu, 1996). Table 2.2 summarizes the variety of pretreatment methods and their applications, as well as pros and cons (Taherzadeh & Karimi, 2008; Kumar, Barrett, Delwich, & Stroeve, 2009; Tomás-Pejó, Alvira, Ballesteros, & Negro, 2011).

Table 2.2: Summary of pretreatment methods.

Pretreatment Method	Process	Technology	Pros	Cons
Physical Pretreatment	Mechanical Comminution	- Chipping - Grinding - Milling	-Reduce cellulose crystallinity - Increase surface area - No chemicals are required	- High energy consumption - Low delignification efficiency - Not recommended for industrial applications
	Irradiation	-Gamma-ray irradiation - Microwave Irradiation - Electron-beam		
	Liquid Hot Water	-Immersion in water at high pressures and elevated temperatures		
Chemical Pretreatment	Ozonolysis	-Use of Ozone as solvent	- Reduce cellulose crystallinity - Increase surface area - High delignification efficiency - Suitable for industrial	-Chemical requirements - Need harsh conditions - Can be economically unviable
	Organosolv	-Use of organic or aqueous solvent		
	Alkali Pretreatment	-Use of alkali solution as solvent		
	Acid Pretreatment	-Use of acid solution as solvent		
Biological Pretreatment	Fungi and Actinomycetes Pretreatment	-Use of microorganism to degrade lignocellulosic material	- Minimal inhibitors production - No chemicals are required - High delignification efficiency - Reduced energy inputs -Environment-friendly	- Very low treatment rate - Not recommended for commercial applications

The efficiency of a pretreatment is related to several factors, such as the cellulose crystallinity level, the superficial area accessible to enzymes, and the natural protection due to lignin, hemicelluloses, and cellulose interaction (Silva, Júnior, & Júnior, 2010). To maximize enzymatic hydrolysis: the loss of fermentable carbohydrates needs to be minimized; the addition of reagents that inhibit the fermentative microorganisms should be avoided; minimize the use of energy, reagents and equipment, and allow for implementation at an industrial scale are relevant requirements for evaluating the efficiency of a pretreatment (Sun & Cheng, 2005).

2.4.1 Alkaline Pretreatment

Alkaline pretreatment refers to the application of bases to remove lignin and other undesirable components of the lignocellulosic matrix, and its effect depends directly on the nature of the biomass feedstock, primarily the lignin content (McMillan, 1994). Alkali pretreatment processes are relatively inexpensive (Xu J. , Cheng, Sharma-Shivappa, & Burns, 2010b) and normally utilize lower temperatures, pressures and residence times compared to other pretreatment technologies (McMillan, 1994). Moreover, they allow for chemical and water recycling, and require lower enzyme loads to convert cellulose to glucose (Sendich, et al., 2008). Disadvantages of the alkaline pretreatment process are the potential corrosiveness of the process and the washing stage required to adjust pH prior to enzymatic hydrolysis and fermentation (Lillo-Ródenas, Cazorla-Amorós, & Linares-Solano, 2003).

During alkaline pretreatment, high concentrations (5-20 wt-%) are used that induced swelling of cellulose (Brown, 2003b) that resulted in an increased internal surface area and a decrease in the degree of polymerization and crystallinity (Fan, Gharpuray, & Lee, 1987), followed by delignification as a consequence of saponification of intermolecular ester bonds crosslinking hemicelluloses and other components. This leads to an increase in the porosity of lignocellulosic materials, in other words, fractions enriched in both cellulose and hemicellulose (Sun & Cheng, 2002).

Calcium, sodium, and potassium hydroxide are suitable alkaline pretreatment agents (Kumar, Barrett, Delwich, & Stroeve, 2009). Among these three alkalis, sodium hydroxide has been the most widely studied, and has been shown to be effective. Xu et al. (2010b) investigated sodium hydroxide pretreatment of switchgrass for ethanol production and reported that at pretreatment conditions of 50 °C, 12 h, and 1.0 % NaOH, and 21 °C, 6 h, and 2.0 % NaOH, the total reducing sugars yield were, respectively, 453.4 and 406.2 mg/g raw biomass, which were 3.78 and 3.39 times that from untreated biomass. The maximum lignin reduction at 50 and 21 °C was 77.8 % and 62.9 %, obtained at 48 h, 2.0 % NaOH and 96 h, 2.0 % NaOH, respectively. Another study with wheat straw resulted in a maximum sugar yield of 667 mg/g pretreated material attained with 2 % NaOH, 90 min, and 60 °C, while delignification was reduced to 42 % of the raw material (McIntosh & Vancov, 2011).

Although sodium hydroxide pretreatment of lignocellulosic biomass results in considerable lignin reduction and high retention of the total reducing sugar content per gram of biomass treated (Sharma, Palled, Sharma-Shivappa, & Osborne, 2013), it presents the disadvantages of being more costly, dangerous to handle, and corrosive than other potential alkaline agents such as calcium hydroxide (lime) (Winugroho, Ibrahim, & Pearce, 1984). Calcium hydroxide usage has several other advantages, including the possibility to easily recover unreacted calcium salt after washing by neutralizing with inexpensive carbon dioxide and, subsequently, employing kiln technology to regenerate calcium hydroxide (Kaar & Holtzapple, 2000). These advantages have led researchers to also investigate the effectiveness of lime as pretreatment agent for lignocellulosic feedstock.

Previous research by Kim & Holtzapple (2005) showed that corn stover pretreated with 0.5 g $Ca(OH)_2$/g raw biomass at 55 °C for 4 weeks achieved 91.3 % of glucan and 51.8 % of xylan converted into glucose and xylose, respectively. Also, 87.5 % of the initial lignin and almost all acetyl groups were removed. Chang et al. (1997) investigated lime pretreatment of switchgrass and reported that at recommended conditions (100 and 120 °C, 2 h, and 10 % $Ca(OH)_2$), the reducing sugar yields were five times that of untreated switchgrass. Xu et al. (2010a) studied switchgrass and obtained reducing sugar yields of 433.4 and 411.7 mg/g raw biomass at pretreatment conditions of 50 °C, 24 h, and 10 % $Ca(OH)_2$, and 21 °C, 96 h, and 10 % $Ca(OH)_{2,}$ respectively. These values were an increase of 3.61 and 3.43 times that of untreated biomass. However, the results showed limited lignin reduction, which can be attributed to the formation of calcium-lignin complexes due to the poor solubility of calcium in water (Xu J. , Cheng, Sharma-Shivappa, & Burns, 2010a; Winugroho, Ibrahim, & Pearce, 1984). Additional disadvantages of utilizing lime as a pretreatment include the tendency of treated materials to become moldy with time and the treatment effectiveness was influenced by the method of treatment and reaction time (Sirohi & Rai, 1998).

A few researchers have evaluated potassium hydroxide for pretreatment of lignocellulosic biomass. Potassium hydroxide could be a prospective pretreatment agent due to its ability to remove acetyl compounds from the lignocellulosic matrix (Sharma, Palled, Sharma-Shivappa, & Osborne, 2013) and to react with carbon nanofibers and carbon nanostructures, degrading highly ordered tubular structures (Raymundo-Piñero, et al., 2005). Sharma et al. (2013) studied switchgrass pretreated at KOH concentrations of 0.5-2% for varying treatment times of 6-49 h, 6-24 h, and 0.25-1 h at 21, 50, and 121 °C, respectively. The highest yield of reducing sugar for 21 and 50 °C treatments were 582.4 and 514.0 mg/g untreated biomass achieved at 0.5 % KOH, 12 h, and 2 % KOH, 24 h, while the maximum lignin reductions of 28.5 % and 41.7 % were observed at 2 % KOH, 48 h, and 2 % KOH, 24 h, respectively. Bales, Kellogg, & Miller (1979) investigated potassium hydroxide pretreatment with milo stalks and reported that in vitro dry matter digestibility (IVDMD) improved by 55.0% for stalks treated with 5 % KOH.

Many researchers have concluded that calcium hydroxide is not as effective of a pretreatment agent as other alkalis such as sodium and potassium hydroxide (Chang, Burr, & Holtzapple, 1997). When potassium and sodium hydroxide effectiveness as pretreatment agents were compared, opinions varied. Bales, Kellogg, & Miller (1979) reported that NaOH appeared superior to KOH, despite some of its disadvantages. Ong, Chuah, & Chew (2010), on the other hand, showed that KOH treatment resulted in significant higher sugars than NaOH treatment at similar conditions when equal hydrolytic enzyme loading was employed.

Most of these comparisons were made considering a combination of treatments using calcium, sodium and potassium hydroxides under the same conditions, specifically using the same amount of the chemicals on weight basis. However, it is an imbalanced comparison. Calcium hydroxide, for instance, is a weaker base and to achieve an equivalent alkalinity requires eight times as much calcium hydroxide as sodium hydroxide (Winugroho, Ibrahim, & Pearce, 1984). An experiment comparing these chemicals on molar basis would contribute to a better understanding of their pretreatment effectiveness.

2.5 Hydrolysis

Hydrolysis is an organic or inorganic reaction between an anion or a cation, and water, with supply of H + or OH- ions to the solution (Russel, 1994). Among the processes of hydrolysis in organic chemistry are saponification of fatty acids and other esters, inversion of sugars and protein breakdown (Barcza, 2010).

There are five known categories of hydrolysis: pure, acid, alkaline, alkaline fusion at high temperatures and enzymatic (Barcza, 2010). For the purpose of second generation fuel production from lignocellulosic feedstock, acid or enzymatic hydrolysis are favored.

Acid hydrolysis can be classified into two groups: (i) concentrated-acid hydrolysis and (ii) dilute-acid hydrolysis. Concentrated acids disrupt glycosidic linkages of polysaccharides under low temperatures, and this process is reported to give a theoretical glucose yield of 90%. Nevertheless, this method requires a high concentration that results in considerable corrosion. In addition, acid recovery is an energy-intensive process. High capital costs for construction and high operating costs have led to decreased interest on a commercial scale (Taherzadeh & Karimi, 2007).

Dilute acid hydrolysis is carried out with low acid concentrations and short reaction time. However, the high temperatures required tend to degrade monomeric sugars, producing liquid streams rich in undesirable byproducts, which lead to inhibitors of the fermentation process (Taherzadeh & Karimi, 2007).

Taherzadeh and Karimi (2007) reported the possibility of optimization of dilute acid hydrolysis when carried out in two stages. The first stage is the degradation of the hemicellulose to monosaccharides while the second stage is the hydrolysis of the remaining cellulose. This method decreased the number of fermentation inhibitors formed and achieved greater production of hexose sugars in the second stage, minimizing energy consumption.

Enzymatic hydrolysis refers to the utilization of a complex of enzymes to cleave the cellulose and hemicellulose polymers into fermentable sugars. Due to mild temperatures during the process and the specificity of enzymes, degradation of glucose is prevented, and, thus, fewer undesirable byproducts are generated. Hence, high yields of fermentable sugars are obtained without separation processes that reduced the cost (Rabelo, 2010; Contiero, 1992)

2.5.1 Enzymatic Hydrolysis of Lignocellulosic Material

The pretreated lignocellulosic material is hydrolysed to monomeric sugars by the action of an enzyme complex known as cellulase. Cellulase usually contains cellobiohydrase I and II or exoglucanase, endoglucanase I and II, β-glucosidase, and hemicellulase. Cellobiohydrates work on cellulose to yield cellodextrins that will further be hydrolyzed into cellobiose by endoglucanases. β-glucosidase is responsible for breaking down cellobiose into glucose. Hemicellulase, on the other hand, degrades hemicelluloses, resulting in a variety of five carbon monomers (Aguiar & Ferraz, 2011; Jorgensen, Kristensen, & Fekby, 2007; Brown, 2003b).

One of the main challenges faced by researchers regarding the enzymatic hydrolysis of lignocellulosic materials is to discover organisms capable of producing enzymes specifically adapted for cellulose and hemicellulose degradation. Several studies have been conducted with bacteria and fungi with the aim of isolating and characterizing cellulolytic enzymes. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic (Sun & Cheng, 2002). Among the bacteria species commonly used are *Clostridium*, *Cellulomonas*, and *Bacillus,* while *Trichoderma*, *Aspergillus* and *Penicillium* are the fungi species that have mainly caught the attention of researchers (Sun & Cheng, 2002).

Several structural and compositional factors affect the enzymatic hydrolysis of cellulose – substrates, cellulase activity, presence of inhibitors, and reaction conditions. The first factor in with enzymatic hydrolysis is substrate level. At low substrate levels, substrate concentration and the yield and reaction rate of hydrolysis are usually directly correlated. However, high substrate concentration can cause product inhibition by the substrate, which consequently decreases the yield of the hydrolysis (Ong, Chuah, & Chew, 2010). Structural features of the substrate such as cellulose crystallinity, cellulose degree of polymerization, surface area, and lignin and hemicellulose content are factors that influence the susceptibility of cellulosic substrates to cellulases (Alvira, Tomás-Pejó, Ballesteroa, & Negro, 2010; Sun & Cheng, 2002). A second factor in enzymatic hydrolysis is cellulase activity. High cellulase activity results in high yield and rate of hydrolysis, but also increases the final cost of the products. However, cellulases can be recovered from the liquid supernatant or the solids residue. This recycling process can effectively increase the yield and rate of the hydrolysis while reducing the process cost.

Another factor is the presence of inhibitors. Inhibiting compounds such as weak acids, furan derivatives, and phenolic compounds could also be formed during the pretreatment and hydrolysis steps, decreasing the ethanol yield and productivity of the overall process. To enhance the efficiency of the process, detoxification methods and strategies are required (Palmqvist & Hahn-Hargedal, 2000). Finally, a fourth factor to be considered in enzymatic hydrolysis is reaction conditions. Residence time, pH of the medium, concentration of substrate in the medium, and agitation rate impact the yield of the enzymatic hydrolysis by influencing the generation of fermentation inhibitors.

2.6 Fermentation

After hydrolysis of the pretreated biomass, the hydrolysate is fermented to alcohol, which is then purified through distillation or filtration to obtain the desired fuel-grade quality control (Lin & Tanaka, 2006). Fermentation is the biological process of transforming simple compounds such as sugars into alcohol and carbon dioxide by the action of yeast or alcoholic ferment (Silva, Jesus, & Rodrigues, 2011).

In order to be considered suitable for the alcoholic fermentation process, microorganisms must support a wide range of temperature and acidic levels, and high concentration of alcohol (Silva, Jesus, & Rodrigues, 2011). Several fungi, bacteria, and yeasts have been used for the production of ethanol. Yeasts that belong to the *Saccharomyces* genus, mainly *Saccharomyces cerevisiae*, have been the most reportedly applied in fermenting sugars (Pinal, Cedeño, Gutiérrez, & Alvarez-Jacobs, 1997; Ansanay-Galeote, Blondin, Dequin, & Sablayrolles, 2001; Yu & Zhang, 2003). Among *S. cerevisiae*'s advantages, the literature cities its high inhibitor tolerance; and capacity of producing ethanol in concentration as high as 0.18 times of the fermentation broth, as well as of growing both on the monosaccharide glucose and the disaccharide sucrose (Lin & Tanaka, 2006; Hahn-Hagerdal & Palmqvist, 2000). In addition, *Saccharomyces cerevisiae* is a hardy organism that makes it a good fit for industrial applications.

Among the bacterial sources suitable as a biocatalyst for ethanol production a commonly studied organism is *Zymomonas mobilis*. In comparison to *Saccharomyces cerevisiae*, *Zymomonas mobilis* yields from 5 to 10% more ethanol per fermented glucose, and its specific ethanol productivity is 250% higher (Lin & Tanaka, 2006). Moreover, it is able to tolerate high concentrations of ethanol. However, the microorganism holds a narrow substrate range, since it only ferments glucose, fructose, and sucrose (Dien, Cotta, & Jeffries, 2003).

Escherichia coli is another specie of bacteria with potential for alcoholic fermentation that features numerous benefits, including the ability to ferment a wide spectrum of sugars, no requirements for complex growth factors, and prior industrial use. Nevertheless, some of its disadvantages are a limited pH growth range, and are less hardy compared to yeast (Dien, Cotta, & Jeffries, 2003).

Several other microbes have been considered, although unsuccessfully, for the cellulose-to-ethanol biotransformation, including the anaerobic thermophilic bacteria Clostridium thermocellum (Zertuche & Zall, 1982), as well as some filamentos fungi, such as Arpegillus sp. (Nakamura, Ogata, Hamada, & Ohta, 1996), Trichoderma viride (Iyayi & Aderolu, 2004; Gervais & Sarrette, 1990), and Neurospora sp. (Yamauchi, et al., 1989). Fermentation processes utilizing these microorsganisms have resulted in low ethanol yields and long residence time, besides the production of various byproducts, primarily acetic and lactic acids (Lin & Tanaka, 2006).

The fermentation and hydrolysis processes can be performed either sequentially (Separated Hydrolysis and Fermentation, SHF) or concurrently (Simultaneous Saccharification and Fermentation; SSF). The difference between SHF and SSF lies mainly in the processing steps. When the hydrolysis and fermentation are performed separately (SHF), both steps may occur at their optimum temperature, which maximizes the efficiency of the hydrolysis and fermentation. However, product inhibition from hydrolysis could occur because of the accumulation of glucose in the reactor. Constant sugar removal by a yeast during hydrolysis, in other words a SSF type of process, could avoid such product inhibition. The combination of hydrolysis and fermentation should lead to less risk of contamination due to the fact that a higher ethanol concentration is obtained during hydrolysis. However, both the hydrolysis and the fermentation are performed at some temperature that is not optimal for either process. Moreover, yeast recirculation is more difficult when mixed with lignin residues (Drissen, Maas, Tramper, & Beeftink, 2009; Öhgrena, Burab, Lesnickic, Saddlerb, & Zacchia, 2007).

Chapter Three: EXPERIMENTAL PROCEDURE

3.1 Biomass Preparation

Miscanthus, corn stover, switchgrass, and wheat straw were harvested locally, air-dried, and ground to pass through a 2 mm screen with a knife mill (Retsch GmbH, type SM1 # 70947). To minimize changes to the feedstocks during storage, the feedstocks were dried at 44.5 +-0.2°C in a Thelco Model 6 drying oven for 48 hours and left at room temperature for 24 hours to cool. Moisture content was less than 10% for all feedstocks. After cooling, the samples were stored in zipper plastic bags and placed in a refrigerator at 4 °C until further testing.

3.2 Pretreatment

Prior to testing, subsamples of the feedstocks were taken and the moisture content measured using an oven at 105°C for 24 hours (Rabelo, 2010). Based on the measured moisture content, twenty four samples of ten grams (0% moisture dry matter) of each biomass were weighed using an analytical balance (Denver Instrument, serial # PI214069010). The samples were placed in 250 ml Erlenmeyer flasks that were autoclaved (Steris Amsco Lab 250, Model # 0333808-21) for 30 min at 121°C and 15 psi. Distilled water was added to each flask to achieve a 60% moisture content (see appendix A for calculation). This resulted in a solids loading content of 0.4 g biomass/g of total material. The flasks were randomly separated into two groups for pretreatment at two temperature levels. Twelve flasks were placed at 50°C and shaken at 150 rpm (Innova 4200 Incubator Shaker, New Brunswick Scientific). The other twelve flasks were placed into an incubator at 20°C and shaken at 150 rpm (Innova 4200 Incubator Shaker, New Brunswick Scientific). The flasks at each temperature level were again divided into four subgroups: three would be pretreated with different sources of hydroxyl (OH^-) and one control (distilled water). The experimental design is shown in figure 3.1 and all treatments were performed in triplicate. The three hydroxyl sources applied were sodium hydroxide (Fisher Chemical, Lot 128098), potassium hydroxide (Sigma-Aldrich, Lot #MKBP7659V), and calcium hydroxide (Acros Organics, Lot #A0323480). The molecular formulas for sodium hydroxide, potassium hydroxide, and calcium hydroxide are NaOH, KOH, and $Ca(OH)_2$, respectively. The control flask had no additional chemical added. The appropriate amount of each chemical used was calculated in order to equalize the concentration of hydroxyl per unit mass of biomass (mole/g) in all treatments. Calcium hydroxide was used as the base of calculation. The calculations to determine the appropriate quantity of calcium, potassium, and sodium hydroxide are shown in appendix B. The pretreatments were conducted for seven days. The quantities of calcium, potassium, and sodium hydroxide applied during pretreatment were 1.000 g, 1.515 g, and 1.079 g, respectively. This resulted in a loading of 0.100, 0.151, and 0.108 g of pretreatment chemical per gram of dry matter for calcium, potassium, and sodium hydroxide, respectively.

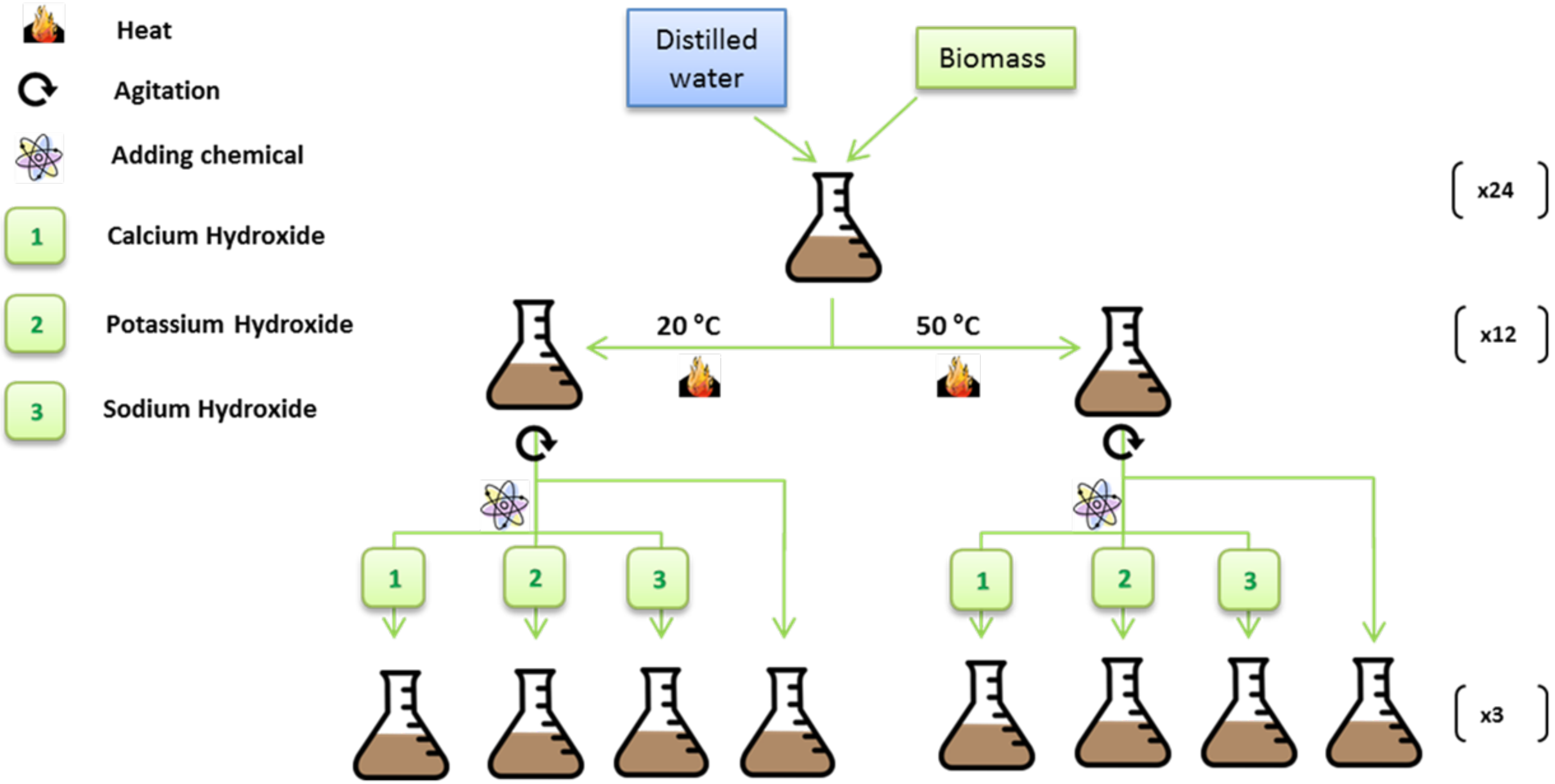


Figure 3.1: Pretreatment set up.

