ABSTRACT

ANSANAY, YANE OKTOVINA. Niobium Oxide Catalyst for Delignification of Switchgrass for Fermentable Sugar Production. (Under the direction of Dr Praveen Kolar.)

Switchgrass is a promising lignocellulosic feedstock for bioethanol production. Typically, pretreatment of switchgrass is required to release sugars needed for fermentation into ethanol. Most commercial pretreatment processes use dilute sulfuric acid to alter the lignin structure and to remove hemicelluloses almost completely. Although effective, liquid acid pretreatment results in additional downstream processing such as neutralization. In addition, the acid itself cannot be recycled easily making the process expensive. One approach to minimize the use of liquid acids is to use solid acid catalysts for pretreatment. Hence, in this study, niobium oxide (Nb_2O_5) was evaluated as a solid acid catalyst to pretreat *Alamo* switchgrass. The objectives were to: (1) determine the effects of temperature, catalyst loading, and pretreatment time on delignification and enzymatic hydrolysis of switchgrass, (2) evaluate reusability of the catalyst, and (3) investigate the mechanism of niobium oxide pretreatment.

Batch experiments were performed using a Box–Behnken statistical model to study the effects of temperature (30 ^{0}C, 60 ^{0}C, 90 ^{0}C), pretreatment time (30 min, 75 min, 120 min), and catalyst loading (0.25 g/g raw switchgrass, 0.625 g/g raw switchgrass, 1 g/g raw switchgrass). Subsequently, the pretreated switchgrass samples were hydrolyzed using 40 % and 57.26 % (g enzyme/g dry biomass) of Cellic®Ctec2 (Novozymes). Further, the catalyst was characterized via scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and Brunauer-Emmett- Teller (BET) surface area.

Results indicated that niobium oxide pretreatment was able to reduce lignin concentrations up to 60.95% (acid soluble lignin and acid Insoluble lignin combined). In

addition, there was an effect on the carbohydrate content in switchgrass. Results from 72-hour hydrolysis experiments (40% enzyme loading) indicated that a maximum glucose yield of 0.169 g g^{-1}(59.94% conversion) was obtained when the switchgrass was pretreated at 60 °C, for 120 min and using 0.25 g g^{-1} of catalyst loading. When higher enzyme loading (57.26%) was employed, a maximum glucose yield of (81.28% conversion) was achieved. In addition, longer hydrolysis time (168 h) resulted in enhanced glucan digestibility. However the xylose yields were lower (0.011- 0.040) g g^{-1} at all pretreatment conditions suggesting that Cellic®Ctec2 was inadequate in converting niobium oxide-treated xylan into xylose. Catalyst reusability studies suggested that niobium oxide was able to pretreat four separate batches of switchgrass without losing activity.

Analysis of the switchgrass samples using SEM indicated that niobium oxide pretreatment was able to disrupt the external structure of switchgrass. The structural disruption was also supported by the BET analysis which revealed that the specific surface area of switchgrass was increased by 31% as a result of pretreatment. In addition, XPS analysis of the pretreated surface indicated an increase in surface oxygen content which suggests that niobium oxide was able to delignify switchgrass via selective oxidation. Solid catalysts such as niobium oxide are expected to lower pretreatment costs and eventually reduce the costs of biomass to ethanol processes and make ethanol production green and environmental friendly.

Niobium Oxide Catalyst for Delignification of Switchgrass for Fermentable Sugar Production

by
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BIOGRAPHY

Yane Oktovina Ansanay, was born on the 4th of January 1986, in Jayapura, Indonesia. She is the second child from the total 6 children in the family. Her family background was not rich, but she has a very work hard family. Her family's vision is really attached to her as she was taught to do the study or work faithfully. She got a scholarship when she was in 11th grade that brought her to study far away from her hometown, Jayapura. She moved to Karawaci when she was 17 and had studied there until she completed her college in 2008 with majoring in Physics. After finished college, she worked as a part time math and physics teacher. In 2010, she has received a Fulbright scholarship and she moved to the US to pursue her graduate studies at the Biological and Agricultural Engineering department at North Carolina State University in August 2010. Right now she is continuing her doctoral study in the Biological and Agricultural Engineering department at North Carolina State University. After finishing school, she is planning to go back to her hometown Jayapura and dedicate her works in that place.

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CHAPTER 1: INTRODUCTION

1.1 Background

Due to the increase in energy consumption, it is predicted that dependence upon crude oil as a primary energy source might need to be reconsidered for future uses. Thus, exploring other possible energy sources is the best alternative solution to overcome a constantly growing need for energy. Switchgrass is a promising energy feedstock as identified by the U. S Department of Energy as a research model for bioenergy production (McLaughlin, 1992). Specifically, it is stated that switchgrass shows great potential and is a promising feedstock due to its high productivity, low water and nutritional requirements. In addition, switchgrass is well adapted to marginal land quality (McLaughlin, 1992) and may be able to reduce pollution to a certain level (Bransby et al., 1998). While this plant can potentially be converted to ethanol, there is, however, an issue related to its complex structure, just like any other lignocellulosics biomass, that might pose a problem in converting promising components such as cellulose and hemicelluloses to produce energy. Several studies conducted previously have indicated that the presence of lignin inside the lignocellulosic biomass makes the structure of biomass difficult to be broken and as a result the efficiency of energy conversion was found to be very low. Lignin has to be removed in order to allow enzymes to obtain more access to the polysaccharides and boost the sugar conversion.