After pretreatment, the biomass was placed on cheese cloth and washed with distilled water until the pH was between 7 and 8. Subsequently, the solids were dried at 44.5 +-0.2°C in a Thelco Model 6 drying oven for 48 hours, then left at room temperature for 24 hours and weighted using an analytical balance (Denver Instrument, serial # PI214069010). Prior to compositional analysis and enzymatic hydrolysis, they were stored in plastic zipper bags and placed into refrigerator at 4 °C.

3.3 Biomass Characterization

Untreated (raw) and pretreated materials were prepared and submitted to compositional analysis following the National Renewable Energy Laboratory (NREL) Analytical Procedure "Determination of Structural Carbohydrates and Lignin in Biomass" (2012) with the exception of the extractives. Samples were analyzed in duplicate by HPLC using a Bio-Rad Aminex HPX-87H (order #125-0140) (ion exclusion) column at 50°C with 0.40 mL min^{-1} flow rate and a 5mM sulfuric acid (Fisher #AC124645001) mobile phase. Chromeleon 7.1 software processed the HPLC data. Glucose and xylose were the only structural carbohydrates reported.

3.4 Enzyme Activity

Enzymes were obtained from American Laboratories Incorporated (Lot # ALI 14175-04) that were produced using *Trichodema longibrachiatum*. The cellulase activity was determined according to the NREL Laboratory Analytical Procedure (LAP) "Measurement of Cellulase Activities" (006, 1996) and expressed in terms of filter paper units (FPU). The concentration protein per milliliters of cellulase was determined according to a modified Bradford method provided in Carey Jr. (2014). Cellobiase activity was measured following a pNPG method for B-glucosidase described in Carey Jr. (2014).

3.5 Enzymatic Hydrolysis of Biomass

After the pretreated biomass and control were dried, enzymatic hydrolysis was carried out in 125 ml Erlenmeyer flasks. For each one of the four biomass types, samples of each pretreated biomass that corresponded to 0.5 g of cellulose were added to six flasks to allow for triplicate in the measurements, including control for each pretreated biomass. Untreated biomass was also enzymatically hydrolyzed as a control. Figure 3.2 shows the experimental design for the enzymatic hydrolysis. The hydrolysis liquid was prepared with a 50% volume of 0.1 M sodium citrate buffer, 1% volume of 2% sodium azide solution, and enough distilled water to complete a final 50 mL volume of hydrolysis liquid. All solutions and the substrates were assumed to have a specific density of 1.000 g/mL. The pH of the samples with the hydrolysis liquid was measured in order to verify that the pH was 5 ± 0.2 which was the ideal pH required to perform the enzymatic hydrolysis. The flasks were sealed, and placed into a shaker at 150 rpm

(Innova 4200 Incubator Shaker, New Brunswick Scientific) for 72 hours at 50°C. The enzyme loading was 60.0 FPU/g cellulose

During hydrolysis, 1.2 mL aliquots of the reaction mixture were collected at sampling times of 0, 1, 3, 6, 12, 24, 36, 48, 60, and 72h to evaluate the effect of reaction duration with the different pretreated substrates. Microcentrifuge tubes containing the aliquots were then immersed in boiling water for 15 min to stop the reaction. The liquid phase was separated from the solid residue by centrifuging twice (Marathon 21000, serial # 64640263, Fischer Scientific) at 10,000 rpm for 15 min, and analyzed in duplicate by HPLC to determine glucose and xylose content.

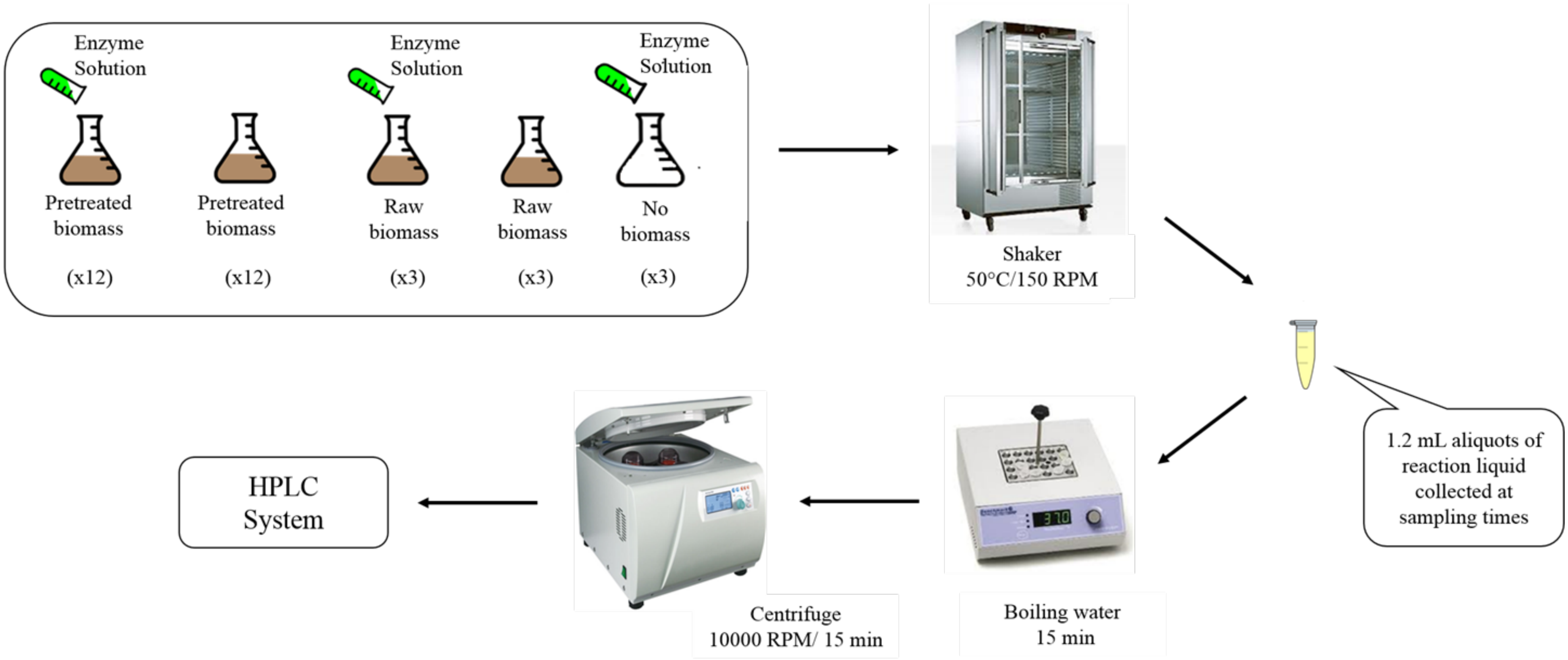


Figure 3.2: Hydrolysis set up.

3.6 Statistical Analysis

All treatment in this study were conducted in triplicate. The experiment design was balanced and completely randomized. SAS 9.3 software was used to perform analysis of variance (ANOVA) on the experimental results to analyze the statistical significance of the data at 95% confidence level. ANOVA was also used to verify the effect of the treatment factors (pretreatment agent and temperature) on the response variables (lignin, carbohydrates, ash, reaction rate, and cellulose conversion rate).

Chapter Four: RESULTS

Numerous factors influence the suitability of biomass as a feedstock for biofuel production. Composition (lignin, cellulose, hemicellulose, and ash) of the biomass plays an important role when it comes to evaluating the potential of a feedstock for biofuel production. The change in composition of the biomass before and after pretreatment can be used to evaluate the effectiveness of the pretreatment. Ultimately, the enzyme hydrolysis after pretreatment is a strong indicator of pretreatment effectiveness. Changes in composition before and after pretreatment and the enzyme hydrolysis of the biomass were evaluated in this study.

4.1 Biomass Composition

The average composition and standard deviation of unextracted raw corn stover, miscanthus, switchgrass, and wheat straw used in this project are shown in Table 4.1.The results in the table below represent the mean of three replications per biomass in the study. The mass balance was determined by summing the average composition and the standard deviation for the overall mass balance was determined by adding individual standard deviations in quadrature. Overall, 95% of the composition was quantified for the wheat straw, switchgrass, and miscanthus. However, only 86.4% of the corn stover composition was accounted for.

Table 4.1: Average composition and standard deviation of unextracted raw biomass; corn stover, miscanthus, switchgrass, and wheat straw. (Average of three replications).

Component	**Dry weight (%)**			
	Corn stover	**Wheat straw**	**Switchgrass**	**Miscanthus**
Acid Soluble Lignin	1.9± 0.14	1.4± 0.04	2.0± 0.15	1.3± 0.03
Acid Insoluble Lignin	32.9± 0.57	34.3± 0.21	27.8± 0.51	30.9± 0.61
Cellulose	35.0±1.45	40.43±1.1	44.4±3.70	45.7± 4.79
Hemicellulose	12.8±2.17	18.4±0.72	22.8±3.19	19.6±0.90
Ash	3.8±0.13	1.1± 0.04	1.0± 0.14	2.9± 0.56
Mass balance	86.4±2.68	95.6±1.33	98.0±4.92	100.4±4.94

4.2 Effect of Pretreatment on Biomass Composition

The change in composition of lignin, carbohydrates (cellulose and hemicellulose), and ash of all four feedstocks due to the three pretreatments plus the control (only water added for pretreatment) at 20°C and 50°C is summarized below. The results shown are the mean of three repetitions and the standard deviation is shown.

The results indicated that the three alkaline pretreatments applied (calcium hydroxide, sodium hydroxide and potassium hydroxide) to the four feedstocks (corn stover, wheat straw, miscanthus, and switchgrass) at both temperatures (20 and 50°C) created some differences in the composition between raw and pretreated biomass. Although, the controls that were pretreated with water also had some influence on the change in composition depending on the feedstock and temperature.

4.2.1 Lignin Changes Due to Pretreatment

The reduction in total lignin (acid insoluble plus acid soluble lignin) after pretreatment is summarized in Table 4.2 and Figure 4.1. Controls that were treated with water also resulted in lower quantities of lignin than the untreated feedstock, although the reduction observed was smaller than for the alkaline treated samples in most cases. The temperature effect on biomass degradation and the effect of different pretreatment chemicals on the degradation are summarized below.

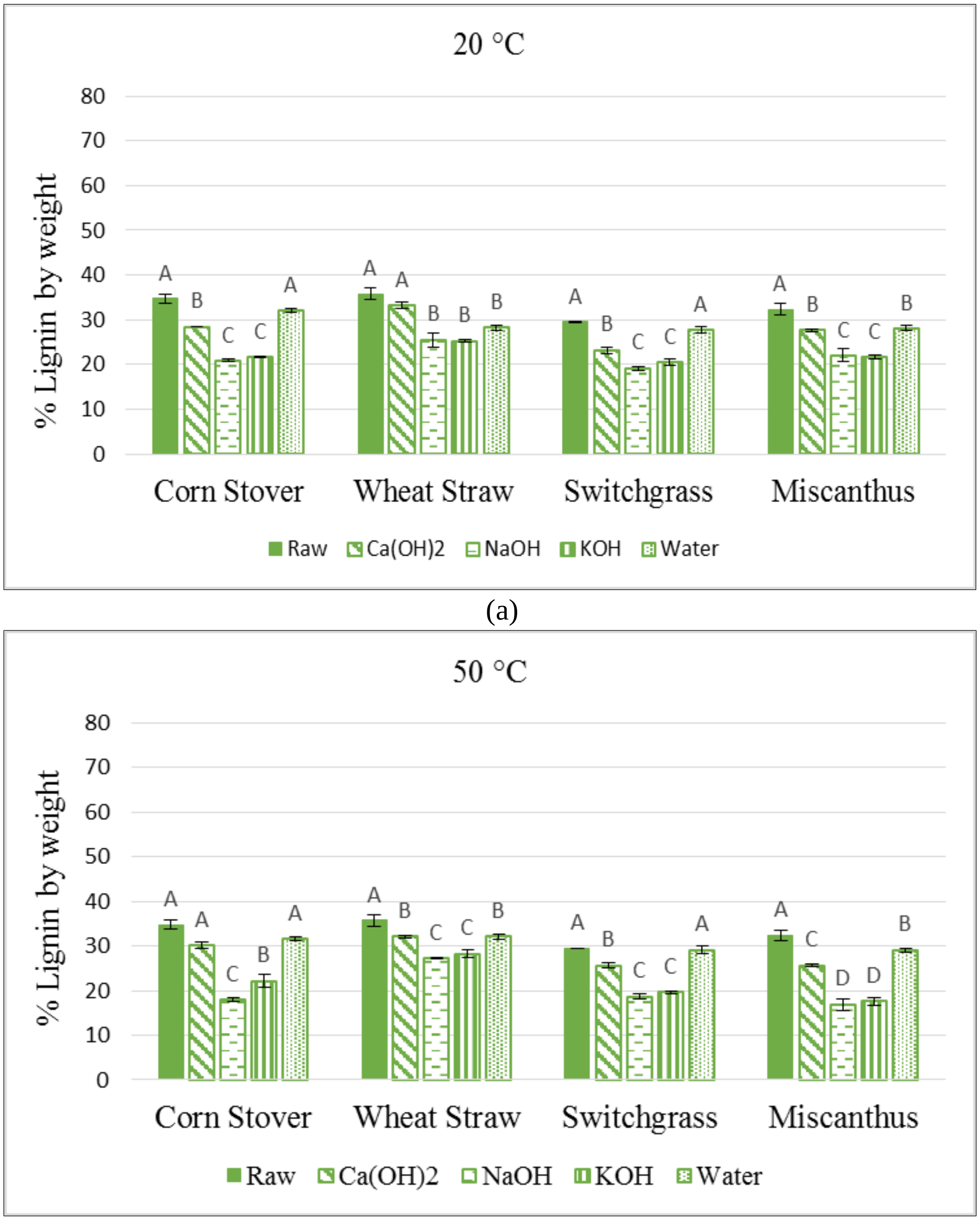
Sodium hydroxide and potassium hydroxide resulted in the largest amount of delignification for the pretreatment chemical agents with all four feedstocks and both temperatures (Figure 4.1). The change in lignin composition of samples treated with sodium and potassium hydroxides at 20°C were statistically not different from each other and were both significantly different than the raw biomass based on the Tukey's correction test at a level of significance of 5%. At 50°C, sodium and potassium hydroxide were statistically not different, except with corn stover where sodium hydroxide had a higher delignification. At 50°C, the potassium and sodium hydroxide resulted in a statistically significant delignification of the raw biomass. Sodium and potassium hydroxide resulted in a 30.2 to 48.3% reduction in lignin with corn stover, miscanthus, and swithgrass (Table 4.2). Delignification of wheat straw was observed to be between 20.9 and 29.2% with sodium and potassium hydroxide relative to the raw biomass (Table 4.2).

Calcium hydroxide was the least efficient in removing lignin among the three alkaline compounds evaluated in this project. In fact, in the case of corn stover pretreated at 50°C and wheat straw pretreated at 20°C, calcium hydroxide had no statistically significant effect on lignin content relative to the raw biomass. In other scenarios, pretreatment with calcium hydroxide led to mixed results compared to the control. Calcium hydroxide had a statistically significant reduction in lignin at 20°C for corn stover and switchgrass. With miscanthus at 20°C, calcium hydroxide did not have a statistically significant delignification relative to the control. At a temperature of 50°C, there was no statistically significant difference between the control and calcium hydroxide for corn stover and wheat straw. However, switchgrass and miscanthus did show a statistically significant delignification at 50°C between calcium hydroxide and the control.

The pretreatment control was water only. In 50%, of the circumstances analyzed, it had no statistically significant effect on lignin reduction relative to the raw biomass. This was expected under the pretreatment conditions utilized in these experiments.

Table 4.2: Percent change in total lignin content due to pretreatment relative to raw unextracted biomass.

Chemical	**Temperature [°C]**	**Feedstock**			
		corn stover	**wheat straw**	**miscanthus**	**switchgrass**
$Ca(OH)_2$	**20**	18.1	6.9	14.2	21.3
	50	13.3	9.6	20.6	13.1
NaOH	**20**	40.0	28.5	31.7	34.9
	50	48.3	23.5	47.6	36.7
KOH	**20**	37.7	29.2	33.0	30.2
	50	36.2	20.9	45.5	33.5
Water	**20**	7.8	21.6	12.9	4.7
	50	8.9	10.1	9.9	1.4



(b)

Figure 4.1: Change in lignin content for the raw biomass, three pretreatments and control (water) at 20°C (a) and 50°C (b). Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

4.2.2 Cellulose Changes Due to Pretreatment

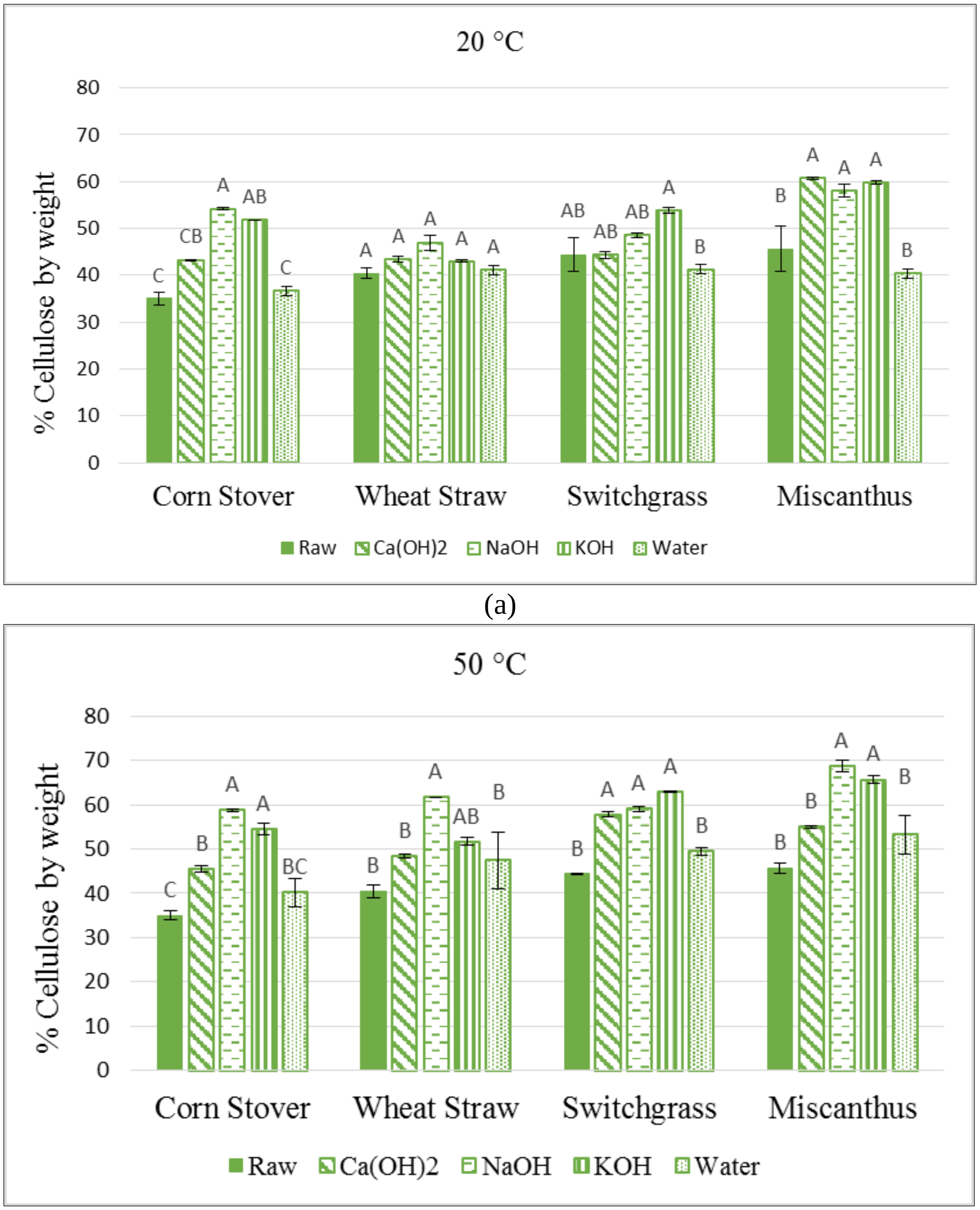
The change in cellulose content due to pretreatment is shown in Figure 4.2 and Table 4.3. Controls that were treated with water did not show a significant difference in the quantity of cellulose in the pretreated solids compared to the untreated biomass.

As observed for the change in lignin content, potassium hydroxide and sodium hydroxide resulted in the largest change in cellulose content after pretreatment. In the case of cellulose, the concentration in the pretreated solids increased after pretreatment. The change in cellulose content of the solids pretreated with sodium and potassium hydroxides at either 20°C or 50°C showed to be statistically not different by the Tukey-Kramer adjustment test at the 95% confidence interval. Sodium and potassium hydroxides increased the amount of cellulose by 19.5 to 23.7% in corn stover and swtichgrass when pretreated at 50°C. (Table 4.3). The lowest increase in cellulose content (2.5 to 6.6%) after pretreatment with sodium and potassium hydroxide was in the case of wheat straw pretreated at 20°C (Table 4.3). At 20°C, no statistically significant change in cellulose content was observed for wheat straw relative to the raw material.

Calcium hydroxide in most cases, had similar performance to sodium and potassium hydroxide in increasing cellulose content, with the exception of miscanthus and corn stover pretreated at 50°C. Calcium hydroxide frequently did not improve cellulose content in comparison to pretreatment with water or the untreated biomass for wheat straw at 50°C, and wheat straw, corn stover and switchgrass at 20°C. The worst performance of calcium hydroxide occurred with switchgrass pretreated at 20°C where no change in cellulose composition was achieved.

Table 4.3: Percent gain in total cellulose content due to pretreatment relative to raw unextracted biomass.

Chemical	**Temperature [°C]**	**Feedstock**			
		corn stover	**wheat straw**	**miscanthus**	**switchgrass**
Ca(OH)2	**20**	8.2	2.9	15.0	0.0
	50	10.6	8.0	13.4	9.4
NaOH	**20**	19.2	6.6	12.4	4.1
	50	23.7	21.2	14.7	23.1
KOH	**20**	16.7	2.5	14.2	9.5
	50	19.5	11.2	18.5	20.0
Water	**20**	1.7	0.7	-3.2	-5.2
	50	5.2	7.1	5.1	7.6



(a)

(b)

Figure 4.2: Change in cellulose content for the raw biomass, three pretreatments and control (water) at 20°C (a) and 50°C (b). Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey’s correction test at a significance level of 5%.

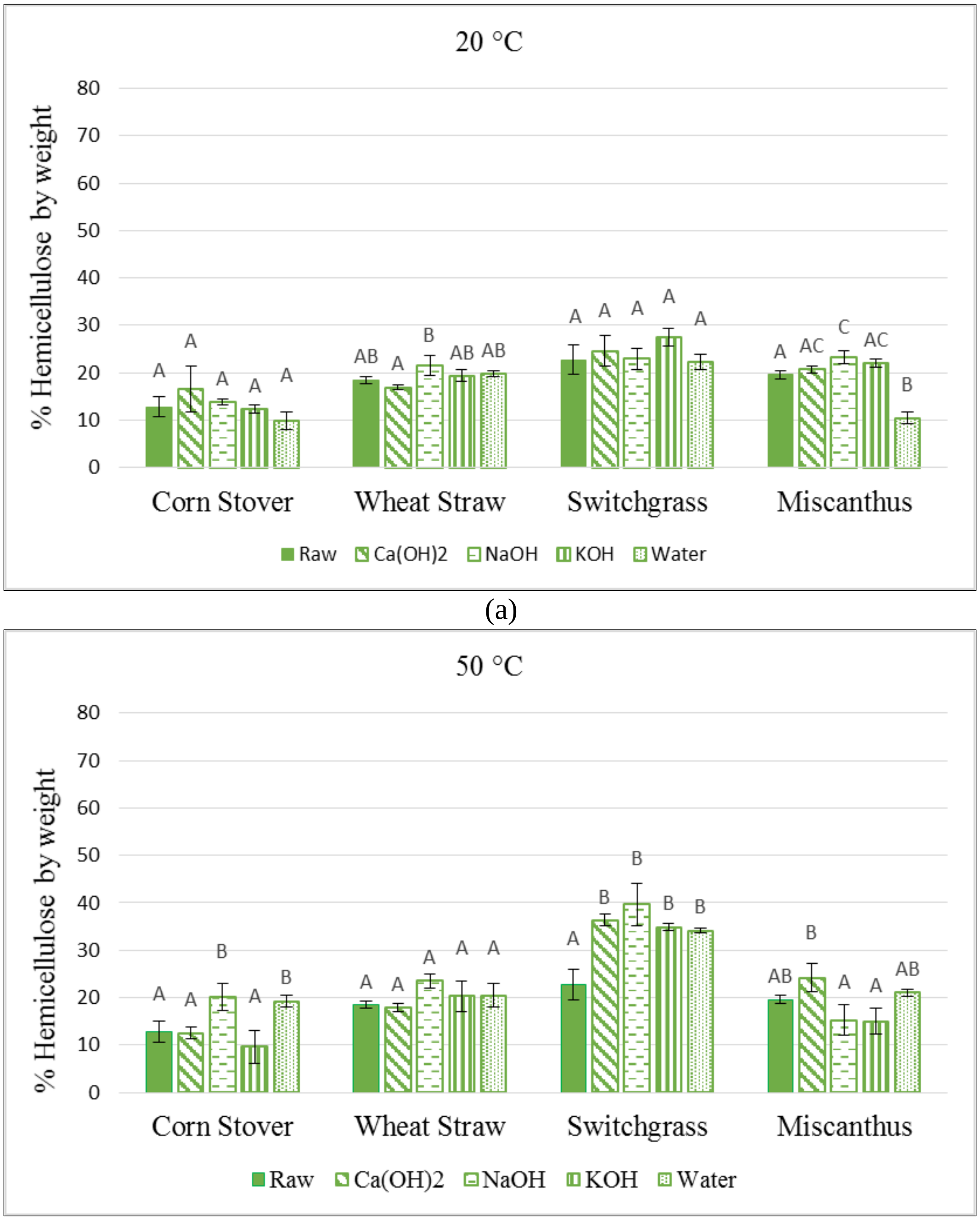
4.2.3 Hemicellulose Changes Due to Pretreatment

The change in hemicellulose content due to pretreatment is shown in Figure 4.3 and Table 4.4. Controls that were treated with water did not show a significant difference in the hemicellulose content compared with untreated biomass, with the exception of corn stover and switchgrass at 50°C and miscanthus at 20°C. In the majority of the scenarios analyzed, the difference in hemicellulose content with corn stover, wheat straw, switchgrass, and miscanthus resulted in no significant difference based on Tukey's correction at a level of significance of 5%.

With a pretreatment temperature of 50°C, sodium hydroxide had the largest increase in hemicellulose content in corn stover, increasing the amount of hemicellulose by approximately 8 and 10 percentage points in comparison with calcium and potassium hydroxides, respectively, which showed to be not statistically different from each other. On the other hand, no clear trends in hemicellulose content were observed between calcium and potassium hydroxide for wheat straw and switchgrass relative to the control. With corn stover and switchgrass pretreated at 20°C and wheat straw pretreated at 50°C, no difference in hemicellulose content was observed between the control, three pretreatment chemicals, and the raw material.

Table 4.4: Percent change in total hemicellulose content due to pretreatment relative to raw unextracted biomass.

Chemical	**Temperature [°C]**	**Feedstock**			
		corn stover	**wheat straw**	**miscanthus**	**switchgrass**
Ca(OH)2	**20**	3.7	-1.5	1.1	1.9
	50	-0.3	-0.6	4.5	13.6
NaOH	**20**	1.0	3.1	3.7	0.2
	50	7.2	5.1	-4.4	16.9
KOH	**20**	-0.5	0.9	2.5	4.7
	50	-3.2	1.8	-4.7	12.1
Water	**20**	-2.9	1.3	-9.1	-0.4
	50	6.4	2.0	1.5	11.4



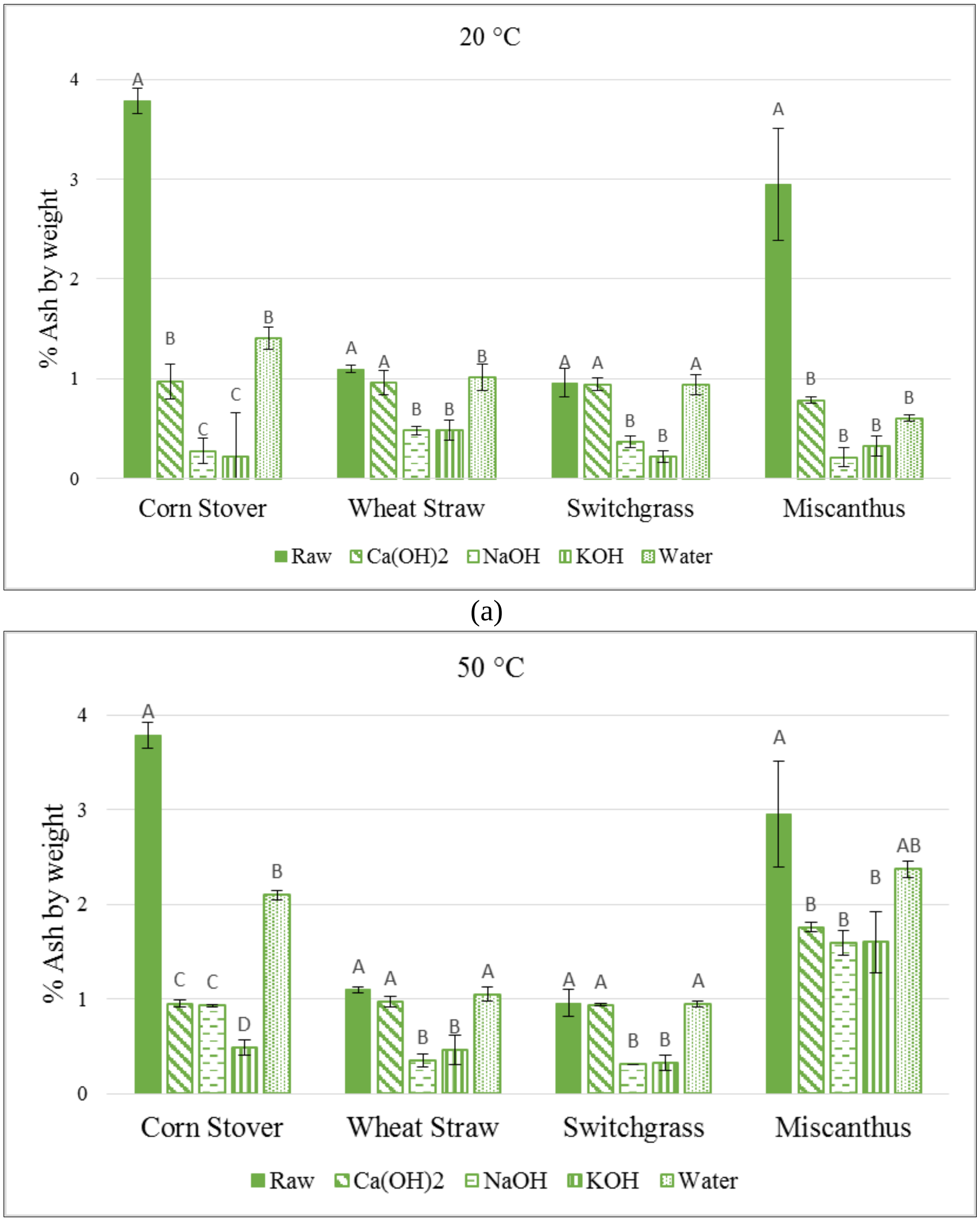
(a)

(b)

Figure 4.3: Change in hemicellulose content for the raw biomass, three pretreatments and control (water) at 20°C (a) and 50°C (b). Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

4.2.4 Ash Changes Due to Pretreatment

In terms of ash removal, sodium and potassium hydroxides achieved the largest reductions irrespective of the reaction temperature (Figure 4.4). The Tukey's correction test at a level of significance of 5% resulted in no statistically significant difference in ash content between sodium and potassium hydroxide with the exception of corn stover pretreated at 50°C, to which potassium hydroxide removed 11.7% more ash than sodium hydroxide. In all cases, but corn stover and miscanthus pretreated at 50°C, calcium hydroxide had statistically equivalent performance to water.



(a)

(b)

Figure 4.4: Change in ash content for the raw biomass, three pretreatments and control (water) at 20°C (a) and 50°C (b). Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

4.2.5 Temperature Effect on Pretreatment of Corn Stover

The compositional changes with pretreatment of corn stover in terms of lignin (Figure 4.5), cellulose (Figure 4.6), hemicellulose (Figure 4.7) and ash (Figure 4.8) are shown below. Regarding the effect of temperature on the pretreatment of corn stover, it was observed that there was a statistically significant influence of temperature in the change of lignin only for samples pretreated with sodium hydroxide. Samples pretreated with calcium hydroxide, potassium hydroxide, and water at 20°C showed no statistically significant difference in delignification from samples pretreated with these chemicals at 50°C. On the other hand, samples pretreated with sodium hydroxide at 50°C resulted in an 8.3% increase in the amount of delignification in comparison with samples pretreated at 20°C.

With respect to the change in cellulose content, the pretreatment temperature was not influential. With all four treatments in this study, the composition of cellulose after pretreatment at 20°C was statistically equivalent to the results found at 50°C.

Hemicellulose content of corn stover samples pretreated with sodium hydroxide, and control were significantly affected by the temperature of pretreatment. The temperature at 50°C resulted in 6.2% and 9.3% higher quantities of hemicellulose when pretreated with sodium hydroxide and water, respectively. No significant changes in quantities of hemicellulose were obtained with either calcium or potassium hydroxides.

There was slightly higher ash removal at 20°C when pretreated with sodium hydroxide 9.0% reduction) and water (18.5% reduction). The reduction in ash content with the alkali pretreated corn stover could have been due to the high quantity of water used in washing or was related to the pretreatment.

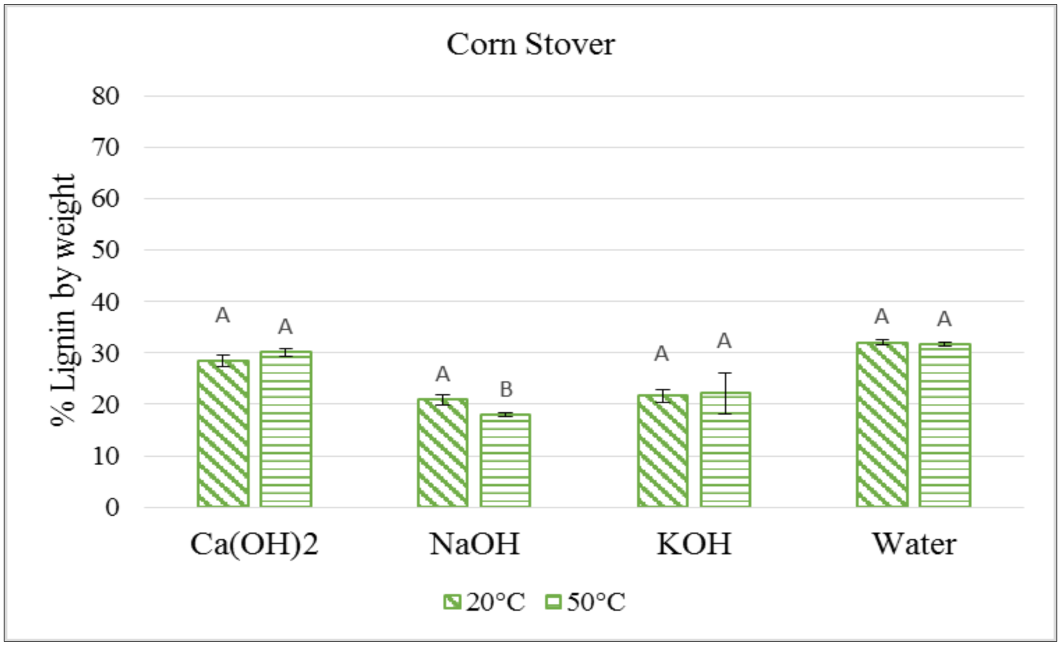


Figure 4.5: Temperature effect on lignin composition of corn stover after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

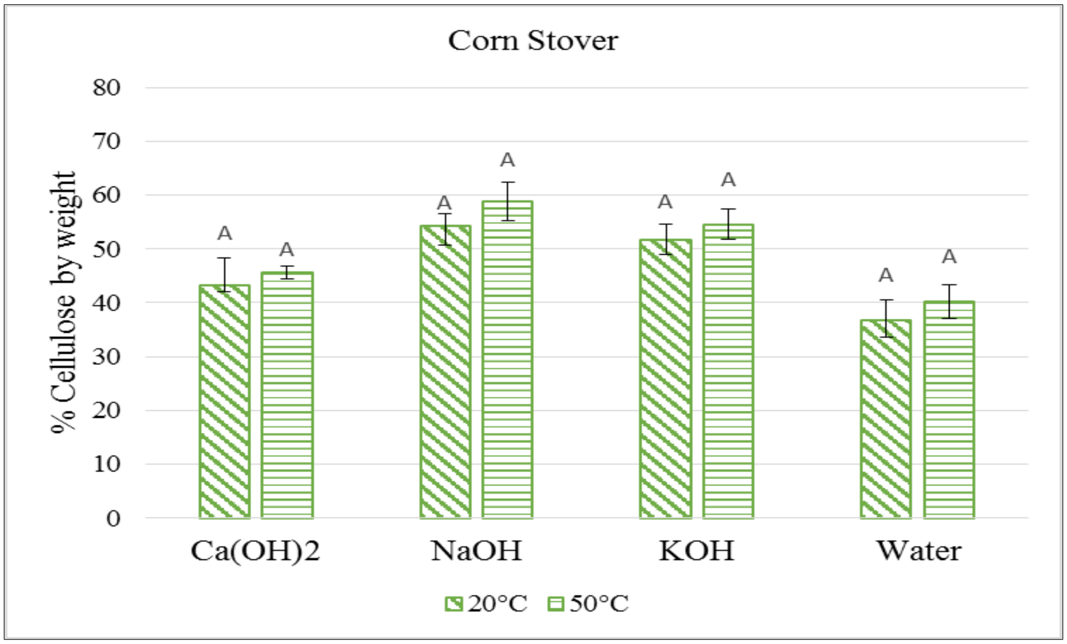


Figure 4.6: Temperature effect on cellulose composition of corn stover after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

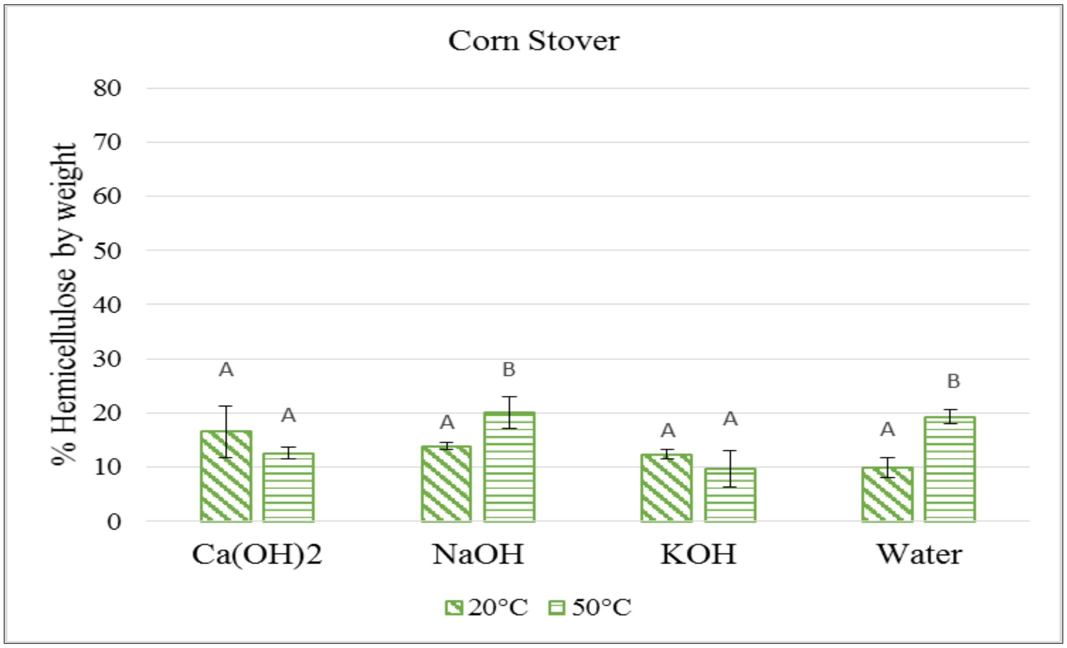


Figure 4.7: Temperature effect on hemicellulose composition of corn stover after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

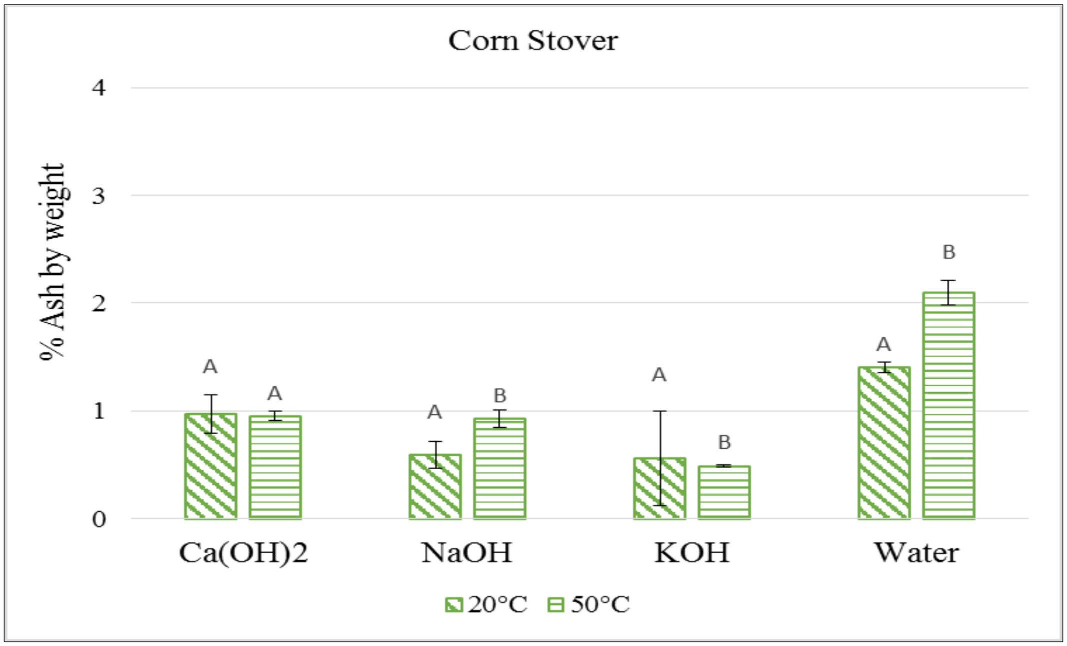


Figure 4.8: Temperature effect on ash composition of corn stover after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

4.2.6 Temperature Effect on Pretreatment of Wheat Straw

The influence of temperature on the change in lignin (Figure 4.9), cellulose (Figure 4.10), hemicellulose (Figure 4.11), and ash (Figure 4.12) with wheat straw are summarized below. Temperature did not influence the delignification of wheat straw with the alkali chemicals investigated. Surprisingly, a lower lignin content was observed at 20°C than 50°C when pretreated with water. The only significant effect of temperature on the cellulose content was with sodium hydroxide. However, the temperature of pretreatment did not affect significantly the change in hemicellulose composition in wheat straw pretreated with all three alkali investigated in this study, as well as in control samples. Sodium hydroxide resulted in a lower ash content when pretreated at 50°C relative to 20°C. There was no significant effect of temperature on the ash content when pretreated with water, calcium and potassium hydroxides.