Thus, pretreatment is a necessary step conducted to alter the structure of lignin to increase carbohydrate accessibility to enzymatic attack (Alvira et al., 2010). Mosier et al. (2005)

stated that pretreatment is an important tool to improve cellulose conversion. In summary, pretreatment is an essential step in the production of ethanol from lignocellulosic feedstock (Xu at al., 2009, Mosier et al., 2005, Alvira et al., 2010). A study conducted in 2006 summarized that the overall objective of different studies on pretreatment and states the need to find the most appropriate method for each of the feedstock, in order to provide an approach for economic success upon scaling up (Ragauskas et al., 2006). Physical, chemical and biological concepts have been applied in the pretreatment of biomass (Wyman et al., 2011, Kim et al., 2011). Every pretreatment method has its own advantages and disadvantages. For example acid pretreatment using H_2SO_4 has been used as a powerful treatment method over the years and attracted much attention (Mosier et al., 2005). However, sulfuric acid requires special downstream processing in the form of neutralization of process liquid and it also requires corrosion resistant material. Alkali-based pretreatment are too expensive and it is difficult to recover and reuse the pretreatment agent (Karunanithy and Muthukumarappan, 2011, Mosier et al., 2005). While considering all the aforementioned facts it is an obvious conclusion that pretreatment of lignocellulosic biomass is one the most challenging steps in the production of sugar for ethanol production (Yang and Wyman, 2008, Alvira et al., 2010, Chiaramonti et al., 2012).

Hence, a proposed pretreatment study has to be able to minimize the degradation of sugar, improve sugar yields, be environmentally benign, and economically feasible. Apparently, a solid acid catalyst tends to overcome these issues. Additional advantages of a solid catalyst are that these particles can be easily separated via filtration, and the catalyst can be used

repeatedly for the reaction without neutralization, minimizing energy consumption and waste. Solid niobium oxide (Nb_2O_5) is one of the promising natural acidic materials that reportedly have high catalytic activity (Ziolek 2003, Braga et al., 2007) and 100 % selectivity for the esterification reaction using hydrated niobium oxide $Nb_2O_5.nH_2O$ (Lisuka et al., 1986). Since niobium oxide has both high catalytic activity and selectivity, it is worthwhile to explore these properties by applying them as the catalyst in pretreatment of biomass for ethanol production.

1.2 Objectives

The main goal of this research project was to evaluate the effectiveness of niobium oxide (Nb_2O_5) as a solid acid catalyst for treatment of switchgrass for fermentable sugar production. The objectives were to: (1) determine the effects of temperature, catalyst loading, and pretreatment time on delignification and enzymatic hydrolysis efficiency (2) evaluate reusability of the catalyst, and (3) study the mechanism of the NO pretretament.

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CHAPTER 2: LITERATURE REVIEW

2.1 Biomass for renewable energy

Biomass is one of the alternative energy resources which can reduce consumption of petroleum and other fossil fuels. Recently, the term of biomass has been used to describe all biological materials that are capable of being converted to produce clean energy. To be more specific, energy from biomass can be derived from either plant based material or animal waste. Up until now, material normally used to produce energy from biomass has been corn for ethanol production, algae, and waste oil for biodiesel production. However, a study by Sun and Cheng, 2002 observed that converting corn as feedstock, into bio-ethanol may not be the best approach because corn is used primarily for food and in the long run more land will be needed to provide enough feedstock. In addition, Sun and Cheng, 2002 mentioned that for corn as a feedstock to produce bio-ethanol, may not be the best approach due to its large foot print. When choosing a feedstock basic idea such as availability and environmental friendliness are to be considered. To meet these requirements, lignocellulosic feedstocks have been considered (Mousdale, 2008) for energy production, mostly for ethanol production. The materials that are classified as lignocellulosic feedstocks are agricultural residues, waste paper, herbaceous and woody crops (Gray et al., 2006, Kumar et al., 2009). Some of the previous studies mentioned that, in general, these kinds of lignocellulosic biomass are underutilized and usually burnt producing little energy (Pang et al., 2006; Rezende et al., 2002).

2.2 Lignocellulosic as a bioenergy resources

2.2.1 Structure of lignocelluloses

As reported previously, lignocellulosic biomass is the most widely available renewable natural resource for second generation bioethanol production (Mousdale, 2008). It is important to understand the structure of feedstocks. Generally, the major components of lignocellulosic biomass are cellulose, hemicelluloses and lignin. Cellulose and hemicelluloses are potential sources of fermentable sugars for biofuel production. However, unlike corn which contains starch that is easily converted into fermentable sugar, the carbohydrates fraction (cellulose and hemicelluloses) in lignocellulosic feedstocks are not easily converted into fermentable sugar. The presence of lignin in the cell wall inhibits cellulolytic enzymes from working at the hydrolysis stage and reduces the efficient release of sugars needed for subsequent fermentation (McMillan, 1994). Figure 2.1 briefly explains the presence of lignin in the cell wall, which poses problems in converting the cellulose and hemicelluloses. Hence it is important to disrupt the structure of biomass so that enzymes will get an access to cellulose and hemicelluloses (Zaldivar et al., 2001).

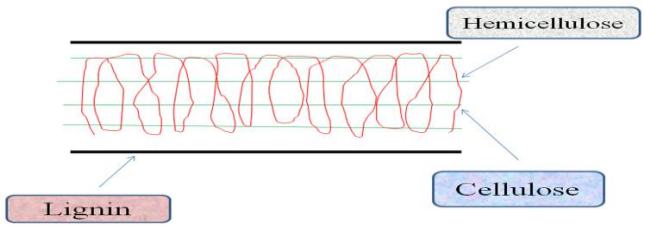

Figure 2.1. Structure of lignocellulosic biomass.

2.2.2 Alamo switchgrass as a promising feedstock

The U. S Department of Energy has identified switchgrass as a model herbaceous energy crop (McLaughlin, 1992). The study stated that switchgrass as an alternative energy crop shows a promising potential and should be considered for research, as this crop has high productivity, matches well with marginal land quality and also is environment friendly. It is also important to note that, while improving the effectiveness of switchgrass as a feedstock for energy, this plant could also reduce the amount of CO_2 emissions and at the same time improve quality level of soil (Bransby et al., 1998). In addition, switchgrass has an abundance of cellulose and hemicelluloses which could be convertible to ethanol (Keshwani and Cheng, 2009). A study conducted by Schmer et al., 2008 stated that switchgrass-based ethanol produced 94% less gas emissions than those created from gasoline.