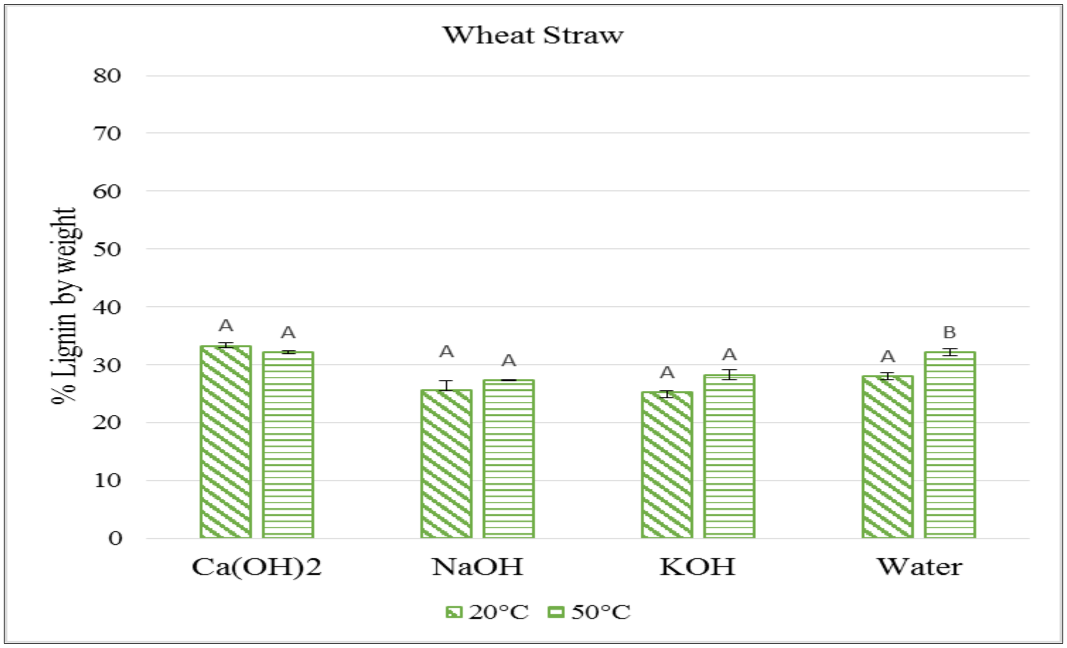


Figure 4.9: Temperature effect on lignin composition of wheat straw after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

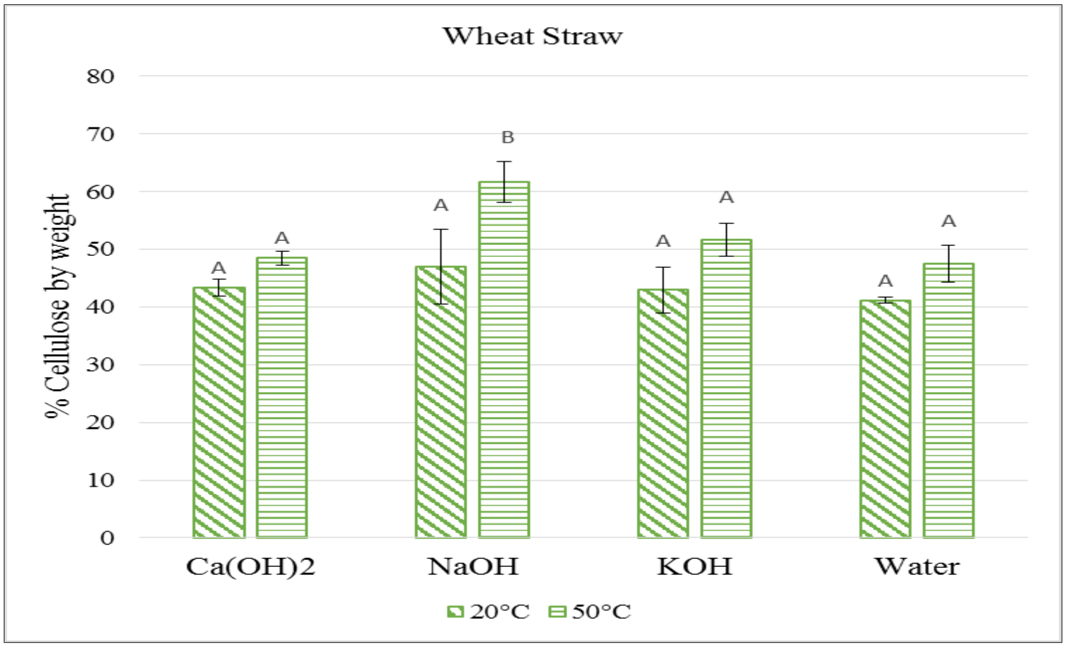


Figure 4.10: Temperature effect on cellulose composition of wheat straw after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

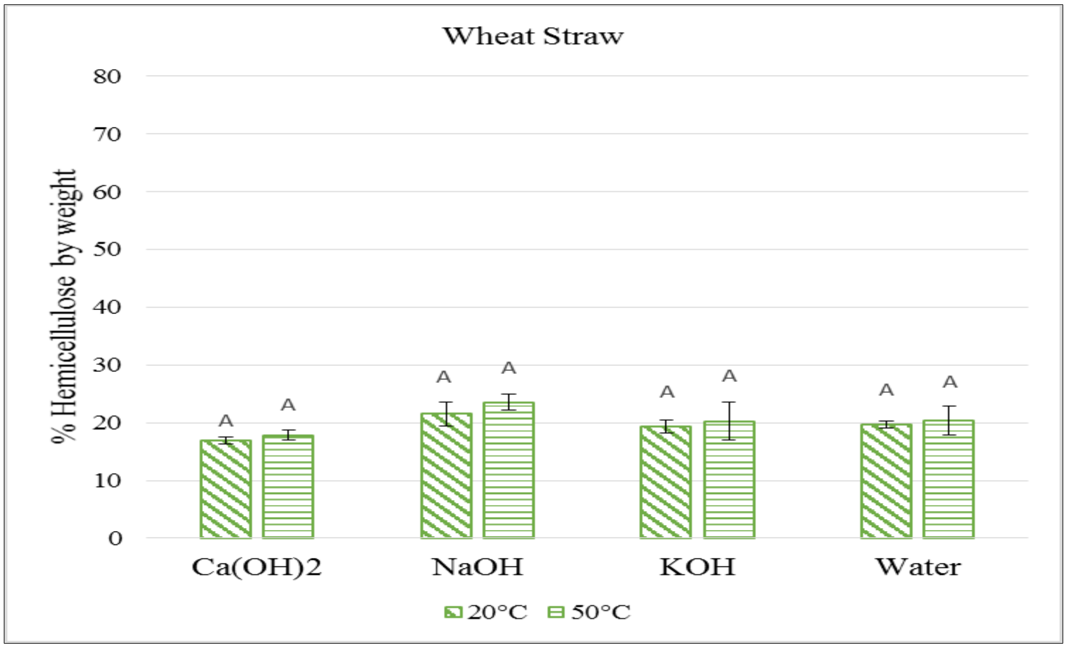


Figure 4.11: Temperature effect on hemicellulose composition of wheat straw after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

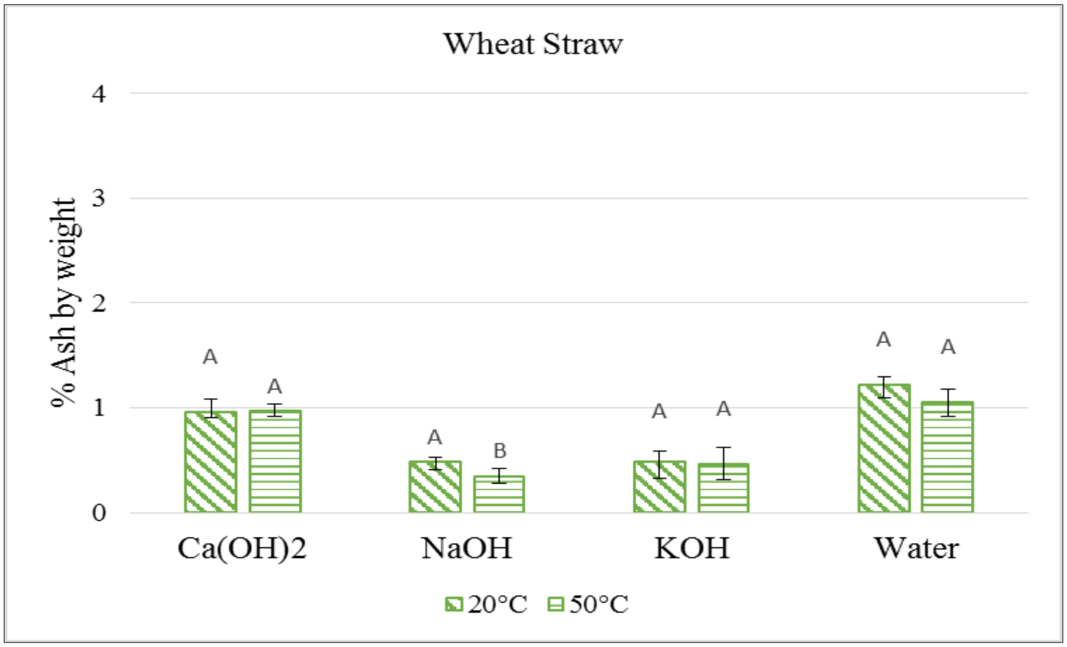


Figure 4.12: Temperature effect on ash composition of wheat straw after pretreatment with alkali and water**.** Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

4.2.8 Temperature Effect on Pretreatment of Switchgrass

The influence of temperature on the pretreatment of switchgrass in terms of lignin (Figure 4.13), cellulose (Figure 4.14), hemicellulose (Figure 4.15), and ash (Figure 4,16) content are shown. No temperature influence was observed in the change in lignin content of switchgrass. Cellulose and hemicellulose content were significantly increased when the pretreatment temperature was 50°C for all pretreatments investigated. There was no effect of temperature on the ash content due to pretreatment.

The highest quantities of cellulose were attained at a temperature of 50°C. The highest increase in cellulose content (13 percentage points) occurred with calcium hydroxide. Sodium hydroxide and potassium hydroxide also increased the cellulose content by 11 and 9 percentage points, respectively.

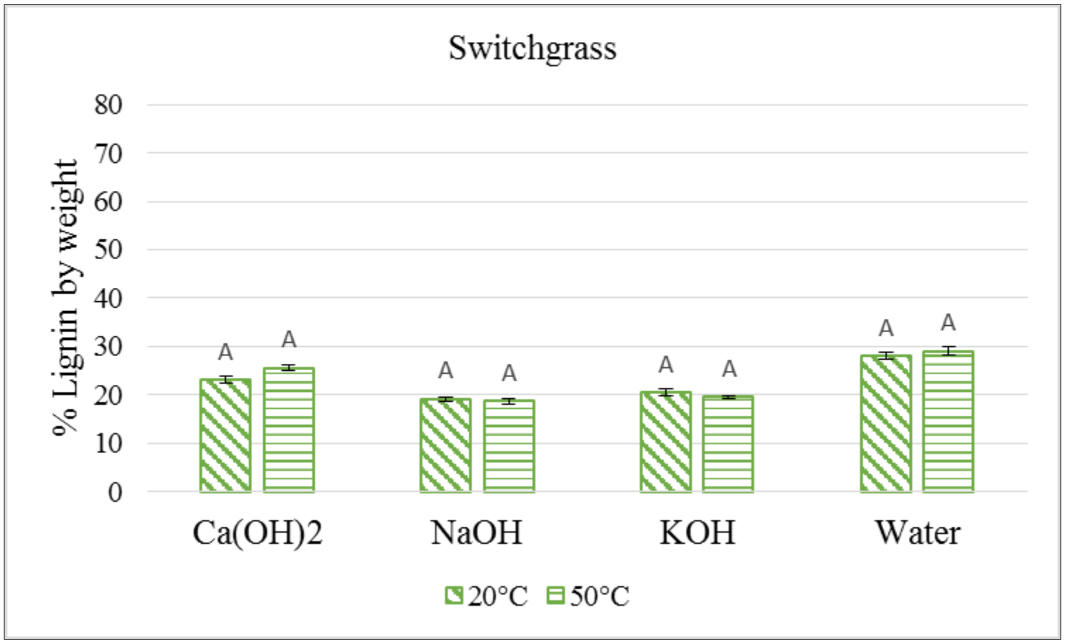


Figure 4.13: Temperature effect on lignin composition of switchgrass after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

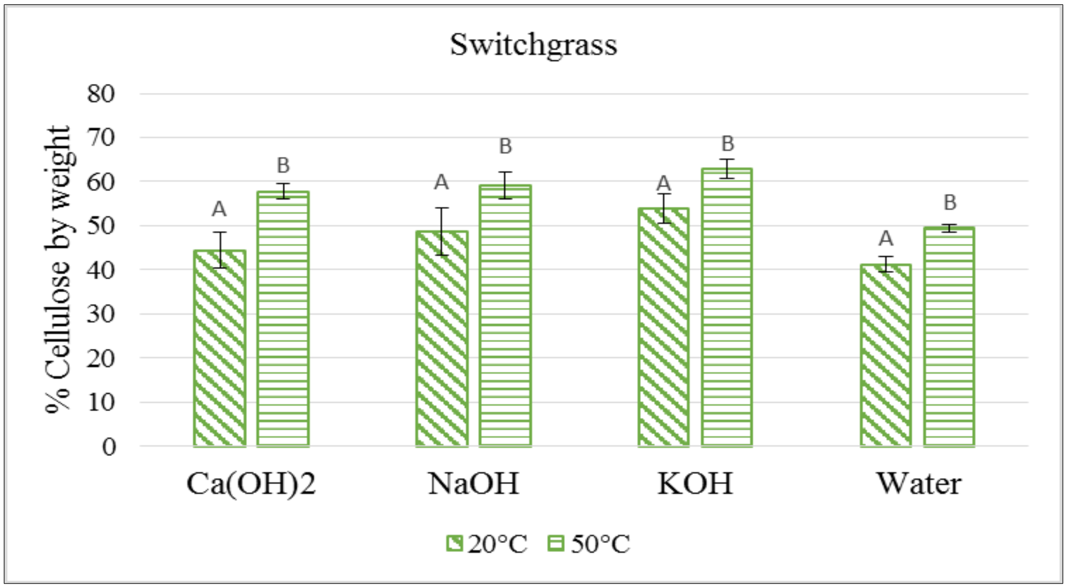


Figure 4.14: Temperature effect on cellulose composition of switchgrass after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

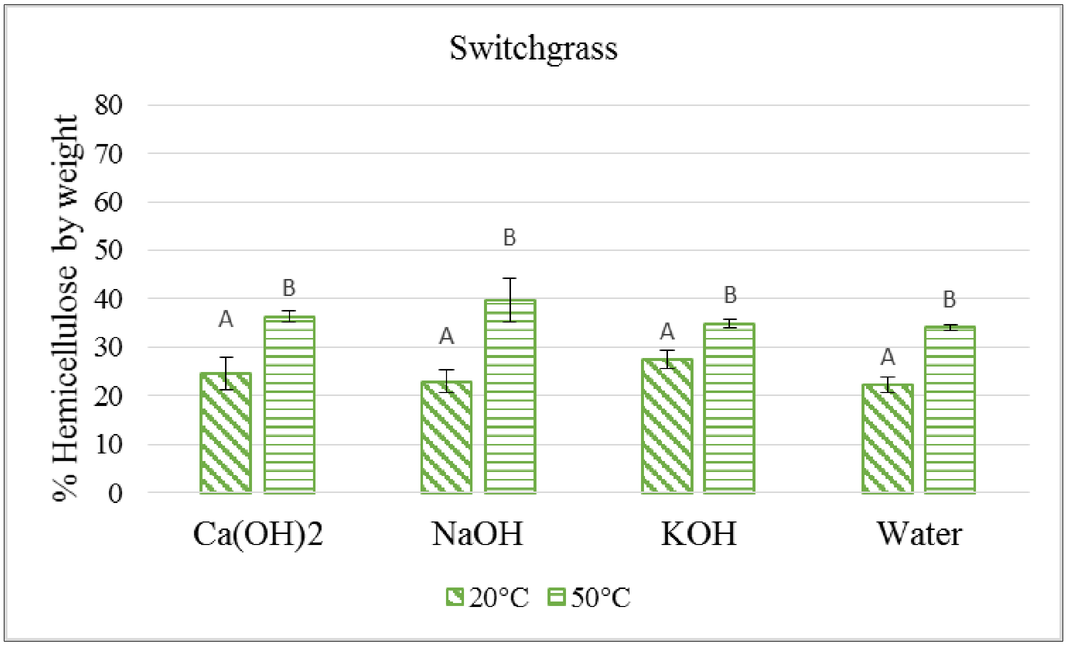


Figure 4.15: Temperature effect on hemicellulose composition of switchgrass after pretreatment with alkali and water**.** Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

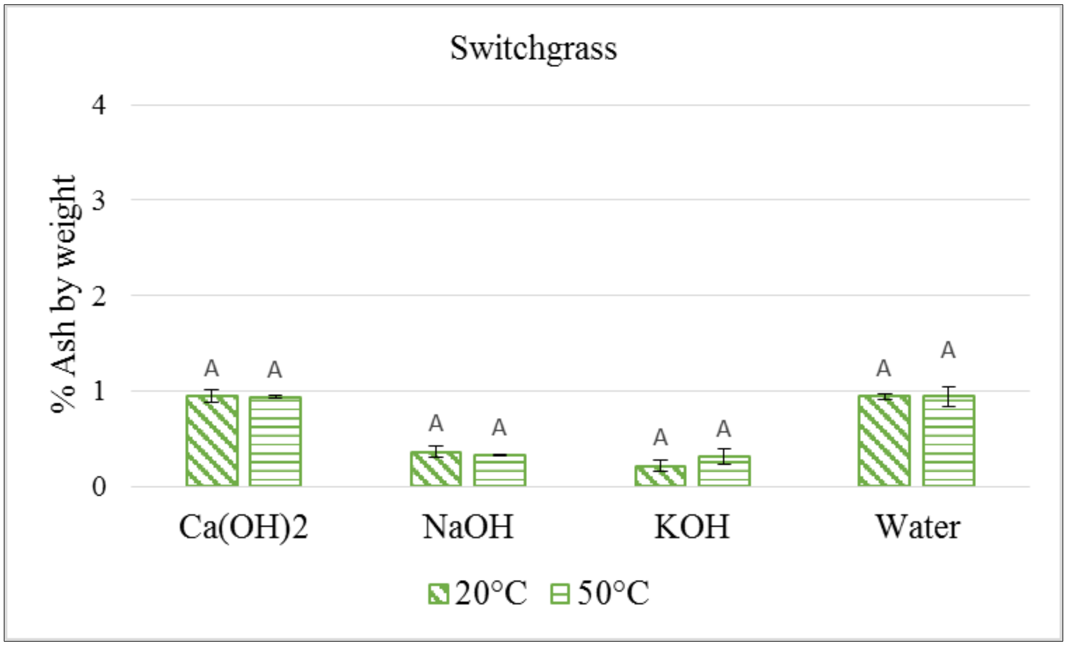


Figure 4.16: Temperature effect on ash composition of switchgrass after pretreatment with alkali and water**.** Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey’s correction test at a significance level of 5%.

4.2.7 Temperature Effect on Pretreatment of Miscanthus

For miscanthus, the pretreatment temperature had a statistically significant influence in delignification for all three alkali conditions investigated (Figure 4.17). However, there was no significant difference in lignin content with the control. As expected, the lignin content decreased with the three chemicals when the temperature increased. In terms of cellulose content, water, sodium and potassium hydroxide had a statistically significant increase in cellulose content as the temperature increased (Figure 4.18). At a temperature of 50°C, the cellulose content decreased relative to 20°C when pretreated with calcium hydroxide.

Significant changes in hemicellulose content due to temperature differences were observed with sodium and potassium hydroxides, and the control samples (Figure 4.19). With the sodium and potassium hydroxide pretreatment at 50°C led to an 8 and 7 percentage point decrease in hemicellulose relative to 20°C. While the control at 50°C resulted in an 11 percentage point increase in hemicellulose content. No significant changes in quantities of hemicellulose were noticed in calcium hydroxide pretreated.

Ash contents had a statistically significant increase when pretreated at 50°C (Figure 4.20). This was not expected and was only observed with miscanthus.

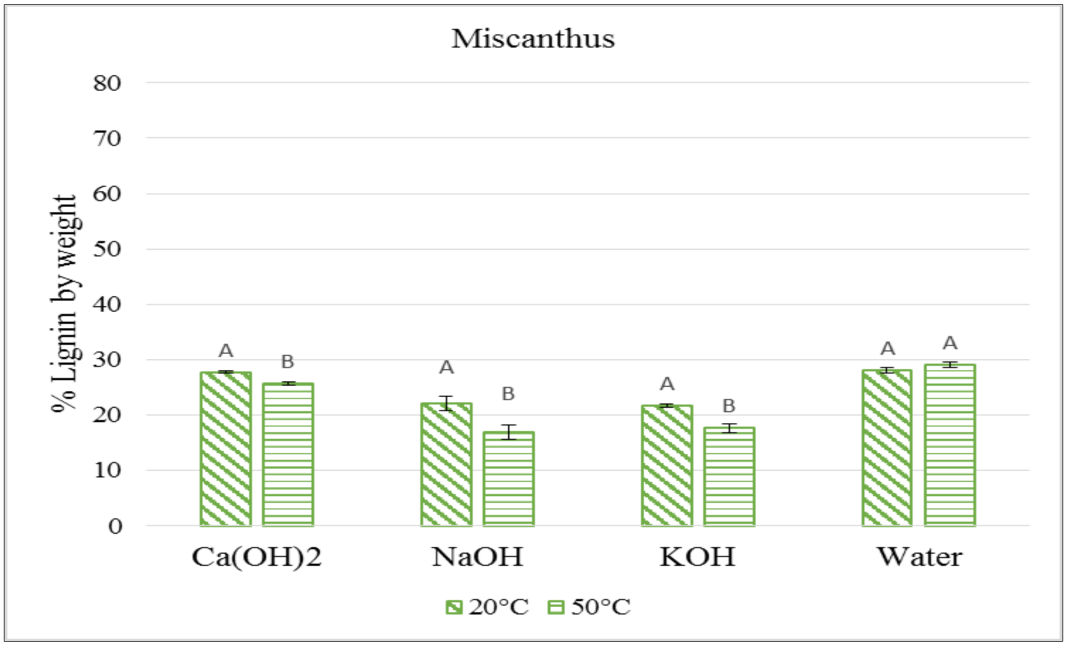


Figure 4.17: Temperature effect on lignin composition of miscanthus after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey’s correction test at a significance level of 5%.

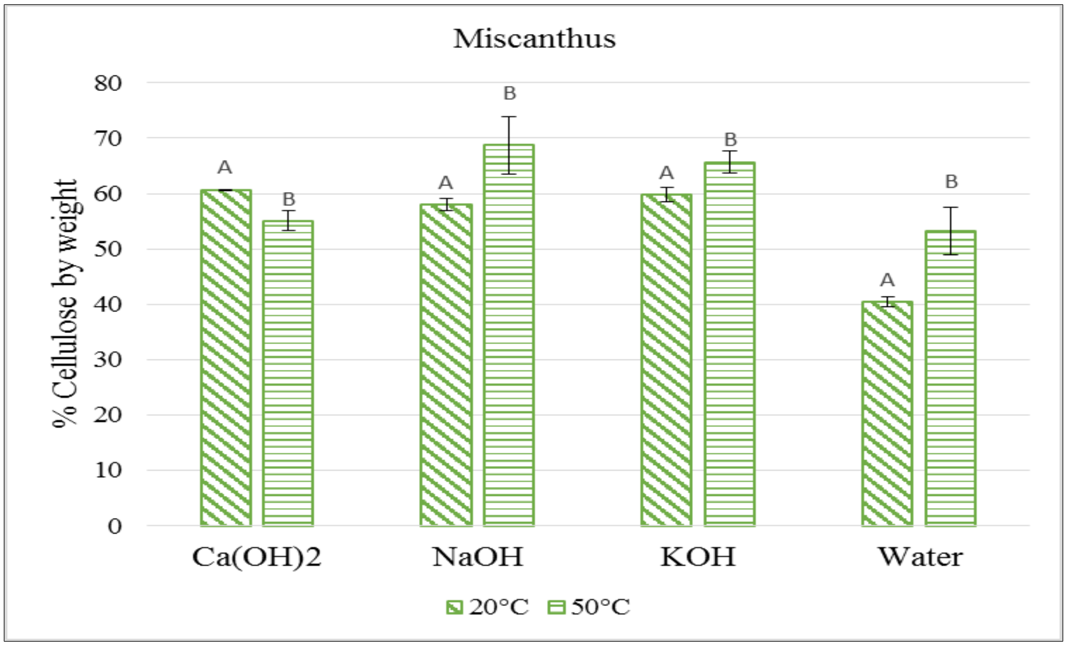


Figure 4.18: Temperature effect on cellulose composition of miscanthus after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey’s correction test at a significance level of 5%.

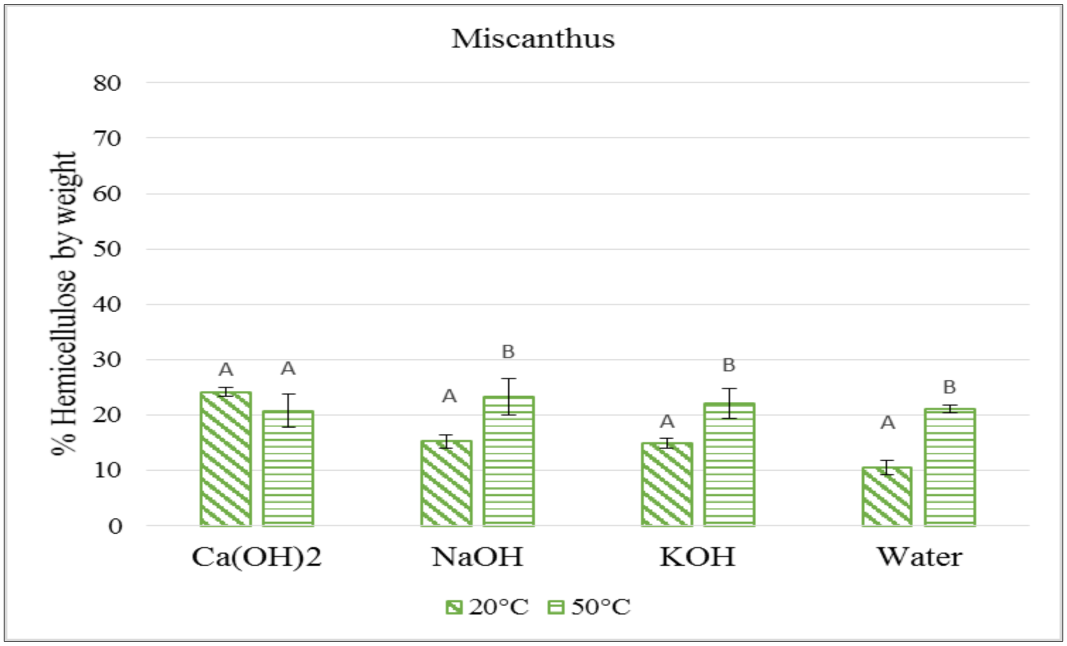


Figure 4.19: Temperature effect on hemicellulose composition of miscanthus after pretreatment with alkali and water**.** Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

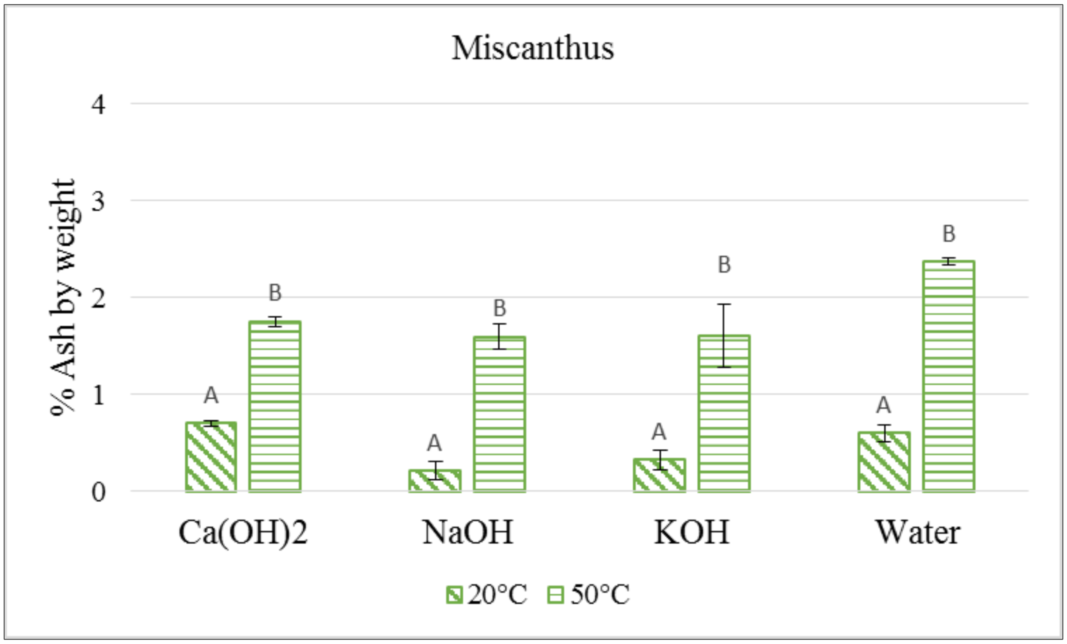


Figure 4.20: Temperature effect on ash composition of miscanthus after pretreatment with alkali and water. Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

4.3 Effect of Hydrolysis on Carbohydrates Digestibility

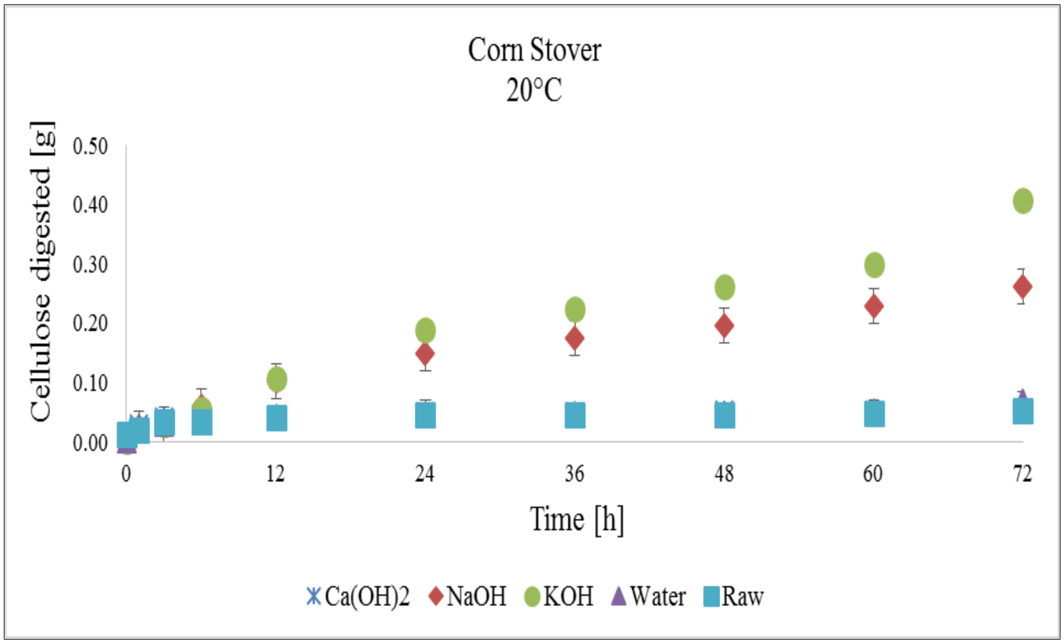
After pretreatment, samples were enzymatically hydrolyzed. The sugars released after enzymatic hydrolysis were measured for all four feedstocks pretreated at the two temperatures and are summarized below. The results shown are the mean and standard deviation of three repetitions.

The effectiveness of hydrolysis was evaluated by calculating the maximum reaction rate observed during hydrolysis and the percentage of cellulose converted into monomeric sugars.

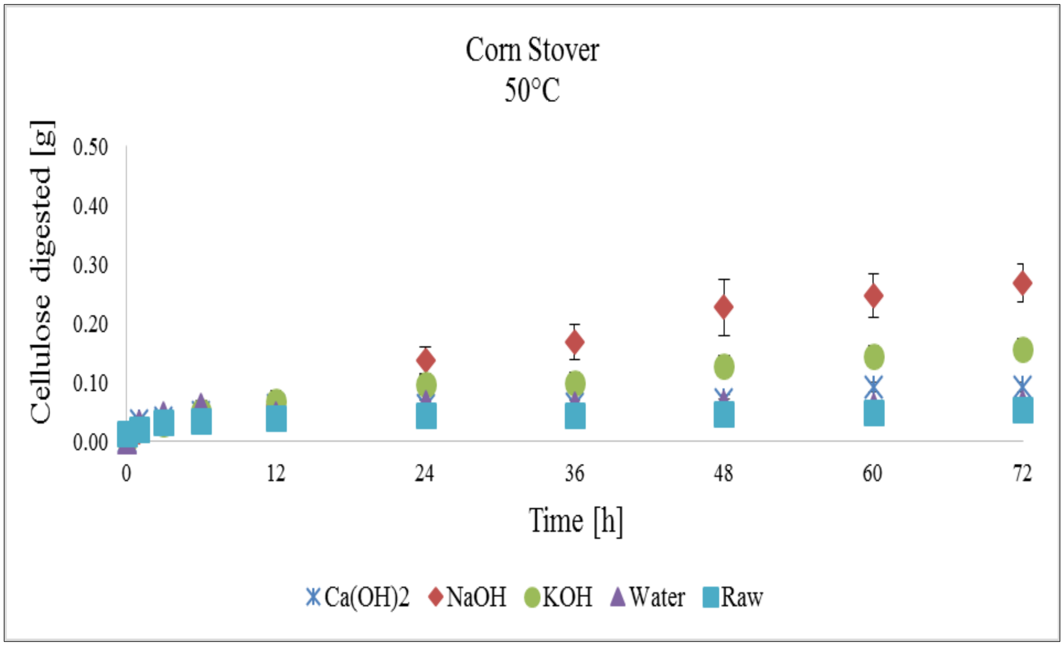
4.3.1 Hydrolysis Profile

A complete hydrolysis profile was measured for all four feedstock and two temperatures. Plots of cellulose conversion as a function of time are shown in Figure 4.21, Figure 4.22, Figure 4.23, and Figure 4.24. The cellulose digested shown is a mean of three replications.

It can be inferred from the figures below that the glucose concentration during enzymatic hydrolysis varied in a similar way for the biomass pretreated with sodium hydroxide and potassium hydroxide in the majority of the cases analyzed. Pretreatment with calcium hydroxide led to a lower glucose concentration over the time allowed for hydrolysis. This may be attributed to the composition changes observed during pretreatment with sodium and potassium hydroxide. Moreover, in corn stover, there was no variation in the hydrolysis profile for pretreatment with calcium hydroxide, the water control, and untreated biomass.

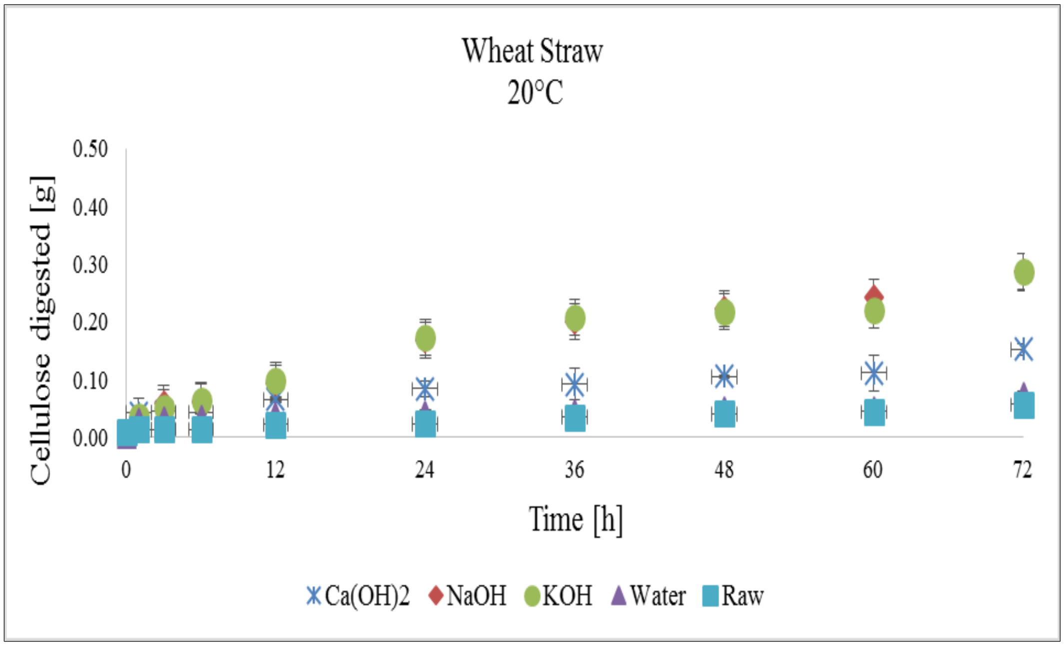


(a)

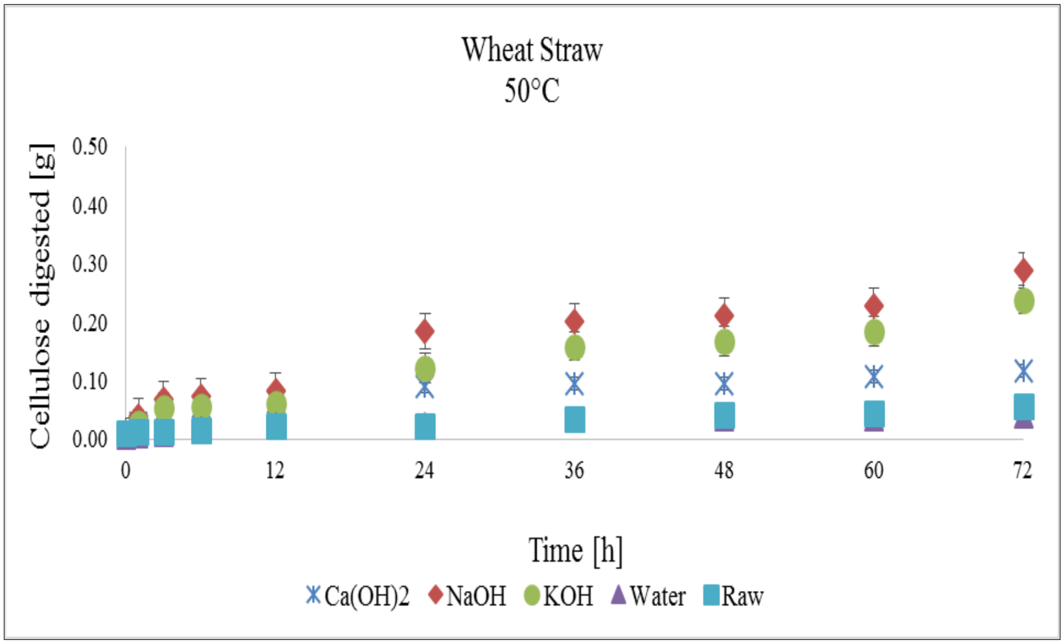


(b)

Figure 4.21: Cellulose digested after enzymatic hydrolysis of untreated corn stover, water control, and pretreated with three alkali at 20°C (a) and 50°C (b).Error bars are the standard deviations of three replications.

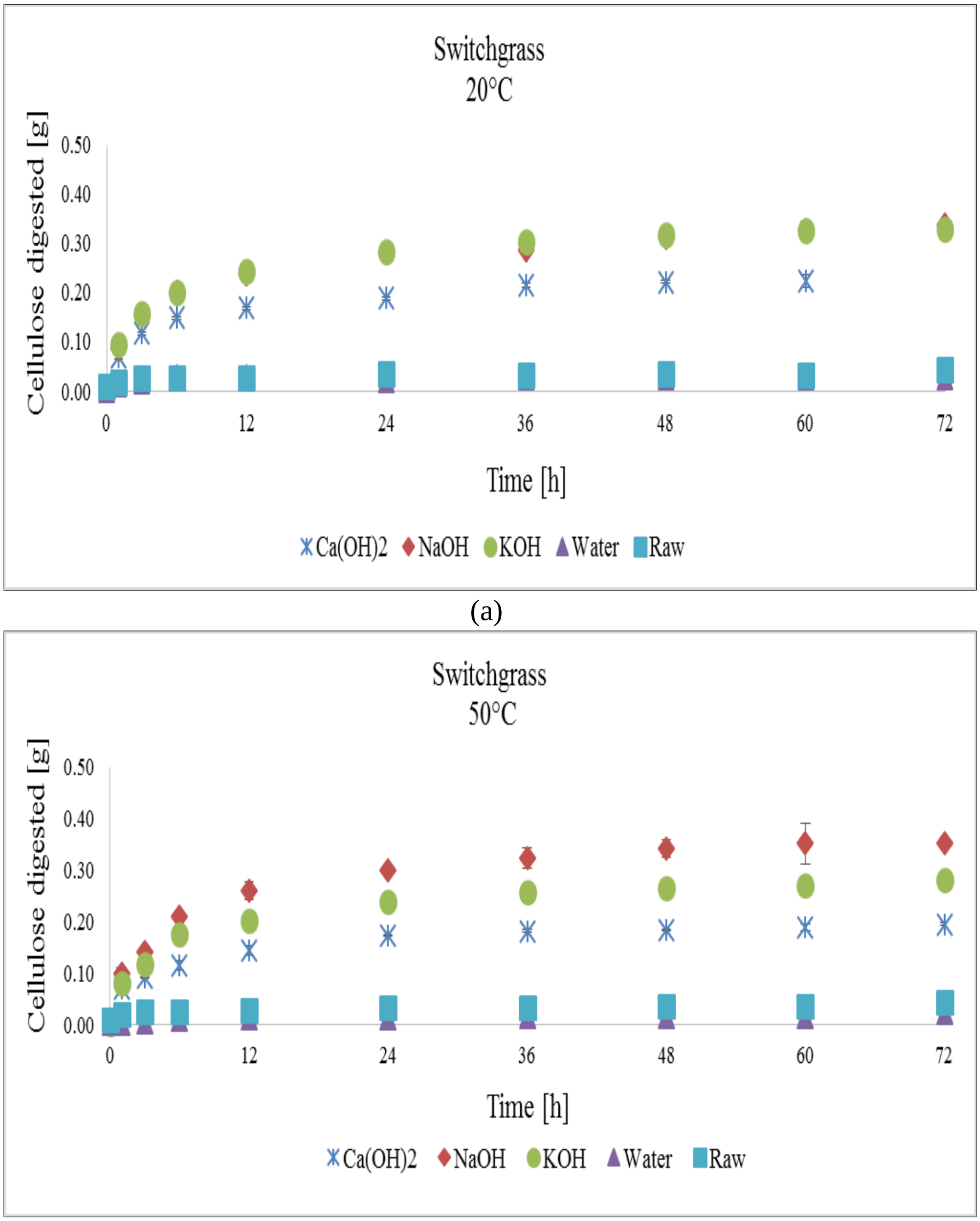


(a)



(b)

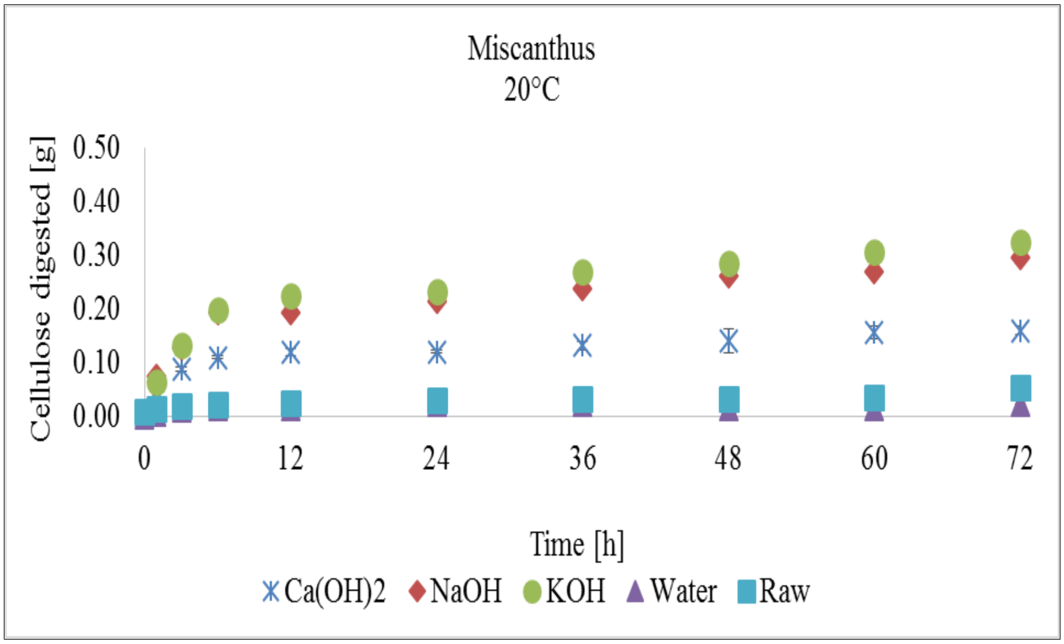
Figure 4.22: Cellulose digested after enzymatic hydrolysis of untreated wheat straw, water control, and pretreated with three alkali at 20°C (a) and 50°C (b).Error bars are the standard deviations of three replications.



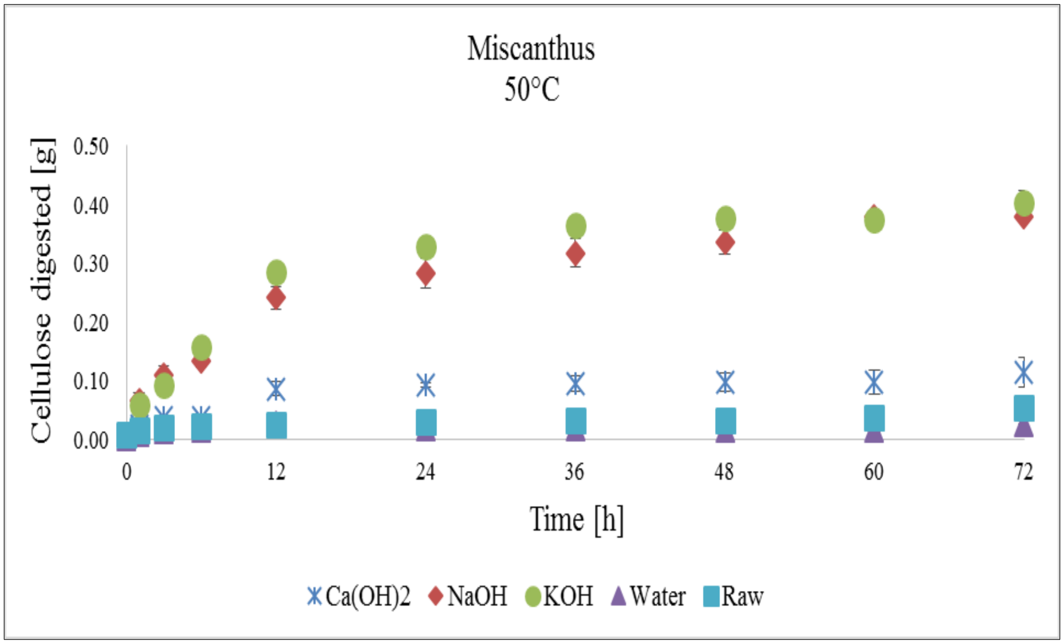
(a)

(b)

Figure 4.23: Cellulose digested after enzymatic hydrolysis of untreated switchgrass, water control, and pretreated with three alkali at 20°C (a) and 50°C (b).Error bars are the standard deviations of three replications.



(a)



(b)

Figure 4.24: Cellulose digested after enzymatic hydrolysis of untreated miscanthus, water control, and pretreated with three alkali at 20°C (a) and 50°C (b).Error bars are the standard deviations of three replications.