2.3 Conversion of switchgrass to bioethanol

Overall, the conversion of lignocellulosic biomass to ethanol requires 4 steps: pretreatment, hydrolysis, fermentation and high level of purification. The very first step for the conversion of lignocellulosic biomass to ethanol is pretreatment which is reportedly as one of the most challenging steps (Yang and Wyman, 2008, Alvira et al., 2010). Pretreatment is required to alter the biomass structure so that hydrolysis of the carbohydrate component can be achieved easily and can produce higher sugar yields. Three major hydrolysis processes generally

conducted to produce sugars for ethanol production: dilute acid, concentrated acid or enzymatic hydrolysis (Kumar et al., 2009). The next step is fermentation of glucose to ethanol which normally uses either yeast or bacteria, however, for fuel ethanol, yeast is more commonly used. Following fermentation, ethanol must be separated from water and has to reach the purification level of > 99% in order to be used as a biofuel- especially if used in automobiles.

2.4 Pretreatment

2.4.1 Introduction of pretreatment

The primary reason for conducting pretreatment is to disrupt the structure of the cell wall. Specifically, to break down the structure so that lignin is broken down and cellulose and hemicelluloses are converted by enzyme to fermentable sugars that are needed for alcohol production (Wyman, 1994, Mosier et al., 2005). While the main objective of pretreatment is to disrupt the cell wall cell, it is also expected that pretreatment will enhance enzymatic hydrolysis, have minimal effect on carbohydrate and minimize production of inhibitory compounds. In addition, overall cost of pretreatment is also important (Wyman et al., 2005).

Numerous pretreatment methods have been studied extensively. Most of the biomass pretreatment procedures involve treatment with harsh chemicals such as acids (Wyman et al., 1992, Chung et al., 2005, Dien et al., 2006, Wyman et al., 2011 and

Zhou et al., 2011), bases (Chang et al., 1997, Wang et al., 2008 and Xu et al., 2008), ammonia (Alizade et al., 2005, Kurakate et al., 2001), and ozone (Vidal and Molinier., 1998). Additional information on pretreatment of lignocellulosic biomass can be researched from several studies conducted previously (Sun and Cheng, 2002, Keshwani and Cheng, 2009, Kumar et al., 2009, Mosier et al., 2005, Kim et al., 2011, Agbor et al., 2011, Chiaramonti et al., 2012). In summary, the overall objective of conducting pretreatment is to find appropriate methods which satisfy the requirements mentioned previously and contribute to a better economic conversion (Ragauskas et al., 2006). In general, there are three main categories of pretreatments: physical, chemical and biological.

2.4.2 Physical pretreatment

Generally, physical pretreatment involves breakdown of the physical structure of biomass. The following are some available options to physically alter the biomass structure:

2.4.2.1 Mechanical comminution

In this particular pretreatment the biomass is reduced to a certain size by grinding, milling and chipping. The primary purpose of this size reduction is to reduce the crystallinity of cellulose fibers. Earlier studies reported that size reduction tends to avoid heat transfer issues during the hydrolysis process (Schell and Harwood, 1994).

It is reported that size reduction of materials is normally between 10-30 mm after chipping and 0.2-2 mm after milling or grinding (Sun and Cheng, 2002, Kumar et al., 2009). A recent study has performed to determine the effect of size reduction of switcgrass obtained by applying ball milling (Brigeman et al., 2007). Several studies were also conducted to examine the overall energy requirements for size reduction of switchgrass (Samson et al., 2000, Mani et al., 2004, Igathinathane et al., 2008). In overall, a study reported high energy requirements for mechanical comminution pretreatment is not economically feasible as the size requirement would lead to great energy cost (Hendriks and Zeeman, 2009)

2.4.2.2 Ammonia fiber explosion (AFEX)

This particular treatment is technically classified as physico-chemical pretreatment where the biomass is exposed to liquid ammonia at high temperature and pressure for short periods of time, followed by dropping the pressure abruptly. Normally, ammonia is introduced to the process between 1-2 kg/1kg of dry biomass, temperature at about 90 ^{0}C and reaction time 30 min (Mackie et al., 1985). One study reported that AFEX does not solubilize hemicelluloses (Vlasenko et al., 1997). In addition, AFEX is reportedly not too effective for treating biomass with high lignin content such as woodchips and newspaper (Agbor et al., 2011, McMillan 1994). Sun and Cheng, 2002 reported that for environmental safety reasons and economic considerations, ammonia has to be recovered after the completion of pretreatment.

2.4.2.3 Steam explosion

Steam explosion is one of the common methods used for pretreatment of lignocellulosic feedstocks. The procedure for pretreatment with steam explosion is generally the same as AFEX. The biomass with a reduced size is exposed to saturated steam in a high-pressure chamber for a short period of time after which pressure is released. Typically, common conditions for steam explosion are pretreatment conducted at temperature 160 ^{0}C-206 ^{0}C and pressure 0.69-4.83 MPa (McMillan, 1994). It is reported that four major factors affect the steam explosion process: length of pretreatment, moisture content of the feedstock, temperature, and size of material (Duff and Murray, 1996). Steam explosion mainly contributes to hemicellulose degradation. In addition, since steam explosion doesn't use any chemical product, it is friendlier to the environment. However, during the steam explosion process, intermediate compounds are normally generated that may inhibit and prevent cellulolytic enzymes from working optimally at enzymatic hydrolysis and fermentation stages (Mackie et al., 1985). To solve this problem, the pretreated sample needs to be washed to get rid of unwanted compound.

2.4.3 Chemical pretreatment

Typically, chemical pretreatment of lignocellulosic feedstock is conducted by applying certian chemicals such as acids, alkali, ozone, organic solvents and peroxides.

2.4.3.1 Acid pretreatment

Acid pretreatment has been traditionally used over the years (Mosier et al., 2005). Concentrated acid such as H_2SO_4 and HCl have been extensively used in the past and it is reportedly a powerful mediator for cellulose hydrolysis. However, concentrated acids also have side effects that are harmful to the environment, may cause corrosion and the process is not economically feasible (Sivers and Zacchi, 1995). Hence, dilute acid pretreatment has been studied as an alternative. It is reported that dilute acid pretreatment was an effective pretreatment which removed the hemicelluloses, but unfortunately failed to remove lignin (Keshwami and Cheng, 2009). It is reported that the first study using dilute acid pretreatment of switchgrass for bioethanol production was conducted by Wyman's group (Wyman et al., 1992). In that particular study, the treatment was conducted at 140 ^{0}C for 1 hour using low concentration of sulfuric acid (up to 0.5 %v/v) and resulting biomass yielded up to 70% conversion of cellulose into glucose after 5 days of process. Another study reported that 90 % of cellulose in the pretreated sample was converted into reducing sugars during 72 h enzymatic hydrolysis (Chung et al., 2005). The optimal conditions suggested from that particular study are concentration at 1.2 % (w/w) at 180 ^{0}C. Since dilute acid pretreatment is not efficient for removing lignin in lignocellulosic feedstock, it will lead to a problematic enzymatic hydrolysis stage (Wu and Lee, 1997). However, another study reported that although lignin is not significantly removed in acid pretreatment, the structure of lignin is interrupted thus making the carbohydrates more accessible to

enzymes (Yang and Wyman, 2004). Typically, two kinds of dilute acid pretreatments are employed: high temperature pretreatment (T>160 C) for continuous flow of low solids (5% -10%), and low temperature pretreatment (T<160 C) for batch process high solids (20%-40%). Although, dilute acid pretreatment efficiently improves cellulose hydrolysis, it can be costly; the pH of the treated biomass at the end of the process needs to be neutralized in order to get the treated biomass ready for enzymatic hydrolysis and fermentation (Sun and Cheng, 2002). Recently, Zhou et al., 2012 reported the highest conversion of carbohydrate of transgenic swicthgrass- corresponding to 82.6 %.

2.4.3.2 Alkali pretreatment

Bases are also used for pretreatment of biomass. A study conducted in 1994 stated that the effect and efficiency of alkaline pretreatment depends on the lignin content of the materials (McMillan, 1994). Alkali pretreatment is conducted at lower temperature and pressure compared to acid pretreatment (Mosier et al., 2005). However, in this particular treatment, time is a major factor to consider as this alkali pretreatment tends to consume more time for the treatment of lignocellulosic feedstock (Kumar et al., 2009, Wyman et al., 2005, Alvira et al., 2010). It is reported that the major effect of alkali pretreatment is saponification of intermolecular ester bonds which crosslink lignin and carbohydrates (Sun and Cheng, 2002). As a result there was an increased porosity and internal surface area of the biomass as well as a

decreased degree of crystallinity of cellulose (Sun and Cheng, 2002). It is also reported that alkaline pretreatment was able to maintain a higher percentage of sugar available after the treatment (Xu et al., 2010, Keshwani and Cheng, 2010). In addition, and less inhibitory compounds were created during the treatment process (Gaspar et al., 2007). There are several alkaline pretreatment agents that have been introduced such as sodium, ammonium, potassium, and calcium hydroxides. Of these four, sodium hydroxide (NaOH) and calcium hydroxide (widely known as lime) are commonly used as agents in alkaline pretreatments. It is reported that NaOH is an agent for removing lignin from lignocellulosic feedstock at 121 ^{0}C for short periods of time (30min) (Wang et al., 2008). NaOH also reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24%-55% to 20% (Kumar et al., 2009). Lime pretreatment of switchgrass was investigated by Chang et al (1997). In that study, the group concluded that with a lime loading of 0.1 g g^{-1} of dry switchgrass, with a pretreatment time of 2 h at 100 ^{0}C, the study showed that after 72h enzymatic hydrolysis, the pretreated biomass was able to produce five times higher total reducing sugar than untreated switchgrass.

Ammonia, another alkaline agent, has also been studied for the pretreatment of the biomass. The ammonia concentration used for the ammonia recycled percolation process for the pretreatment of corn cobs/ stover mixtures and switchgrass was 2.5 %-20%, with the temperature 170 ^{0}C and reaction time was 1 h and flow rate of 1

mL/min (Iyer et al., 1996). The report mentioned that the efficiency of delignification was 65% - 85% for switchgrass and 60%-80% for corn cobs.

2.4.3.3 Ozonolysis pretreatment

Ozone has potential for the pretreatment of lignocellulosic biomass. Ozone was found to be an effective agent for removing lignin without creating any by-product or inhibitory compound (Vidal and Moliner, 1988). In general the reaction of pretreatment using ozone is carried out at room temperature. Feedstocks that normally use ozone pretreatment are wheat straw, bagasse (Ben-Ghedalia et al., 1984), green hay, pine, peanut (Neely et al,.1984) and poplar (Vidal and Molinier, 1988). Several advantages have been listed for the ozonolysis pretreatment, such as effectiveness in removing the lignin, no toxic residues at the end of the process (and hence recovery is not necessarily needed), and the process takes place at room temperature and low pressure (Vidal and Molinier, 1988). However, the process requires high amounts of ozone and could be costly (Sun and Cheng, 2002).