4.3.2 Maximum Reaction Rate of cellulose digestibility

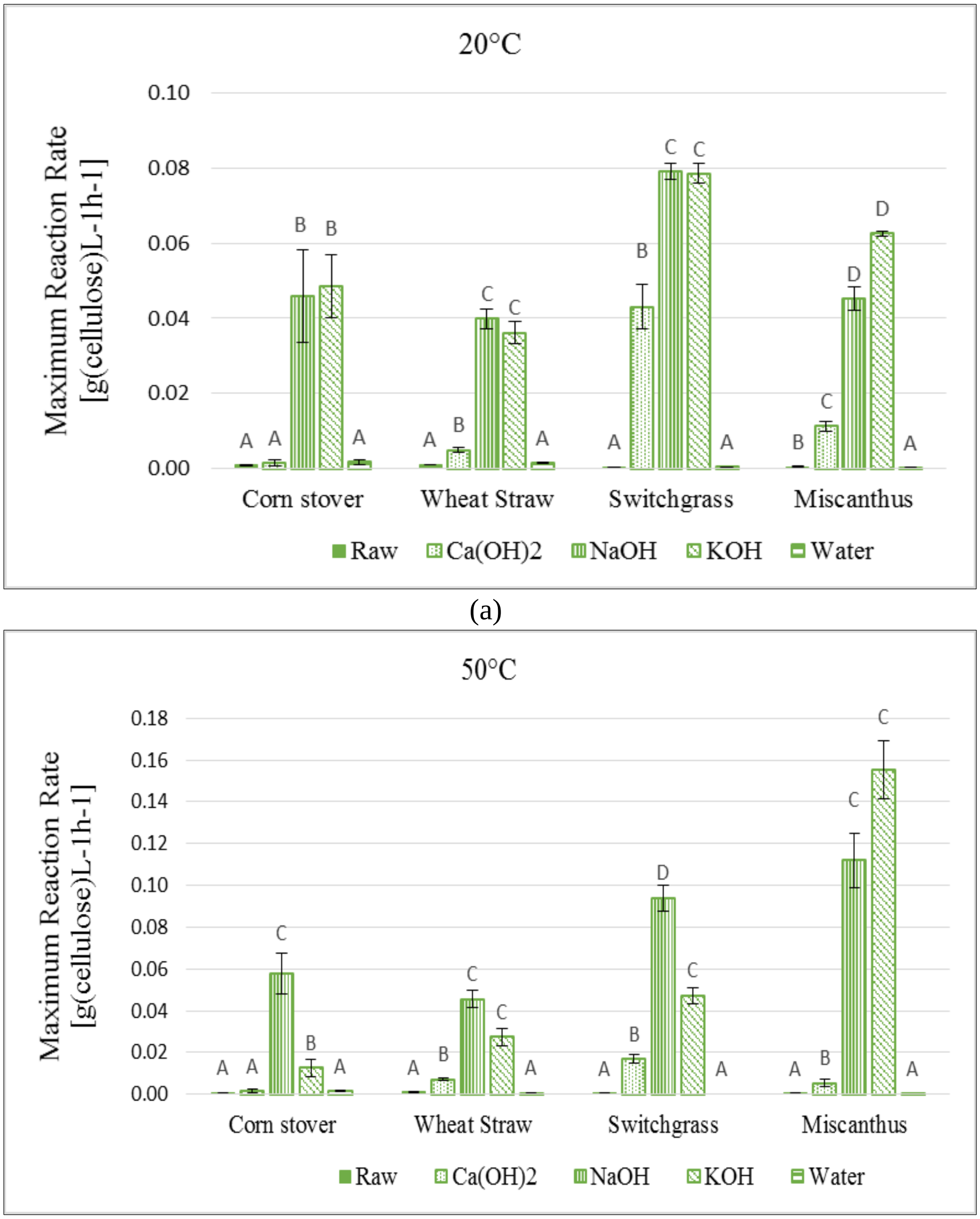
Figure 4.25 and Table 4.5 summarize the maximum reaction rate on each treatment investigated for all four biomass types and the two temperature levels. The maximum reaction rate of cellulose digestion, expressed in terms of $gL^{-1}h^{-1}$, was calculated applying Michaelis-Menten model for a batch reactor, as demonstrated in appendix C.

At 50°C, sodium hydroxide had the highest hydrolysis reaction rate among the three chemicals utilized as pretreatment agents for switchgrass, corn stover, and wheat straw. With miscanthus, sodium and potassium hydroxide had the highest and statistically equivalent reaction rates. Samples pretreated with calcium hydroxide resulted in the lowest hydrolysis reaction rate among the alkali pretreated samples for all biomass types studied.

At 20°C, for all feedstocks the hydrolysis reaction rate when pretreated with sodium and potassium hydroxides resulted in no statistically significant difference based on the Tukey's correction test at 95% confidence. Samples pretreated with calcium hydroxide had the lowest hydrolysis reaction rate among the alkali pretreated samples at 20°C. In switchgrass, miscanthus and wheat straw, all chemicals had a positive effect on the reaction rate in comparison with water, which did not show significant difference from the untreated samples for the two temperatures investigated and all four biomass. In corn stover, calcium hydroxide had a statistically equivalent reaction rate to water and untreated feedstock.

Table 4.5: Maximum reaction rate of cellulose digestibility for the chemically pretreated biomass, raw biomass with no pretreatment, and samples pretreated with water and no chemical addition.

Treatment	**Maximum Rate of cellulose digestibility [$gL^{-1}h^{-1}$]**							
	corn stover		**wheat straw**		**switchgrass**		**miscanthus**	
	20°C	**50°C**	**20°C**	**50°C**	**20°C**	**50°C**	**20°C**	**50°C**
Raw	0.0009	0.0009	0.0010	0.0010	0.0004	0.0004	0.0005	0.0005
Ca(OH)2	0.0015	0.0017	0.0049	0.0071	0.0431	0.0168	0.0113	0.0053
NaOH	0.0460	0.0578	0.0399	0.0455	0.0790	0.0941	0.0452	0.1119
KOH	0.0485	0.0126	0.0362	0.0274	0.0785	0.0473	0.0626	0.1554
Water	0.0017	0.0016	0.0016	0.0006	0.0004	0.0000	0.0002	0.0002



(a)

(b)

Figure 4.25: Maximum reaction rate of cellulose digestion for the raw biomass, three pretreatments and control (water) at 20°C (a) and 50°C (b). Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

4.3.3 Carbohydrate Conversion

The percentage of polysaccharides converted into monosaccharides is one method to evaluate the efficiency of a pretreatment and the success of a bioconversion process. Figure 4.26 and Table 4.6 summarize the percentage of cellulose converted for all four feedstocks and the two temperatures investigated in this study. Two controls were utilized; raw feedstock and feedstock pretreated with water.

Cellulose conversion rates between 31.4 and 81.4% were achieved with samples pretreated with either sodium hydroxide or potassium hydroxide. With the exception of switchgrass samples pretreated at 20°C (where a conversion of 77.6% was measured), calcium hydroxide led to the lowest conversion for the other treatment conditions (between 10.5 and 38.9%).

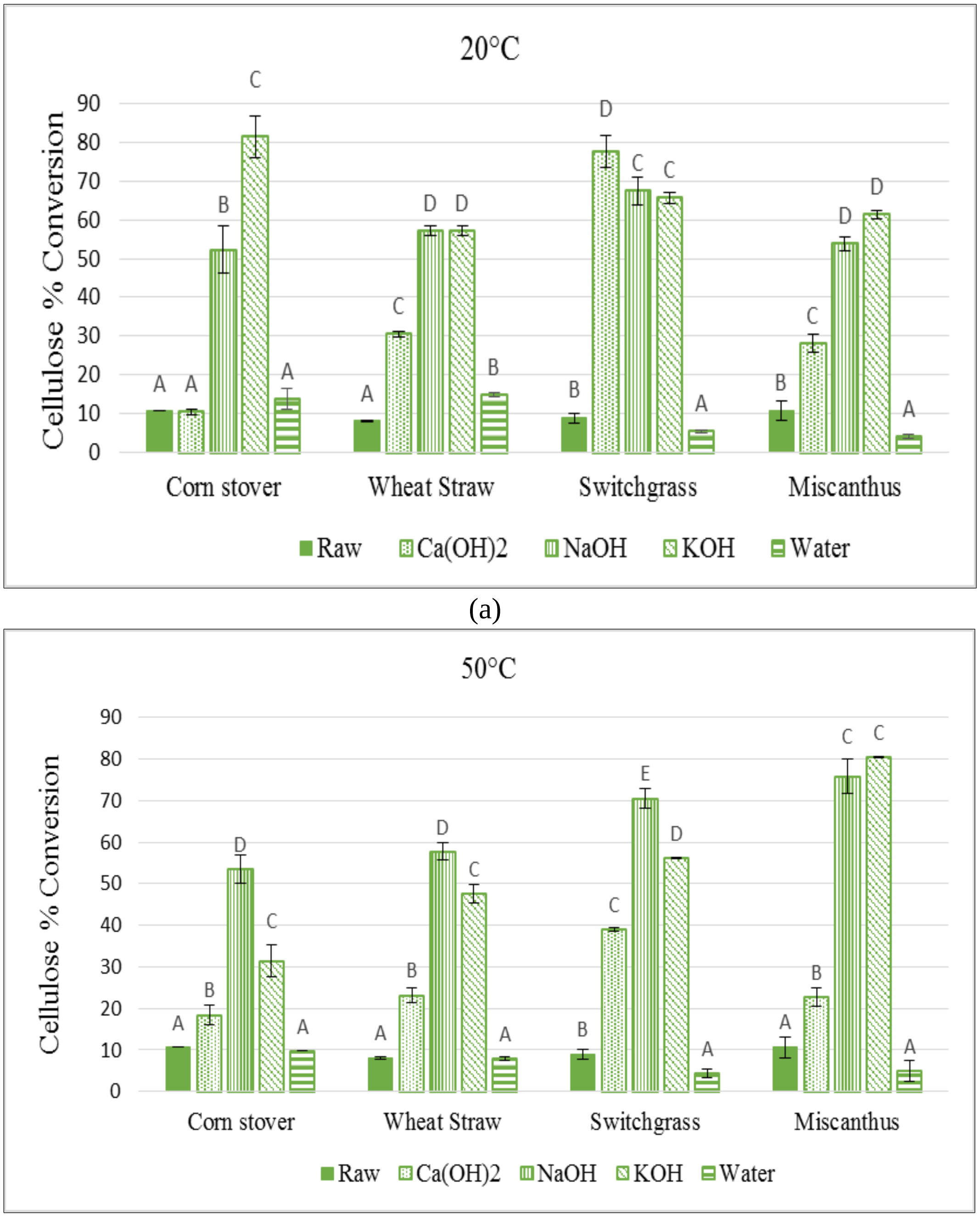
Cellulose conversion for switchgrass, corn stover, and wheat straw samples pretreated at 50°C showed that all three alkali released different concentrations of glucose based on the adjusted Tukey’s test at a level of significance of 5%. A constant cellulose loading of 0.5 g per flask was used for the enzymatic hydrolysis experiments. Sodium hydroxide released the highest concentration of glucose after 72 hours of hydrolysis (7.83 g/L, 5.94 g/L, and 6.41 g/L, respectively), followed by potassium hydroxide (6.22 g/L, 3.49 g/L, 5.28 g/L, respectively) and calcium hydroxide (4.32 g/L, 2.04 g/L, and 2.57 g/L, respectively).

For switchgrass and wheat straw, at a pretreatment temperature of 20°C, sodium and potassium hydroxides had the same cellulose conversion that varied between 47.5 and 70.5%. Switchgrass pretreated with calcium hydroxide led to a 77.6% cellulose conversion at 20°C, although only 38.9% at 50°C. For corn stover, samples that were pretreated at 20°C with potassium hydroxide lost 81.4% of the initial cellulose after 72 hours of enzymatic hydrolysis, which corresponded to 29.2 and 70.9 percentage points more cellulose digested than observed with sodium hydroxide and calcium hydroxide pretreated corn stover. The low conversion percentage achieved with calcium hydroxide pretreated samples showed to be statistically not different to the control and untreated corn stover.

For miscanthus, no statistically significant difference was observed for cellulose conversion due to the use of either sodium or potassium hydroxides at both pretreatment temperatures after 72 hours of enzymatic hydrolysis that varied between 53.8 and 80.5%. Samples pretreated with calcium hydroxide resulted in approximately a 30 to 55 percentage point difference in cellulose conversion compared to the other two alkali. Control samples, in most cases, did not show a statistical difference in cellulose conversion compared to untreated feedstock.

Table 4.6: Percent cellulose converted into glucose after 72 h of enzymatic hydrolysis.

Treatment	**Cellulose % Conversion**							
	corn stover		**wheat straw**		**switchgrass**		**miscanthus**	
	20°C	**50°C**	**20°C**	**50°C**	**20°C**	**50°C**	**20°C**	**50°C**
Raw	10.6	10.6	8.1	8.1	8.9	8.9	10.7	10.7
Ca(OH)2	10.5	18.4	30.5	23.1	77.6	38.9	28.1	22.7
NaOH	52.2	53.5	57.2	57.7	67.4	70.5	53.8	75.8
KOH	81.4	31.4	57.2	47.5	65.7	56.1	61.3	80.5
Water	13.9	9.7	14.9	8.0	5.4	4.4	4.2	5.0



(a)

(b)

Figure 4.26: Cellulose % conversion for the raw biomass, three pretreatments and control (water) at 20°C (a) and 50°C (b). Error bars are the standard deviation of three replications and treatments with the same letter are not statistically significant based on Tukey's correction test at a significance level of 5%.

4.3.4 Effect of Pretreatment Temperature on Hydrolysis of Corn Stover

A Tukey's correction test revealed that increasing pretreatment temperature increased the maximum reaction rate only for the sodium hydroxide samples (Figure 4.27). There was no difference in the maximum reaction rate for the calcium hydroxide and a negative impact on the reaction rate for potassium hydroxide.

Pretreatment with sodium hydroxide at 20°C and 50°C did not result in any significant difference in cellulose conversion (Figure 4.28). The results were mixed with calcium and potassium hydroxides. Increasing the temperature with corn stover led to a significant increase in cellulose digested with calcium hydroxide. The biggest effect of temperature of pretreatment on cellulose digestion of corn stover occurred with potassium hydroxide. Samples of the potassium hydroxide group pretreated at 20°C led to double the amount of cellulose converted and a reaction rate approximately 3.8 times greater.

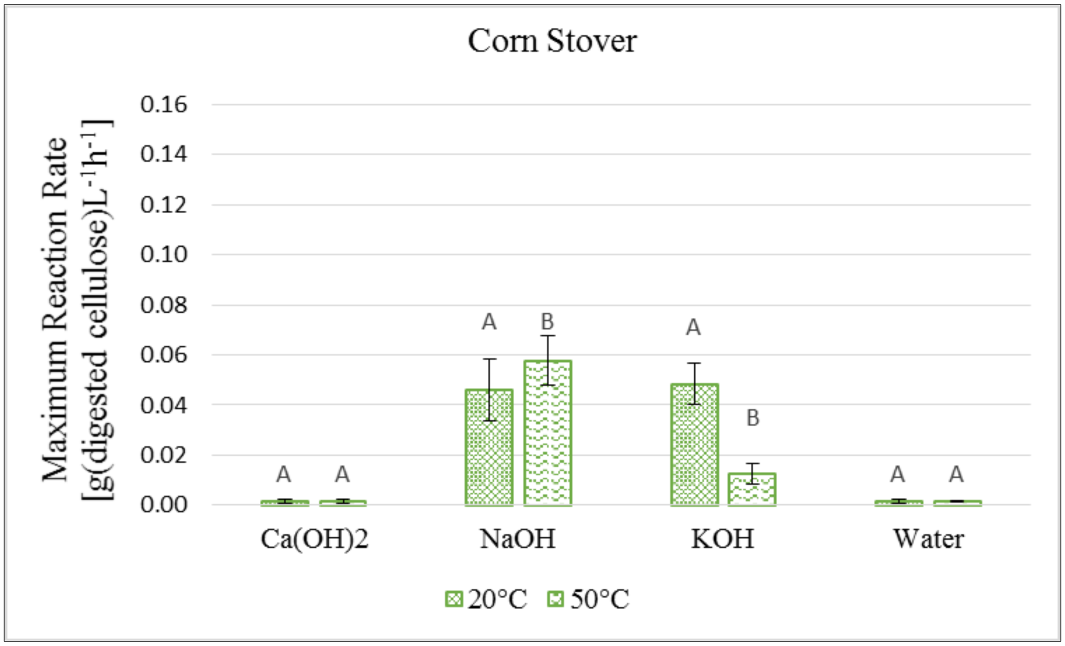


Figure 4.27: Pretreatment temperature effect on maximum rate of digested cellulose of corn stover pretreated with three alkali and water control. Error bars are the standard deviations of three replications.

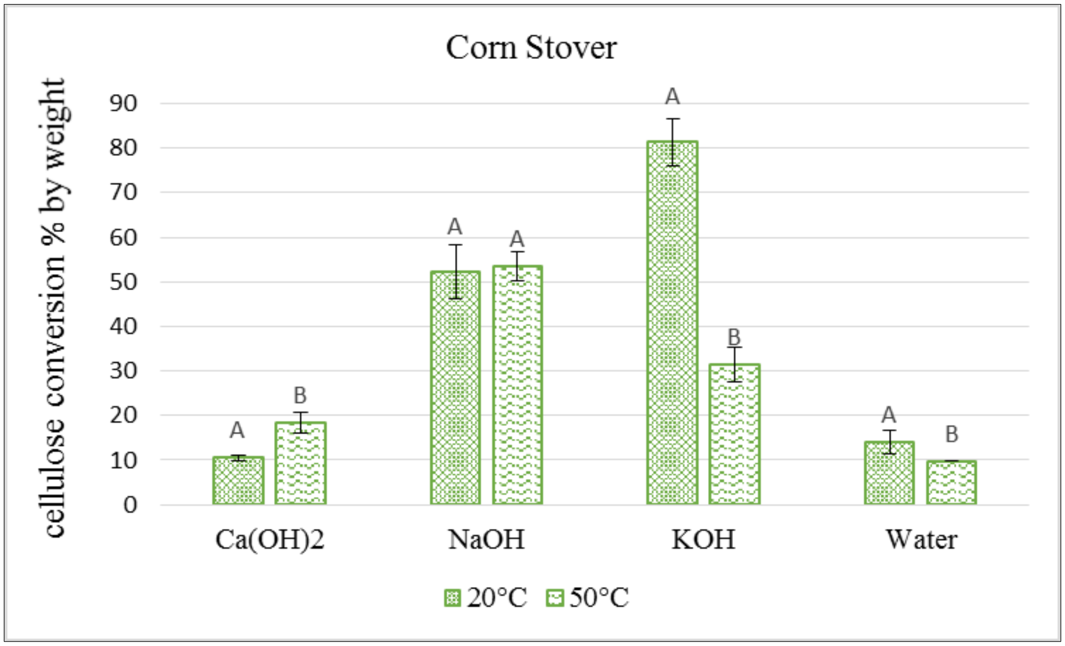


Figure 4.28: Pretreatment temperature effect on cellulose digestion of corn stover due to enzymatic hydrolysis. Error bars are the standard deviations of three replications.

4.3.5 Effect of Pretreatment Temperature on Hydrolysis of Wheat Straw

A positive influence on the maximum reaction rate due to pretreatment temperature on wheat straw pretreated with calcium and sodium hydroxide was observed (Figure 4.29). However, the temperature did not positively influence the reaction rate when potassium hydroxide was used. No significant differences in the measured hydrolysis reaction rate between both temperatures in the study were observed with samples pretreated with sodium and potassium hydroxides. On the other hand, samples pretreated with calcium hydroxide at 20°C had a 1.45 times slower reaction rate than the ones pretreated at 50°C. Wheat straw pretreated with water showed a similar trend to calcium hydroxide; a lower pretreatment temperature resulted in a higher reaction rate.

In terms of cellulose conversion, the difference in the total cellulose hydrolyzed after 72 h when wheat straw was pretreated with sodium hydroxide at 20°C and 50°C was not significant (Figure 4.30). In the case of calcium and potassium hydroxides, a lower pretreatment temperature resulted in a higher cellulose conversion. The maximum reaction rate for calcium hydroxide was achieved at 50°C, although a higher total cellulose conversion was obtained at 20°C were contradictory. However, potassium hydroxide followed the trend of increased reaction rate led to a higher total cellulose conversion.

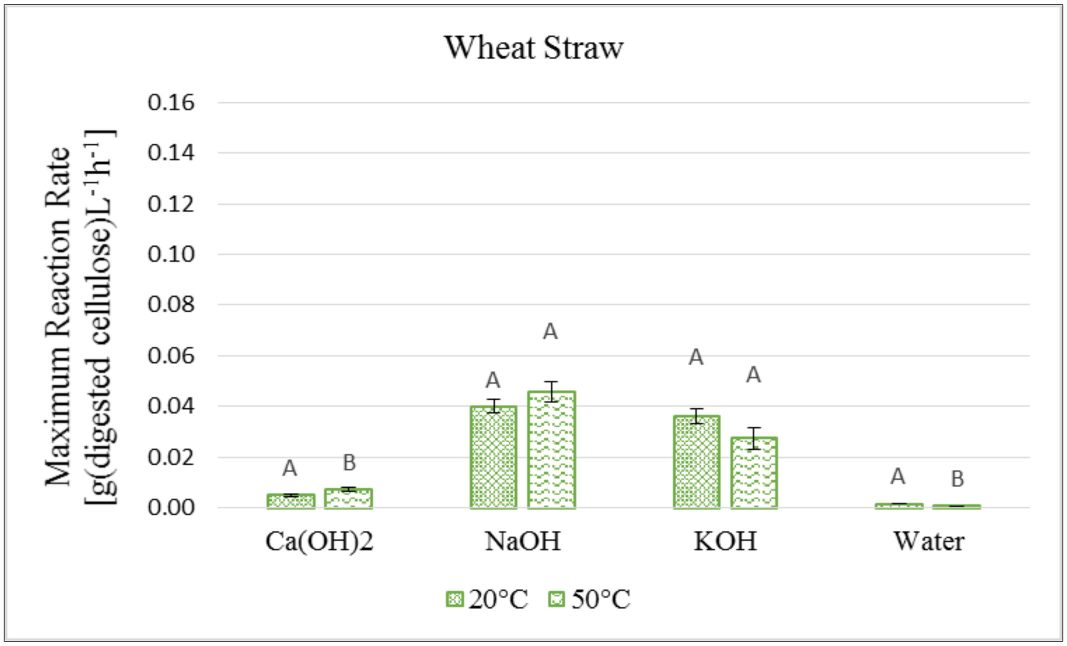


Figure 4.29. Pretreatment temperature effect on maximum rate of digested cellulose of wheat straw pretreated with three alkali and water control. Error bars are the standard deviations of three replications.

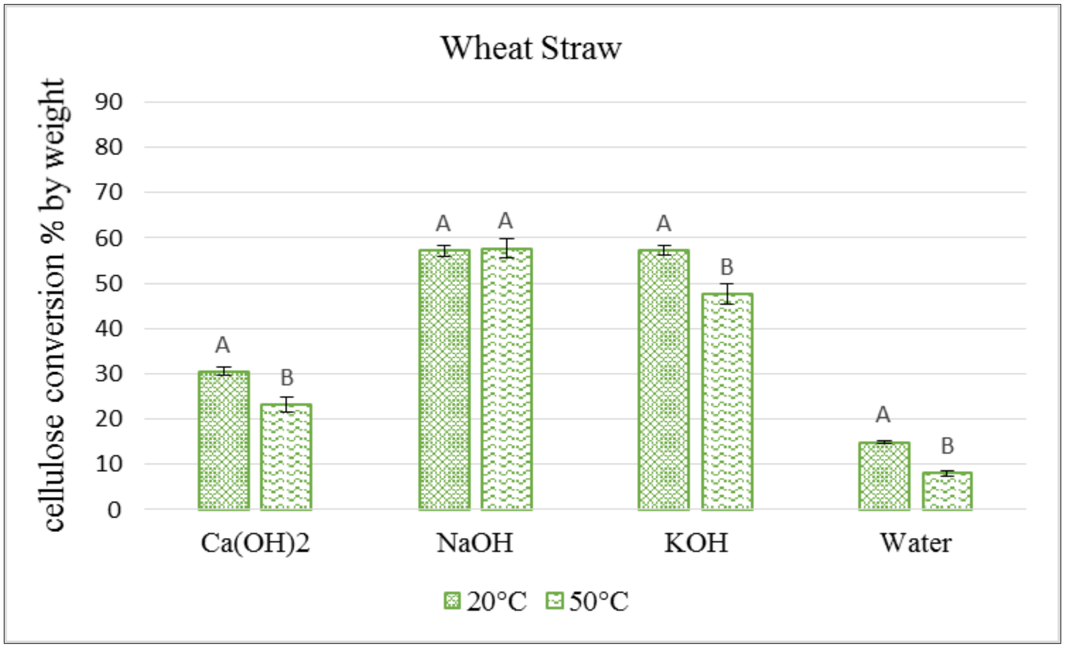


Figure 4.30: Pretreatment temperature effect on cellulose digestion of wheat straw due to enzymatic hydrolysis. Error bars are the standard deviations of three replications.

4.3.7 Effect of Pretreatment Temperature on Hydrolysis of Switchgrass

Samples of switchgrass that were pretreated at 20°C with calcium and potassium hydroxides had a higher cellulose conversion compared to pretreatment at 50°C. However, sodium hydroxide was not significantly influenced between temperature levels (Figure 4.31). Considering only alkali pretreated samples, temperature had the greatest influence with the calcium hydroxide pretreated samples where decreasing the temperature increased the velocity of degradation by a factor of 2.57 times (Figure 4.32). With respect to control samples pretreated at 50°C, the measured reaction rate was negligible. Lower pretreatment temperatures also increased the velocity of degradation with potassium hydroxide by a factor of 1.66.

Sodium hydroxide samples that were pretreated at 20°C resulted in a faster cellulose digestion rate after 72 hours. However, no statistically significant difference due to temperature effect was observed with respect to cellulose conversion (Figure 4.32). Control samples also did not show statistically significant difference in cellulose conversion due to temperature severity. For calcium and potassium hydroxides, the results for conversion rate followed the tendency observed in maximum reaction rate since samples pretreated at 20°C conducted to statistically higher conversions. The quantity of cellulose converted followed the trends in delignification observed with pretreatment.

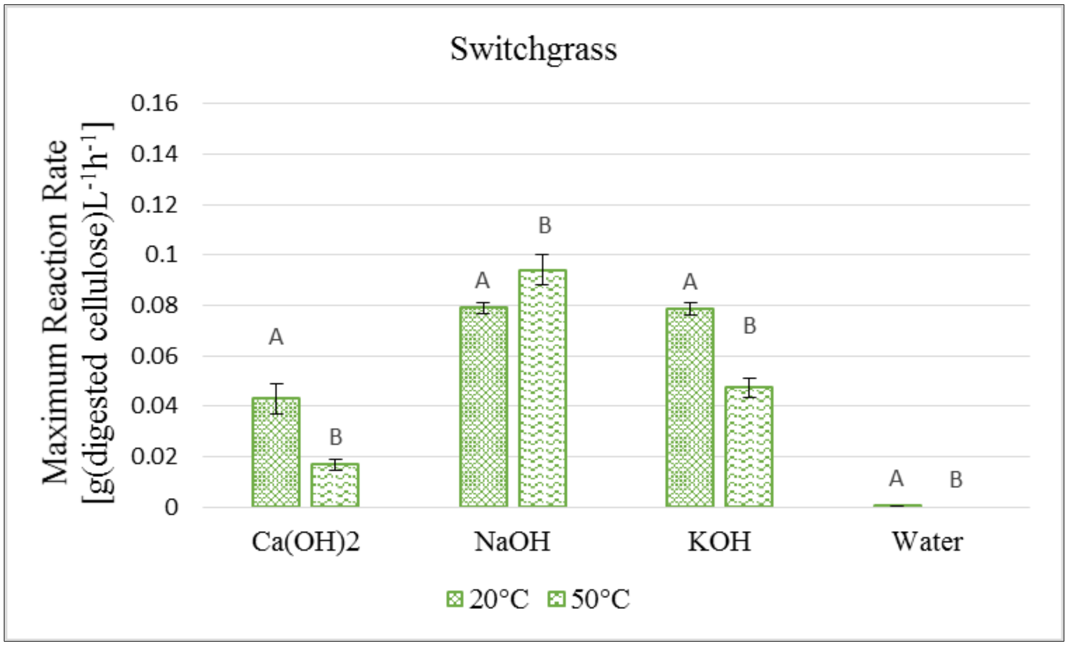


Figure 4.31: Pretreatment temperature effect on maximum rate of digested cellulose of switchgrass pretreated with three alkali and water control. Error bars are the standard deviations of three replications.

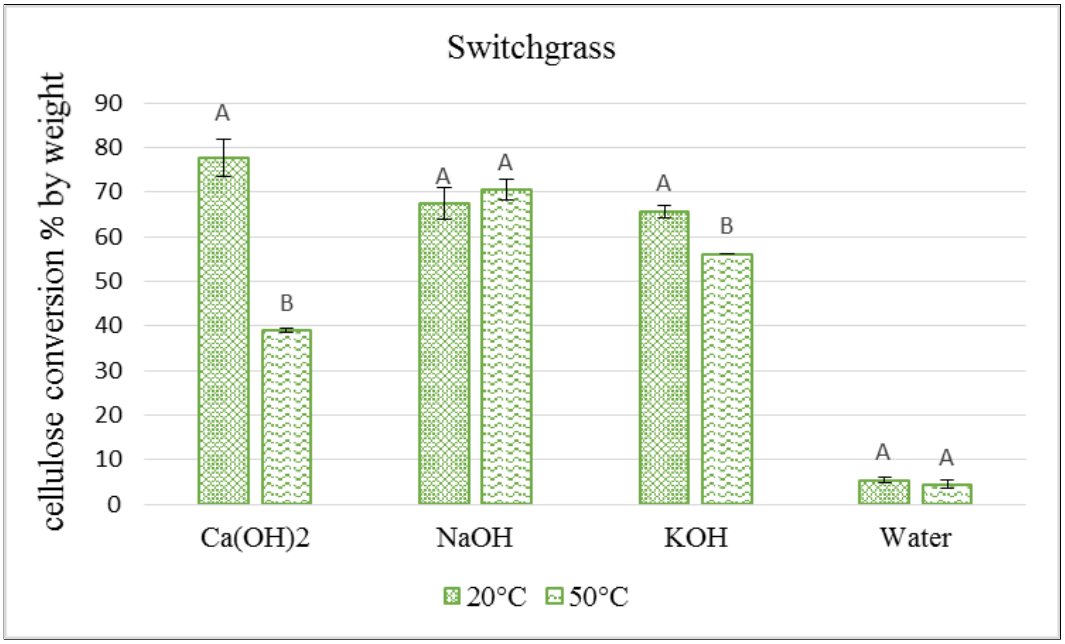


Figure 4.32: Pretreatment temperature effect on cellulose digestion of switchgrass due to enzymatic hydrolysis. Error bars are the standard deviations of three replications.

4.3.6 Effect of Pretreatment Temperature on Hydrolysis of Miscanthus

Regarding the effect of pretreatment temperature on hydrolysis of miscanthus, the results showed a positive interaction due to temperature for samples pretreated with sodium and potassium hydroxides. Significant increases in the hydrolysis reaction rate were observed when the temperature was increased from 20 to 50°C (Figure 4.33). The higher pretreatment temperature increased the reaction by approximately 2.48 times for sodium and potassium hydroxides. On the other hand, calcium hydroxide pretreated samples showed to degrade cellulose 2.13 times faster in a 20°C environment relative to a pretreatment temperature of 50°C. Control samples did not show a significant difference in reaction rate due to the different temperature conditions.

Similar trends were observed for the percentage of cellulose converted into glucose with each pretreatment agent due to pretreatment temperature with miscanthus (Figure 4.34). After 72 hours of hydrolysis, 22.0% and 19.2% more cellulose was degraded in samples pretreated with sodium and potassium hydroxides, respectively, at 50°C in comparison with those pretreated at 20°C.

Although the results for cellulose conversion percentage with sodium and potassium hydroxides followed the measured maximum hydrolysis reaction rate, calcium hydroxide did not follow the same trend.

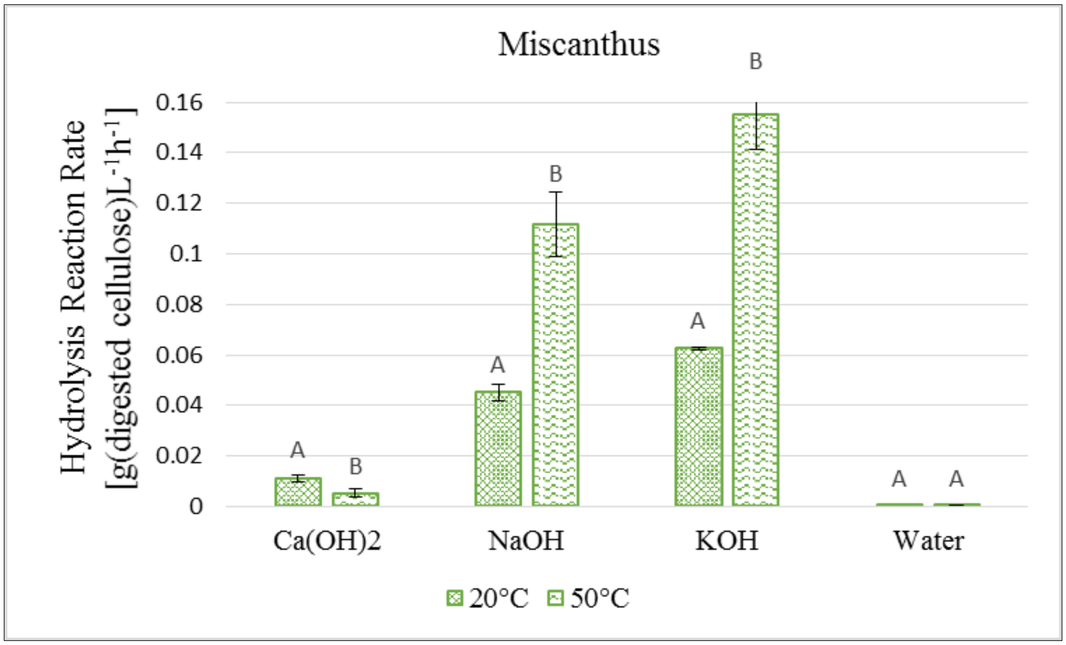


Figure 4.33: Pretreatment temperature effect on maximum rate of digested cellulose of miscanthus pretreated with three alkali and water control Error bars are the standard deviations of three replications.

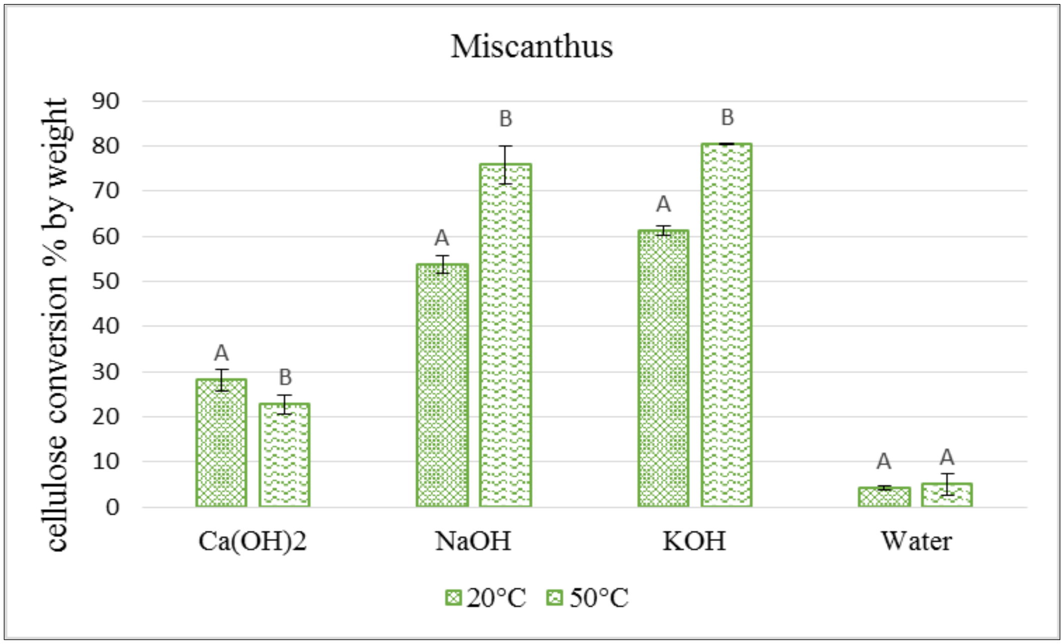


Figure 4.34: Pretreatment temperature effect on cellulose digestion of miscanthus due to enzymatic hydrolysis. Error bars are the standard deviations of three replications.

4.4 Economic and Environmental Viability of Alkali Pretreatment

4.4.1 Economic Viability

The economic viability of the three pretreatment chemicals and four biomass types investigated was determined in terms of U$ dollars per gram of glucose released as following:

$$\frac{CH}{SR * C * G} * \frac{U\$\ dollars}{CH}$$

Where CH is unit mass of chemical, SR is unit mass of solids recovered after pretreatment, C is the unit mass of cellulose after pretreatment, G is unit mass of cellulose converted to glucose expressed as a percentage.

Currently prices per kilogram of potassium, calcium and sodium hydroxides are U$1.00, U$0.40, and U$0.35, respectively (Alibaba, 2015). The quantity of solids recovered after pretreatment with corn stover, wheat straw, switchgrass, and miscanthus was calculated based on the solids yields percentage shown in Table 4.7, and on the initial amount of solids used in the pretreatment step (10 g dry matter). The chemical loadings used in this study were 0.1g Ca(OH)2/g biomass, 0.15 NaOHg/g biomass, and 0.11 KOHg/g biomass, and the total cost per unit mass glucose production is shown in Figure 4.35.

Biomass pretreatment with sodium hydroxide produced the lowest cost per unit mass of glucose with corn stover, wheat straw and miscanthus at the two pretreatment temperatures and with switchgrass at 50°C. Calcium hydroxide pretreatment led to the lowest cost per unit mass of glucose released with switchgrass when pretreated at 20°C. Potassium hydroxide showed to be the highest cost per unit of glucose produced.

Table 4.7. Solids recovery percentage after pretreatment.

Treatment	Solids Yields (%)[a]							
	corn stover		**wheat straw**		**switchgrass**		**miscanthus**	
	20°C	**50°C**	**20°C**	**50°C**	**20°C**	**50°C**	**20°C**	**50°C**
Ca(OH)2	75.7	70.1	76.4	80.8	67.2	70.8	79.6	74.3
NaOH	63.7	56.0	76.4	81.4	62.8	58.1	66.8	66.1
KOH	63.4	60.0	78.3	80.9	62.0	63.6	71.7	64.4

[a] Solid yield is shown as percentage of the initial amount of dry matter.

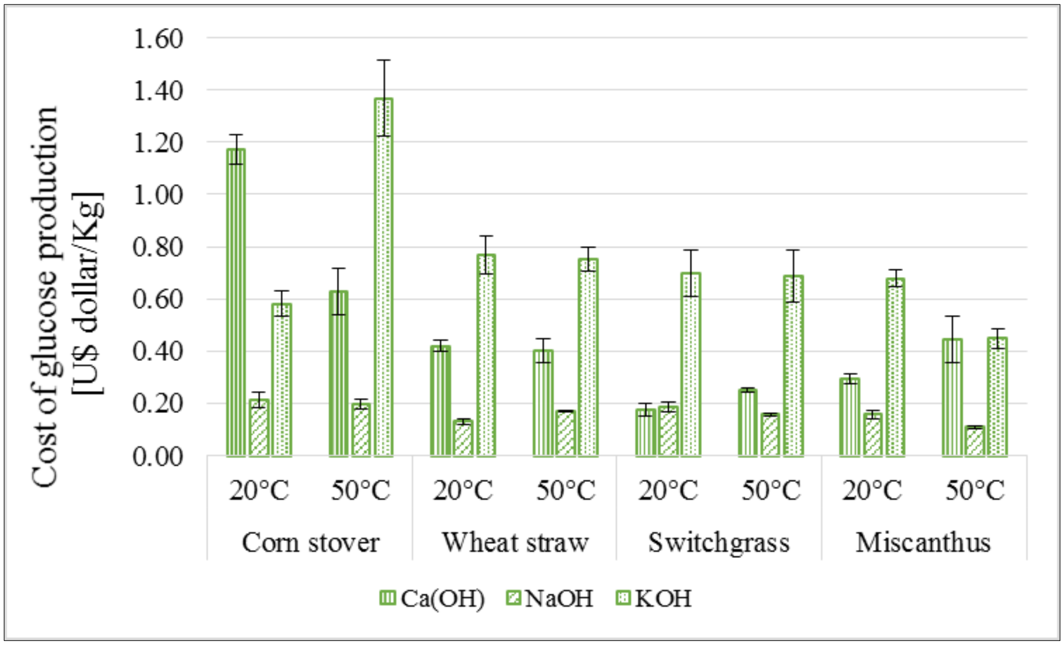


Figure 4.35: Cost of glucose production after enzymatic hydrolysis from pretreated biomass with three alkali and at two temperatures. Error bars are the standard deviation of three replications.

4.4.2 Carbon Dioxide (CO_2) Emissions

The carbon dioxide emissions from the three pretreatment chemicals and four biomass types investigated were estimated in terms of grams of carbon dioxide (CO_2) per gram of glucose released using:

$$\frac{CH}{SR * C * G} * \frac{CO_2}{CH}$$

Where CH is unit mass of chemical, SR is unit mass of solids recovered after pretreatment, C is the unit mass of cellulose after pretreatment, G is unit mass of cellulose converted to glucose expressed as percentage, and CO_2 is unit mass of carbon dioxide emitted.

The CO_2 emissions for sodium hydroxide and calcium hydroxide used were 0.074g CO2/g NaOH, and 0.078g CO2/g $CaOH_2$, respectively (ASSOCIATES, FRANKLIN, 2011) Potassium hydroxide was assumed to have equivalent emission rate to sodium hydroxide (ASSOCIATES, FRANKLIN, 2011).

Biomass pretreated with calcium hydroxide emitted the highest levels of carbon dioxide per unit mass of glucose produced after hydrolysis of corn stover, wheat straw and miscanthus at the two pretreatment temperatures. In the worst scenario, calcium hydroxide led to 80% higher carbon dioxide emissions than sodium hydroxide or potassium hydroxide with corn stover pretreated at 20°C. Potassium hydroxide, which in general showed to be the most environmental friendly chemical in terms of CO_2 emissions, released up to 0.039 grams of carbon dioxide per kilogram of glucose released. Sodium hydroxide was the best choice chemical in terms of carbon dioxide emissions, only with corn stover pretreated at the highest temperature investigated.

Due to its low glucose recovery after enzyme hydrolysis, calcium hydroxide pretreatment consistently led to the highest carbon dioxide emissions. The only exception was during the pretreatment of switchgrass at 20°C where the CO2 emissions were the lowest of the three chemicals.

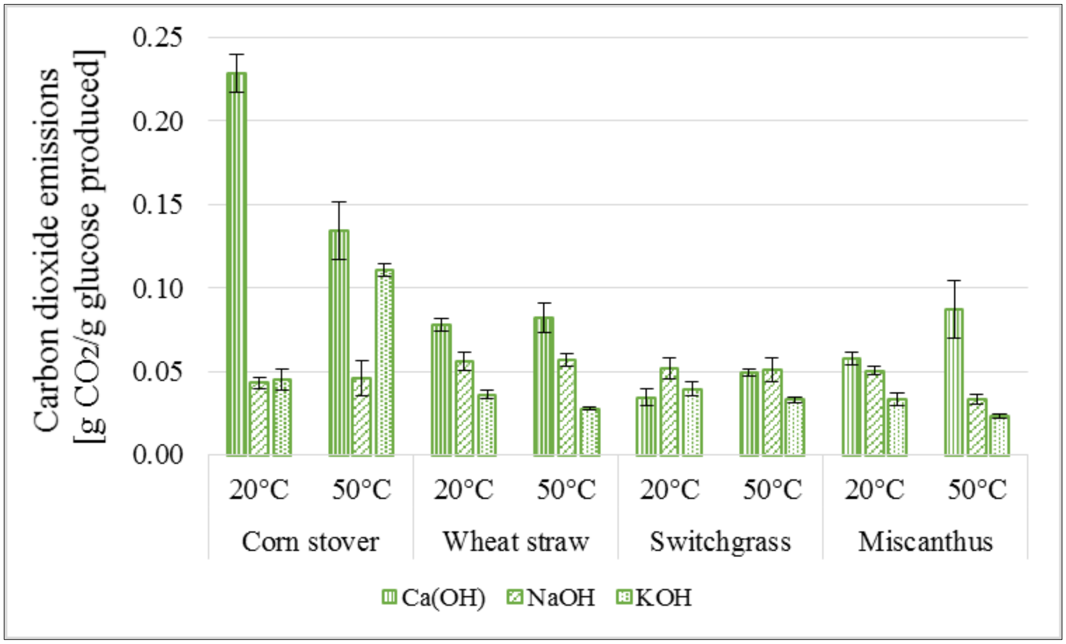


Figure 4.36: Carbon dioxide emission per unit mass of glucose produced due to hydrolysis of pretreated biomass with three alkali and at two temperatures. Error bars are the standard deviation of three replications.

Chapter Five: DISCUSSION

5.1 Biomass Composition

The composition of the biomass used in this study was similar to other literature results. In research conducted by Sharma et al. (2013), the carbohydrate and total lignin (acid soluble and acid insoluble) portions of switchgrass were estimated to be 67.3% and 24.7%, respectively. These contents are comparable to 67.2% carbohydrates and 29.8% lignin reported in this study.

McIntosh & Vancov (2011) reported wheat straw had a composition of cellulose content of 36.0%, hemicellulose content of 26.0%, and an acid insoluble lignin content of 7.6%. Duguid et al. (2007) found a cellulose, hemicellulose, and acid-insoluble lignin content of 33.4, 21.4, and 17.2%, respectively. Although the cellulose and hemicellulose content match the ones used in this study for cellulose and hemicellulose, the lignin content from this study was considerably higher. The straw used in this study was baled and contained a high percentage of whole stalks that have been shown to be higher in lignin relative to the leaves and chaff.