2.4.3.4 Niobium oxide solid acid pretreatment

Generally, chemical pretreatment of lignocellulosic biomass is carried out using liquid acid or base catalysts. However, aqueous acids and base catalysts always require special treatment processing for neutralizing which involves additional cost. Moreover, the separation part also plays an important role. Inefficient catalyst separation results in unfriendly sulfate waste which harms the environment (Clark

2002, Okuhara 2002). A better, friendlier catalyst would contribute to an efficient catalytic process. A solid acid catalyst tends to overcome this issue. It is reported that a solid acid catalyst is non corrosive, less expensive, environmental friendly, easy to handle and most importantly reusable. (Clark 2002, Okuhara 2002, Corma et al., 2003). Hence solid acid catalysts have been widely used and developed for over 40 years (Tanabe and Wolfgang,. 1999). Industrial processes generally use solid acid catalysts for alkylation, cracking, hydrogenation hydration and esterification (Tanabe and Wolfgang,. 1999). However, in the field of renewable energy, Niobium oxide (Nb_2O_5) is not widely used except for esterification and hydrolysis of cellulose (Hara 2010, Suganuma et al., 2008, Kulkarni et al., 2006 and Yamaguchi et al., 2009).

Figure 2.2. Activated carbon

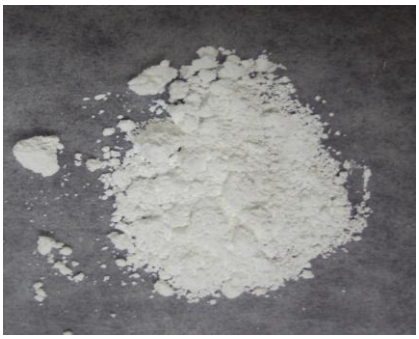

Figure 2.3. Niobium oxide

From the literature, the hydrated niobium oxide ($Nb_2O_5.nH_2O$) is an active species that has an acid strength ($H_0 \leq -5.6$) corresponding to the acid strength of 70% H_2SO_4

(Tanabe 1987). The melting point of Nb_2O_5 is very high at 1785 K (Ziolek 2003). In addition, niobium oxide has been reported as a water tolerant catalyst (Okuhara 2002). When water is involved in the process it is suggested that the reaction should be run at temperatures lower than 773 K (500^0C). One study reported that the surface area of niobium oxide catalyst plays an important role in determining the catalytic activity (Paulis et al., 1999). It has been reported that hydrated niobium oxide shows high catalytic activity and 100 % selectivity for the esterification of ethyl alcohol with acetic acid (Lisuka et al., 1986). Nb_2O_5 is known as a strong solid acid that can be used in various interesting applications (Ziolek 2003, Tanabe 2003).

Although, this catalyst has been used in various ways and the results confirmed its usefulness, it is a fact that this unexplored catalyst might also work well in the pretreatment of lignocelluloses materials for fermentable sugar.

2.5 Enzymatic hydrolysis

Enzymatic hydrolysis of the solid biomass is conducting to break down cellulose and hemmicellulose into fermentable sugar such as glucose and xylose. Enzymatic hydrolysis is affected by several factors, including properties of the subtrates, enzyme activities and reaction conditions such as pH, temperature and reaction time. Unlike the hydrolysis of concentrated acid that has lower pH, for the enzymatic hydrolysis, pH of system has to be maintained at mild condition at pH 4.5-5.0 and the temperature of 45^0C- 50^0C which reported

cost less than acid hydrolysis (Wyman et al., 2005). Spesifically, the conversion of two major promising components of lignocellulosic feedstock named cellulose and hemicelluloses is catalyzed by two different enzymes cellulase and hemicellulase, respectively. Recently, hydrolysis can also be carried out using one enzyme such as *Cellic®Ctec2* from Novozymes. N.A which has been blended already with several components (Aggressive cellulases, high level of β-glucosidases and hemicellulase) that is useful to convert both cellulose and hemicelluloses.

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CHAPTER 3: SOLID NIOBIUM OXIDE ACID CATALYST FOR DELIGNIFICATION OF SWITCHGRASS FOR FERMENTABLE SUGAR PRODUCTION

3.1 Abstract

Pretreatment of switchgrass for bioethanol production involves pretreatment with dilute sulfuric acid. Although effective dilute sulfuric acid is not environmentally friendly. Hence in this research, niobium oxide was evaluated as a pretreatment agent for delignification of Alamo switchgrass. The objectives were to: (1) determine the effects of temperature, catalyst loading, and pretreatment time on delignification and enzymatic hydrolysis of switchgrass and (2) evaluate reusability of the catalyst. Batch experiments were performed using a Box–Behnken statistical model to study the effects of temperature (30 ^{0}C, 60 ^{0}C, 90 ^{0}C), pretreatment time (30 min, 75 min, 120 min), and catalyst loading (0.25 g/g raw switchgrass, 0.625 g/g raw switchgrass, 1 g/g raw switchgrass). Subsequently, the pretreated switchgrass samples were hydrolyzed using 40 % and 57.26 % (g enzyme/g dry biomass) of Cellic®Ctec2 (Novozymes). Results indicated that niobium oxide pretreatment was able to reduce lignin concentrations up to 60.95% (acid soluble lignin and acid Insoluble lignin combined). 72-hour hydrolysis experiments (40% enzyme loading) indicated that a maximum glucose yield of 0.169 g g^{-1}(59.94% conversion) was obtained when the switchgrass was pretreated at 60 °C, for 120 min and using 0.25 g g^{-1} of catalyst loading. When higher enzyme loading (57.26%) was employed, a maximum glucose yield of (81.28% conversion) was achieved. Catalyst reusability studies suggested that niobium oxide was able

to pretreat four separate batches of switchgrass without losing activity. Solid catalysts such as niobium oxide are expected to lower pretreatment costs and eventually reduce the costs of biomass to ethanol processes and make ethanol production green and environmental friendly.

Keyword : Lignocellulosic Pretreatment, Niobium Oxide, Catalyst Reuse, Lignin Degradation, Enzymatic Hydrolysis.

3.2 Introduction

There is a worldwide interest in production of renewable fuels such as ethanol from lignocellulosic biomass (Mousdale, 2008). Switchgrass is one such source of biomass that appears very promising due to high yields in marginal lands even with minimal agronomical inputs (McLaughlin, 1993). Hence, the United States Department of Energy had identified switchgrass as a model crop for bioethanol production (McLaughlin, 1993).

Conversion of switchgrass to ethanol involves pretreatment of the biomass matrix, followed by hydrolysis and fermentation. Pretreatment is necessary to delignify the biomass that would otherwise inhibit the enzymes during subsequent hydrolysis (McMillan 1994). In addition, pretreatment is expected to alter the structure of biomass and allow hydrolytic enzymes and chemicals to access the cellulosic and hemicellulosic portions of the biomass (Wyman 1994, Mosier et al., 2005). Hence, pretreatment is one of the most challenging and expensive processes in the overall bioethanol supply chain.

Most of the biomass pretreatment procedures involve treatment with harsh chemicals such as acids (Wyman et al., 1992, Chung et al., 2005, Dien et al., 2006, Wyman et al., 2011 and Zhou et al., 2011), bases (Chang et al., 1997, Wang et al., 2008 and Xu et al., 2008), ammonia (Alizade et al., 2005, and Kurakate et al., 2001), and ozone (Vidal and Molinier., 1998). Several detailed reports are available on pretreatment of lignocellulosic biomass (Sun and Cheng, 2002, Keshwani and Cheng, 2009, Kumar et al., 2009, Mosier et al., 2005, Kim et al., 2011, Agbor et al., 2011, Chiaramonti et al., 2012). Although these are effective, they

are not environmentally friendly. For the bioethanol processes to become sustainable and green, alternative pretreatment technologies need to be investigated. One approach is to employ solid acid and base catalysts as pretreatment agents. Due to their surface chemistry, such solid catalysts can not only delignify biomass but also can be separated and reused several times.

Niobium oxide is one such solid catalyst that is acidic. Owing to its versatile properties, hydrated niobium oxide has been tested in esterification (Lizuka et al., 1986), and other reactions (Ziolek 2003, Tanabe 2003, Braga et al., 2007). However, to our knowledge very little information is available on the efficacy of niobium oxide as a biomass pretreatement agent. Hence, the goal of this research is to investigate niobium oxide as a solid acid catalyst for pretreatment of switchgrass for delignification and fermentable sugar production. Considering the physio-chemical properties of niobium oxide, we hypothesized that niobium oxide can effectively delignify switchgrass. Our objectives were to: (1) determine the effects of temperature, catalyst loading, and pretreatment time on delignification and enzymatic hydrolysis and (2) evaluate reusability of the catalyst,

3.3 Materials and Methods

3.3.1 Biomass preparation

Switchgrass (*Alamo*) was obtained from North Carolina State University's Reedy Creek Road Field Laboratory. The switchgrass was harvested in mid July 2011, field

cured, and baled with a conventional square hay baler. A subsample was collected, ground to pass through a 2 mm sieve, and stored at room temperature in tightly sealed plastic bags until analyzed for composition and subsequent pretreatment with niobium solid acid.

3.3.2 Niobium oxide pretreatment

Niobium (V) oxide (Nb_2O_5) come in the powder form obtained from Fisher Scientific Co was used as an acid catalyst for pretreatment of switchgrass. Typically, 6 g of switchgrass was mixed with 90 mL deoinized (DI) water (15% loading) in a 120- mL serum bottle (reactor). After adding a predetermined amount of the catalyst to the switchgrass solution, the serum bottles were sealed and the contents of the reactors were stirred (400 rpm) on a temperature controlled hot plate for a predetermined time. After pretreatment, the switchgrass was separated from the slurry via filtration and washing with 200 mL with DI water to remove any catalyst particles. The separated switchgrass was hydrolyzed while the catalyst was stored for subsequent reuse.

3.3.3 Enzymatic Hydrolysis

Enzymatic hydrolysis of the pretreated switchgrass was performed in 50-ml centrifuge tubes placed in automated shaking water bath (50 °C). A 20-mL slurry (pH =5) consisting of 1 g (db) of pretreated switchgrass (equivalent to 5% solid loading), *Cellic®Ctec2* (Novozymes North America, Franklinton, NC) (equivalent to 40% and 57. 26 % (g enzyme/ g dry

biomass) with a density of 1.203 g/ ml along with 0.05 M sodium citrate buffer was agitated at 150 rpm for 72 and 168 hours. In addition, 40 µg/ml of tetracycline hydrochloride was added to the tubes to minimize bacterial growth during hydrolysis. After hydrolysis, the hydrolyzates were centrifuged and the supernatant was stored for a short time period at -4^0C and -80^0C for a long time for sugar analysis.

3.3.4 Experimental design and statistical analysis

A total of 13 experiments were determined using Box-Behnken method design was used to test the effects of catalyst loading (0.25-1 g g^{-1}), pretreatment time (30-120 min), and temperature (30-90 °C) (Table 3.1). Each experiment was performed in triplicates and after each experiment the results for acid soluble lignin (ASL), acid insoluble lignin (AIL) and monomeric sugars were determined via standard analytical methods were analyzed using response surface methodology to determine the important of each parameters selected.