Composition of corn stover showed some variation in terms of hemicellulose and lignin contents from previous work by McDonald et al. (1983), who reported 28.5% and 13% of hemiellulose and lignin contents, respectively. Duguid et al. (2007) found a cellulose, hemicellulose, and lignin content of 36, 18, and 19%, respectively for corn stalks. The corn stover obtained in this study was obtained by using a small square baler after grain harvest and would likely contain a large amount of stalks with a potentially higher lignin content.

5.2 Effect of Pretreatment on Biomass Composition

A number of issues complicate the direct comparison of the results from this study to other studies in the literature. Solids content during pretreatment (g biomass/g of total material), alkali loading (g hydroxyl/g biomass), time, and temperature all influence pretreatment (Rabelo, 2010; Sirohi & Rai, 1998; Nlewem & Thrash Jr., 2010; Kaar & Holtzapple, 2000). Lastly, the mass of biomass prior to pretreatment and after pretreatment are not equal. Some solids are lost during pretreatment making direct comparisons difficult. The results from this study are different from other results presented in the literature, but follow similar trends reported.

Most pretreatments have been developed under low-solids conditions, where pretreatments are most effective (Modenbach & Nokes, 2012). This study utilized high solids that likely reduced the effectiveness of the pretreatments relative to other literature data. This was done to aid in the development of on-farm biomass processing systems where a high solids content is desirable.

5.2.1 Lignin Changes Due to Pretreatment

Potassium and sodium hydroxide performed similiarly in this study, that matched previous studies. Anderson & Ralston (1973) found that there was no difference in delignification between sodium and potassium hydroxide when pretreating rye straw with 0.3 g of chemical/g biomass for 48 h at room temperature. Bales et al. (1979) found that NaOH and KOH had equivalent effect in terms of delignification on a molar basis on milo stalks. Delignification of corn stover with 0.075 g of sodium hydroxide per g of biomass for 24 hours at 20°C resulted in a 46.2% decrease in lignin with a solids content of 50% (Zhu, Wan, & Li, 2010b). Their results were very similar to this study where a 40% decrease in lignin was observed for corn stover.

Although the results obtained in this project show the contrary, Chang et al. (1997) stated that calcium hydroxide has been shown to be as effective as other alkali in improving lignocellulose digestibility when pretreatment conditions compatible with lime were applied. A major difference between their study and this study was the pretreatment conditions used (120°C for 2 hour) and a low solids content (10%). They observed a 29.4% loss in total lignin under their pretreatment conditions with switchgrass (Chang, Burr, & Holtzapple, 1997) that was similar to the 13.1 to 21.3% delignification found in this study.

Calcium hydroxide features low alkalinity and solubility in water (Chang, Burr, & Holtzapple, 1997). Calcium hydroxide solubility is 0.173 and 0.133 grams in 100 ml of water at 20°C and 50°C, respectively. Potassium hydroxide and sodium hydroxide, in comparison, solubilize, respectively, 117.4 and 111.0 grams in 100 ml of water at 20°C and, 138.3 and 106.4 grams at 50°C. Because of calcium hydroxide’s features, it is believed that samples treated with this alkali require longer residence time to achieve the same lignocellulose digestibility rate as samples treated with either sodium (Winugroho, Ibrahim, & Pearce, 1984) or potassium hydroxides. In the interest of lignin reduction specifically, under alkaline conditions, calcium ions tend to link to lignin forming a calcium-lignin complex, which prevents an intense lignin solubilization during pretreatment (Xu J. , Cheng, Sharma-Shivappa, & Burns, 2010a). The time and solids content of the pretreatment conditions in this study likely limited the delignification observed with biomass pretreated with calcium hydroxide.

5.2.2 Cellulose Changes Due to Pretreatment

The overall change in cellulose content obtained in this study share some of the reported values in the literatures. Chang, Burr, & Holtzapple (1997) reported a slight increase in cellulose content when switchgrass was pretreated with lime at 120°C for 2 h in a liquid slurry. Digman et al. (2010) also found higher cellulose, approximately 3.5%, for switchgrass after pretreatment with 0.05 g lime/g biomass for 60 days under anaerobic conditions at 22°C.

Despite the positive changes in cellulose content achieved in this study after pretreatment, there were cases where the cellulose content did not increase with calcium hydroxide. Switchgrass pretreated with calcium hydroxide at 20°C did not show an increase in cellulose content. This could have been due to the intense washing process that the samples went through to reduce the pH to an acceptable level. Samples pretreated with calcium hydroxide required at least three times the volume of water used for the other samples to reach the desired pH value required to perform enzyme hydrolysis.

The data from this research followed the general trend between delignification and increased cellulose content expected due to degradation of the lignocellulose matrix.

5.2.3 Hemicellulose Change Due to Pretreatment

Overall, changes in hemicellulose were not significant from the untreated feedstock, which matched previous research. Chang, Burr, & Holtzapple (1997) and Xu et al. (2010b) attributed the low susceptibility of hemicelluloses to alkaline attack to the high solubilization of hemicellulose.

5.3 Effect of Temperature on Biomass Composition

5.3.1 Lignin Changes Due to Temperature Effect

The majority of the results obtained in this study did not show a significant difference in delignification due to pretreatment temperature. The results obtained with this study did not follow the trend observed by Kim & Holtzapple (2005) and by Xu et al. (2010b). The first reported an increase of 30% in delignification of corn stover pretreated with an excess of calcium hydroxide (0.5g $Ca(OH)_2$/ g raw biomass) for 6 weeks at 55°C in comparison with 25°C. Xu et al. (2010b) pretreated switchgrass with sodium hydroxide at 2% w/v and found 14.9% higher delignification at 50°C in comparison with 21°C. One possible reason for this may be due to the lower alkali loading utilized in this study, the higher solids content in this study, and the influence of pretreatment time.

McIntosh & Vancov (2011) found that the reaction temperature was not a factor in delignification when low loadings of NaOH were used to pretreat wheat straw. In addition, Silverstein et al. (2007) pretreated cotton stalks with sodium hydroxide and concluded that increasing the temperature only enhanced the lignin removal for longer times and higher concentrations of chemical. Moreover, limited lignin reductions that were similar to this study were also reported by Xu et al. (2010a) with lime pretreatment of switchgrass utilizing 0.1 g calcium hydroxide/g raw biomass in a slurry.

5.3.2 Cellulose and Hemicellulose Changes Due to Temperature Effect

The effect of pretreatment temperature on the cellulose and hemicellulose changes were expected to follow the trend observed for delignification. Removal of lignin has a direct influence on the cellulose and hemicellulose available in the pretreated solids (Modenbach & Nokes, 2012; Mosier, et al., 2005). The measured results, however, showed a mixed trend. One plausible explanation for that lies in the loss of solids during pretreatment, which could skew the composition results. It was impossible to recover all of the biomass solids after pretreatment. The chemical composition of the solids lost when transferring between flasks and the soluble material removed during washing were not quantified.

5.4 Enzymatic Hydrolysis

The trend observed for maximum reaction rate from all four feedstocks suggested a relationship between delignification rate (Figure 4.1) and hydrolysis reaction rate (Figure 4.25) for all circumstances analyzed. This relationship follows the conventional wisdom on the role of pretreatment (Modenbach & Nokes, 2012; Mosier, et al., 2005) . With the breakage of the linkages in the lignocellulosic matrix, and the release of the lignin barrier, more cellulose is free to be easily accessed by the enzymes, and so, a faster, more complete conversion occurred.

The cellulose converted in this study was similar to the carbohydrate conversions found by Xu et al. (2010a), Sharma et al. (2013), and Xu et al. (2010b), who also reported different performances with calcium hydroxide, potassium hydroxide, and sodium hydroxide, respectively, for similar hydrolysis conditions. However, looking at those three studies, potassium hydroxide appeared to have the best performance.

The cellulose conversion from corn stover and switchgrass pretreated with calcium hydroxide were somewhat contradictory. It appeared that calcium hydroxide was effective with switchgrass, but did not have a significant difference from the controls with corn stover. Although, both feedstocks had a very similar rate of delignification after pretreatment, but vastly different cellulose conversion.

Glucose yields from untreated switchgrass and switchgrass pretreated with lime for 2 h at 120°C were 12.3 and 58.0%, respectively (Chang, Burr, & Holtzapple, 1997). The yields from the water control and calcium hydroxide pretreatment in this study were approximately 50% lower. This could have been due to inhibitors in the pretreatment solution, differences in enzymes (loading, activity, types of enzymes, etc.), and differences in pretreatment conditions (solids content, time, and temperature).

Results from this study had a similar measured reaction rate with Chang et al. (1997). Chang et al. (1997) concluded that since calcium hydroxide led to lower reaction rates than sodium and potassium hydroxides, a lower conversion was expected for samples pretreated with calcium hydroxide. However, in order to make a fair comparison of the effectiveness of these three alkali as pretreatment agent for bioconversion of lignocellulosic feedstock, residence time compatible with calcium hydroxide reaction rate should be considered.

5.4.1 Effect of Temperature of Pretreatment on Enzymatic Hydrolysis

Overall, the pretreatment temperature had a mixed effect on cellulose conversion. Some conditions achieved better conversion rates with the lower pretreatment temperature, which is in agreement with the study by Sharma et al. (2013). They found higher sugar yields with a lower pretreatment temperature for switchgrass pretreated with 0.5% potassium hydroxide for 24 hours.

Under some conditions, higher conversions with higher temperatures were attained in this study. Kim & Holtzapple (2005) reported higher yields of glucose from corn stover pretreated at higher temperatures with an excess of calcium hydroxide (0.5g $Ca(OH)_2$/ g raw biomass). Xu et al. (2010a) investigate pretreatment of switchgrass with calcium hyroxide at 21, 50, and 121°C and found the 50°C condition optimal in terms of enzymatic hydrolysis.

In general, the maximum reaction rate increased with increased pretreatment temperature. However, this trend did not follow in terms of cellulose conversion. A higher pretreatment temperature did not always increase the amount of cellulose converted to glucose. This anomaly could be attributed to the hydrolysis residence time, which may not allowed for maximum conversion. Other factors could have also influenced the cellulose conversion, such as mixture and activity of the cellulase’s and hemicellulase’s, contamination, solids recovery after pretreatment, sampling errors, and measurement errors.

5.5 Economic and Environmental Viability

Although calcium hydroxide is the least expensive chemical per unit mass of hydroxide, sodium hydroxide resulted in the lowest cost of glucose released in this study. Playne (1984) concluded the opposite. In his study conducted on sugarcane bagasse pretreated with alkali (NaOH, NH_3 (aqueous), NaOH + NH_3, $Ca(OH)_2$, and $Ca(OH)_2$ + Na_2CO_3) at ambient temperature and in combination with steam explosion at 200°C, 6.9 MPa, and 5 min cooking times, he concluded that lime was the least expensive chemical per unit of additional digestible organic matter obtained. These differences were likely due to the stream explosion and other conditions during pretreatment used by Playne (1984).

The environmental analysis done for this study was a simplified study to evaluate potential environmental concerns with the pretreatment chemicals. In terms of waste disposal, calcium hydroxide could be more advantageous because it can be recovered from an aqueous reaction system as insoluble calcium carbonate by neutralizing with carbon dioxide. Subsequently, calcium hydroxide can be regenerated using lime kiln technology (Kaar & Holtzapple, 2000; Carvalheiro, Duarte, & Gírio, 2008). In addition, agricultural fields require large amounts of lime (calcium carbonate) to control soil pH. Reusing the calcium from the pretreatment could lead to lower CO2 emissions and costs if methods were developed to utilize the residual calcium and lignin. Calcium ions tend to crosslink lignin molecules (Xu J. , Cheng, Sharma-Shivappa, & Burns, 2010a) which could lead to benefits as a soil amendment.

Byproducts from potassium hydroxide could also have value as a soil fertilizer. Farmers apply a considerable quantity of potassium chloride every year to manage soil fertility. If the residual potassium was in a plant useable form, or could be converted into one, the cost and environmental impact of potassium hydroxide could be lessened.

Although, sodium hydroxide had the lowest cost per gram of glucose released, it could potentially add to salinity issues and treated products frequently contain little nitrogen (Bales, Kellogg, & Miller, 1979). There could be significant additional environmental impacts with sodium hydroxide that were not considered.

In terms of water consumption, calcium hydroxide was the worst choice since it required at least three times the volume of water for washing relative to the sodium and potassium hydroxide. Although water itself is not expensive, there are environmental concerns with cleanup and disposal after it was used for washing. The results underlined the importance of high glucose recovery from the pretreated solids to minimize costs and potential environmental impacts associated with biomass pretreatment.

Chapter Six: SUMMARY AND CONCLUSIONS

6.1 Composition of Biomass

The change in composition of lignin, cellulose, and hemicellulose of four biomass feedstocks (switchgrass, wheat straw, corn stover, and miscanthus) before and after pretreatment with calcium hydroxide, potassium hydroxide, and sodium hydroxide were measured. All feedstocks were tested at two temperature levels (20 and 50°C), with a constant hydroxyl loading of 0.02701 mol $(OH)^-$ per gram of biomass, and a solids loading of 0.4 g biomass/g of total material. Sodium hydroxide and potassium hydroxide had similar performance in terms of delignification ranging between 20.9 to 48.3%. Calcium hydroxide was statistically lower and reduced the lignin by 6.9 to 21.3%.

Calcium hydroxide increased the cellulose content of the pretreated solids from 0 to 15.0% and often was statistically not different from the control of water only. In many cases, sodium and potassium hydroxide had a slightly higher cellulose content after pretreatment, but was frequently statistically not different from calcium hydroxide.

Overall, no significant changes were observed in hemicellulose content compared to the untreated biomass. For the few cases where there was an increase in hemicellulose content, sodium hydroxide was the pretreatment agent responsible, with exception for miscanthus pretreated at 50°C. Sodium hydroxide and potassium hydroxide were also similar in removing ash in the majority of the scenarios analyzed. They performed better than calcium hydroxide, which did not differ from the control in most cases.

6.2 Enzymatic Hydrolysis

Yields of glucose after pretreatment with the three chemicals and the four biomass types were quantified as a result of enzymatic hydrolysis, and ranged from 1.16 g/L (Corn stover pretreated with calcium hydroxide at 20°C) to 9.04 g/L (Corn stover pretreated with potassium hydroxide at 20°C. Cellulose conversion from calcium hydroxide pretreated material ranged from 10.5 to 77.6%. Sodium hydroxide had a cellulose conversion between 52.2 to 75.8%, while potassium hydroxide varied between 31.4 and 81.4%. In 50% of the cases studied, potassium and sodium hydroxide yielded equivalent glucose concentration.

6.3 Economic and Environmental Viability

Sodium hydroxide, in general, resulted in the lowest cost per gram of glucose released. The lowest cost scenario ($0.11/g glucose) was miscanthus pretreated at 50°C with sodium hydroxide. With sodium hydroxide the highest cost scenario was with corn stover at 20°C where the chemical cost was $0.21 per g glucose. Costs associated with calcium hydroxide were up to $1.18/g glucose for corn stover pretreated at 20°C.

Concerning environmental issues such as carbon dioxide emissions to the atmosphere and water consumption, pretreatment with potassium hydroxide had the best overall performance, although sodium hydroxide was similar in many of the cases studied. It released into the atmosphere 0.2 times the amount of CO_2 emitted with calcium hydroxide per unit mass of glucose produced due to hydrolysis of pretreated corn stover at 20°C, and consumed three times less volume of water during the washing step.

6.4 Overall Conclusion

There was considerable variation in the performance of the source of alkaline pretreatment chemical on biomass (switchgrass, wheat straw, corn stover, and miscanthus) based on compositional changes after pretreatment, sugar yield after enzyme hydrolysis, cost, and potential environmental impacts varied with the temperature of pretreatment and with the feedstock studied.

Under the pretreatment and hydrolysis conditions adopted in this study, potassium hydroxide showed to be the best option for the pretreatment of corn stover at 20°C, and sodium hydroxide at 50°C. In wheat straw pretreated at 20°C, potassium hydroxide was as effective as sodium hydroxide, which had the best performance at 50°C. Sodium hydroxide was also the most effective alkaline compound in the pretreatment of switchgrass at 50°C. At 20°C, however, calcium hydroxide was the most successful pretreatment agent afterwards. With miscanthus, sodium and potassium hydroxides had equivalent performance at both pretreatment temperatures.

However, in order to make a fair comparison of effectiveness of calcium hydroxide, sodium hydroxide, and potassium hydroxide as pretreatment agents, pretreatment conditions compatible with all three alkali must be considered. Pretreatment conditions optimal for calcium hydroxide were not used in this study that lowered the effectiveness relative to sodium and potassium hydroxide.

Chapter Seven: FUTURE WORK

Numerous opportunities for further research could be performed based on the results obtained from this study. One opportunity for future work could be the optimization of conditions for calcium hydroxide pretreatment, development of methods to increase xylose production, quantifying soluble chemicals lost during washing, a deeper analysis of potential use of waste products to decrease costs and environmental issues, and large scale testing.

At first, in order to make a fair comparison of the three hydroxyl sources investigated in this study, it is crucial that the experimental procedure is adjusted to match the optimal pretreatment and hydrolysis condition of all pretreatment agents evaluated. Although the data in this study showed the contrary, calcium hydroxide has been proven to be as efficient as sodium hydroxide and potassium hydroxide where pretreatment and hydrolysis conditions compatible with its needs were considered.

Another aspect to consider is the use of an enzyme complex with higher xylanase activity. The experimental data did not show significant quantities of xylose production due to hydrolysis with the commercial enzyme used. Adjustments should be made to the cellulase complex used to improve the xylanase activity and so increase total sugar yields.

Another aspect would be the evaluation of the soluble chemicals lost during the washing step. This would allow for an accurate analysis of the change in composition of the lignocellulosic compounds. Thus, the pretreatment effectiveness could be better evaluated. In addition to a clearer understanding of the relationship between delignification and carbohydrate conversion.

Despite the efficiency of biofuel production from renewable resources, it is essential the production of the biofuel is commercially and environmentally feasible compared to oil. To achieve an economically viable process, the production cost must decrease to approach the corresponding cost for fossil fuels. By looking at a use for waste products, the cost associated for disposal could be reduced, and so the production cost. Moreover, a functional use of waste products would contribute to diminished environmental impacts.

Finally, this study is part of a project that aims to develop on-farm biomass processing systems where a large production volume is desirable. Scaling up the experiments investigated in this study would give an idea of the feasibility of the alkali pretreatments studied in an overall system analysis of biofuel production from agricultural residues and dedicated energy crops.

APPENDICES

Appendix A: Moisture Content

The moisture content (M) is defined as the direct relationship between the mass of water present in a sample (m_{H2O}) and the total mass of the sample, in other words, the mass of water plus the dry matter (m_{H2O} + dm). The equation below represents the definition of moisture content.

$$M = \frac{m_{H2O}}{m_{H2O} + dm}$$

To calculate the amount of water required to achieve a moisture content of 60%, equation 1 was rearranged into equation 2.

$$m_{H20} = \frac{dm * M}{(1 - M)}$$

Thus, making M = 0.60,

$$m_{H2O} = 1.5 * dm$$

which was the equation employed in the pretreatment step of the experimental procedure of this project.

Appendix B: Determination of Appropriate Quantity of Alkali Compound Applied in Each Pretreatment Method

Molar mass of the individual elements and molecules (Smith, 2011):

Ca:40.0780 g*mol^{-1}

K:39.0893 g*mol^{-1}

Na:22.9898 g*mol^{-1}

O: 15.9994 g*mol^{-1}; O_2 = 31.9988 g*mol^{-1}

H: 1.00794 g*mol^{-1}; H_2 = 2.01588 g*mol^{-1}

$(OH)^-$: 17.00734 g*mol^{-1}

Molar mass of chemical compounds:

$Ca(OH)_2$: 74.09268 g*mol^{-1}

KOH: 56.09664 g*mol^{-1}

NaOH: 39.99714 g*mol^{-1}

Calculations:

Dissociation equation for calcium hydroxide:

$$Ca(OH)_{2_{(s)}} \rightarrow Ca^{2+}{}_{(aq)} + 2(OH)^{-}{}_{(aq)}$$

Stechiometry:

1 mol $Ca(OH)_2$ ——— 2 mol $(OH)^-$

74.09268 g $Ca(OH)_2$ ——— 34.01468 g $(OH)^-$

X g $Ca(OH)_2$ ——— Y g $(OH)^-$

According to (Chang, Burr, & Holtzapple, 1997; Xu J. , Cheng, Sharma-Shivappa, & Burns, 2010a), an ideal loading rate would be 0.1 g chemical* g biomass^{-1}. Thus, 1 g of chemical is required per each 10 g biomass. Taking calcium hydroxide as base for calculation, for X = 1.00000 g $Ca(OH)_2$, Y = 0.45908 g $(OH)^-$.

In order to equalize the concentration of hydroxyl per unit mass of biomass (mole/g) in all treatments, the loading amount of potassium and sodium hydroxides were calculated to contain 0.45928 g $(OH)^-$, or 0.02701 mol $(OH)^-$, in each, as following exemplified.

Potassium Hydroxide:

$$K(OH)_{(s)} \rightarrow K^{+}{}_{(aq)} + \ (OH)^{-}{}_{(aq)}$$

Stechiometry:

1 mol KOH ——— 1 mol $(OH)^-$

56.09664 g KOH ——— 17.00194 g $(OH)^-$

1.51470 g KOH ——— 0.45908 g $(OH)^-$

Sodium Hydroxide:

$$Na(OH)_{(s)} \ \rightarrow \ Na^{+}{}_{(aq)} + \ (OH)^{-}{}_{(aq)}$$

Stechiometry:

1 mol NaOH ——— 1 mol $(OH)^-$

39.99714 g NaOH ——— 17.00194 g $(OH)^-$

1.07999 g NaOH ——— 0.45908 g $(OH)^-$

In conclusion, the quantities of calcium, potassium, and sodium hydroxides applied to each specific pretreatment were 1.00000 g, 1.51470 g, and 1.07999 g, respectively.

Appendix C: Maximum Reaction Rate for Enzymatic Hydrolysis of Biomass

The experimental results were analyzed based on Michaelis Mendem equation by assuming that the cellulose-cellulase system is an uncomplicated one-substrate reaction. It was also assumed a noninhibition mechanism.

Moreover, the linear Lineweaver-Burk method was used to calculate the maximum reaction rate as following (Fan, Gharpuray, & Lee, 1987):

Michaelis Mendem equation, $-r = \frac{V_{max} * S}{K_m + S}$, and mole balance for batch reactor in liquid phase, $-r = -\frac{dS}{dt}$ were combined, rearranged, and integrated to get $t = \frac{K_m}{V_{max}} ln \frac{S_0}{S} + \frac{S_0 - S}{V_{max}}$.

Dividing both sides by $t * K_m / V_{max}$, yields

$$\frac{1}{t} ln \frac{S_0}{S} = \frac{V_{max}}{K_m} - \frac{S_0 - S}{K_m * t}$$

Where t is time; S_0 is the concentration of cellulose digested at time zero; S is the concentration of cellulose digested at time t; K_m is the Michealis Mendem constant, and V_{max} is the maximum reaction rate.

Plotting

$$\frac{1}{t}\ln\frac{S_0}{S} \text{ against } \frac{S_0 - S}{t}$$

gives as intersection and slope

$$\frac{V_{max}}{K_m} \text{ and } \frac{1}{K_m}, \text{respectively}$$

Thus, once the Michaelis Mendem constant was calculated using the scope value, the maximum reaction rate could be determined by multiplying the intersection value by the Michaelis Mendem constant.

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