Table 3.1. Experimental conditions employed during pretreatment of switchgrass using niobium oxide catalyst

Treatment	Temp (^{0}C)	Trt time (min)	Catalyst loading (g/g of raw)
A	30	30	0.625
B	30	120	0.625
C	90	30	0.625
D	90	120	0.625
E	60	30	0.25
F	60	30	1
G	60	120	0.25
H	60	120	1
I	30	75	0.25
J	90	75	0.25
K	30	75	1
L	90	75	1
M	60	75	0.625

3.3.5 Analytical Methods

Moisture, lignin, and sugar contents of the raw and pretreated switchgrass were determined using National Renewable Energy Laboratory (NREL) procedures (Sluiter et al., 2005a, b, 2008). Briefly, for total carbohydrate analysis, switchgrass was hydrolysed in 72% sulfuric acid at 30 ^{0}C for 1 h, followed by 1 h hydrolysis in 4% sulfuric acid at 121^{0}C. The resulting hydrolysate was filtered for the carbohydrate analysis using HPLC and total sugar while acid soluble lignin was measured through absorbance level measurement at 205 nm in UV-Vis

spectrophotometer. Additionally, the remaining solids were gravimetrically analyzed to determine acid insoluble lignin.

Monomeric sugars such as glucose, xylose and arabinose were determined using high-performance liquid chromatography (HPLC) (Dionex UltiMate 3000, Dionex Corporation, Sunnyvale, CA, USA) equipped with a refractive index detector and a Aminex HPX-87H column. Separations were performed at 65 °C and the flow rate of eluant (5 mM H_2SO_4) was set to 0.6 mL min^{-1}. The products were quantified by comparing with standards of glucose, cellobiose, xylose and arabinose (0.625-10 g L^{-1}). Similarly, total reducing sugars in hydrolysis were measured using the 3-5-ditrinocalysic acid method adapted from Miller (1959) and Ghose (1987).

3.3.6 Reusability of Niobium oxide

To determine the reusability of Niobium oxide as a pretreatment agent, we pretreated three an additional batches of switchgrass using the spent catalyst that was separated from the first batch of experiments. All batches of switchgrass were subsequently hydrolyzed using *Cellic®Ctec2* (Novozymes North America) with the enzyme loading (equivalent to 40% and 57.26 % (g enzyme/ g dry biomass) as described in the previous sections.

3.4 Results and Discussions

3.4.1 Delignification of switchgrass

As expected, compositional analysis of switchgrass revealed that glucose and xylose were two major sugar units in switchgrass. The data presented in figure 3.1 are in agreement with several studies conducted previously using *Alamo* switchgrass (Bals et al., 2010, Mann et al., 2009, Marzialetti et al., 2011, Keshwani and Cheng 2010, Karunanithy et al., 2011). The carbohydrate portion consists of glucan, xylan and arabinan was estimated to be 54.81%. From that amount, theoretically there is 95.8 gallons of ethanol yield per dry ton of feedstock can be produced (online ethanol yield calculator). The total lignin (both acid soluble lignin and acid insoluble lignin) was estimated at 26.24% of which AIL was 22.78% and ASL was 3.46 %. Other undefined component was estimated at 16.21 % which believed mainly come from non-structural component including fats, crude protein, extractives and acetyl (Chang et al., 1997, Mann et al., 2009, Keshwani and Cheng, 2010, Kim et al., 2011).

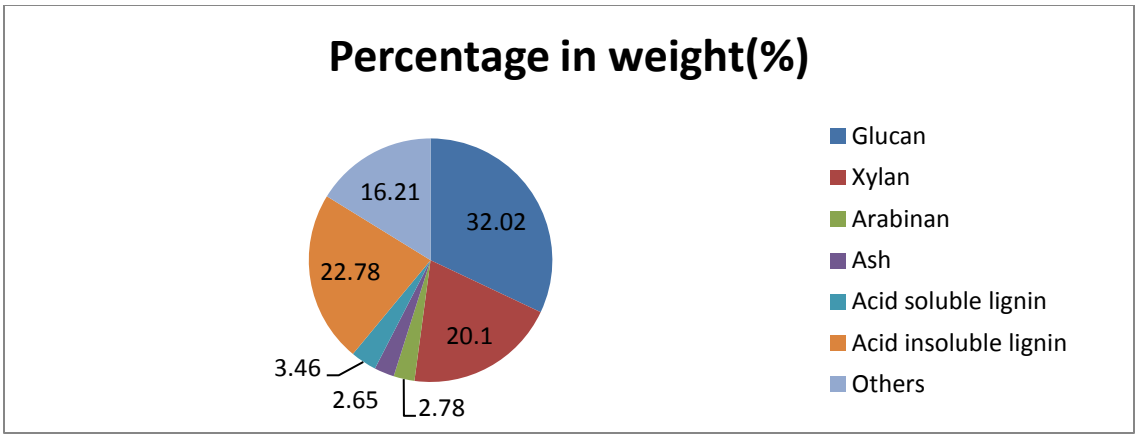

Figure 3.1. Composition of untreated *Alamo* switchgrass

Delignification or extraction of lignin by chemical can cause disruption of lignin structure, biomass swelling, and increases in internal surface area in addition, increased the accessibility of cellulolytic enzyme to cellulose fibers (Agbor et al., 2011).

Although not all pretreatments results in substantial removing lignin, however, the structure of lignin may be broken. Thus, it is believed that during pretreatment, lignin in biomass is impacted (Agbor et al., 2011). Combined effects of temperature, catalyst loading, and pretreatment time on delignification of switchgrass are presented in figure 3.2. Analysis of data suggested that all factors significantly affected delignification of switchgrass **($p < 0.05$).** Overall up to a total of 60% reduction in lignin (ASL+AIL) was observed. Reduction in lignin ranged between 35.26 % and 43.93% for acid soluble lignin **($p < 0.05$)** significant relative to the control whereas 7.82-20.28% of acid insoluble lignin **($p < 0.05$)** was was found partly significant reduced from the switchgrass matrix. When compared to dilute acid

pretreatment, niobium oxide provided higher delignification efficiencies. For example, Li et al. (2010) reported a total lignin decrease of 22.4% correspond to 13.7% of acid soluble lignin and 8.7% of acid insoluble lignin. When treated with 0.5 wt% dilute acid treatment at 160 ^{0}C, acid insoluble lignin was removed by approximately 20% (Kim et al., 2011). Another recent study by Wyman's group compared lignin removal of Dacotah switchgrass using several pretreatments including 1wt% dilute sulfuric acid at 140^{0}C, during which acid insoluble lignin was removed to 16 % (Wyman et al., 2011). In this research, a maximum total lignin removal of 60.95 % was found to occur due to treatment C (30 °C, 90 min, 0.625 g g^{-1}).

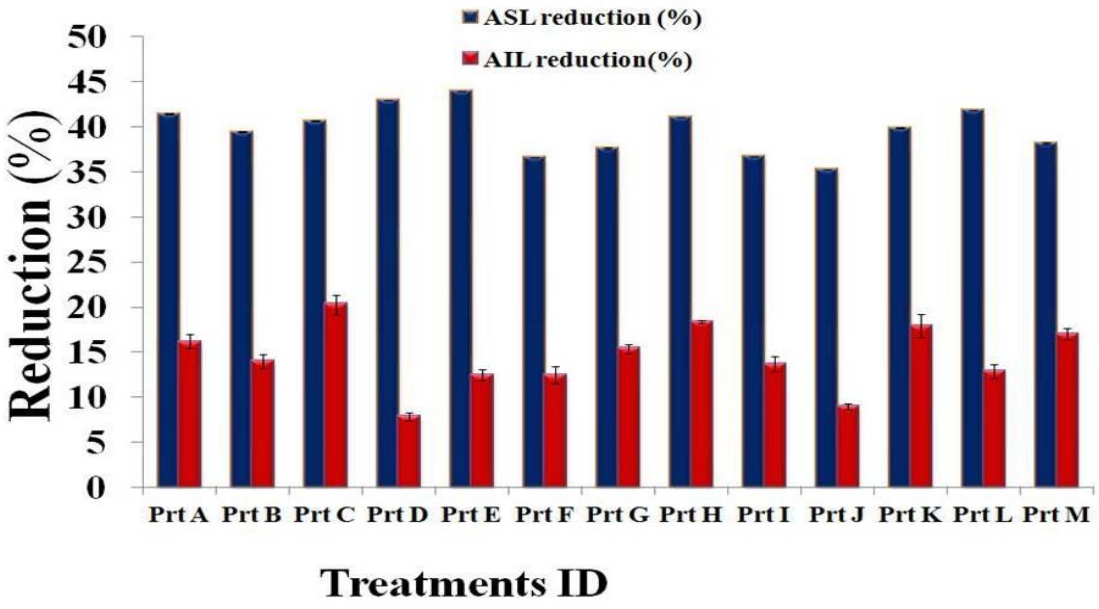

Figure 3.2. Lignin removal after Niobium oxide pretreatment

3.4.2 Effect of Niobium oxide pretreatment on carbohydrate availability

Niobium oxide also impacted the availability of carbohydrate units in switchgrass. The compositions of major carbohydrate in pretreated switchgrass were calculated based on solid recovery for every pretreatment condition. According to figures 3.3 and 3.4, the maximum loss of glucan (28.7%) and xylan (38.3%) occurred due to treatment J (90 °C, 75 min, 0.25 g g^{-1}) and treatment E (60 °C, 30 min, 0.25 g g^{-1}) respectively. However, an average of 77.92 ± 2.03 % and 70.48 ± 1.7%, respectively of the initial glucan and xylan were retained in the solid phase for all pretreatments examined in this study. The results obtained in this study were different from other studies that employed dilute sulfuric acid as a pretreatment agent (1wt% (140^0C), 0.5wt% (160^0C), 1.2% (160^0C)) wherein almost all of the hemicelluloses were removed (Wyman et al., 2011, Kim et al., 2011, Li et al., 2010). Similarly, a recent study by Zhou et al., (2012) also showed that dilute acid (1% (w/v) H_2SO_4 at 150^0C) pretreatment maximized the xylose yield in prehydrolysate. Based on data obtained, it appeared that niobium oxide is more selective towards delignification and does not extract hemicelluloses from biomass as with dilute sulfuric acid pretreatment.

3.4.3 Effect of Niobium oxide pretreatment on enzymatic hydrolysis

The pretreated switchgrass obtained from all pretreatments were hydrolyzed for 72 and 168 h using Cellic®Ctec2 (Novozyme N.A) to investigate carbohydrate digestibility. Figures 3.3 and 3.4 show the yields of glucose and xylose respectively. Maximum glucose yields after 72 h of hydrolysis were found to be 0.169 g g^{-1} (59.94% conversion) from treatment G (60 °C,

120 min, 0.25 g g^{-1}). Even though the glucan conversion after 72 h obtained in our research is comparable with that of ammonia (45-56% conversion), and lime pretreatment (58-67% conversion) (Sun and Cheng 2005, Xu et al., 2010, Chung et al., 2005, Chang et al., 1997), results in our study are slightly lower when compared to several dilute acid treatments conducted previously that provided cellulose conversion of greater than 80 % (Chung et al., 2005, Li et al., 2010, Wyman et al., 2011, Zhou et al 2012).

As expected, when hydrolyzed for 168 h, higher glucose yields were obtained from all the pretreatments as shown from figure 3.3. The glucose yield of 0.196 g g^{-1} (77.51% conversion) was obtained from treatment G (60 °C, 120 min, 0.25 g g^{-1}). Our results with 168 h hydrolysis are comparable with that of dilute acid pretreatment, enzymatic hydrolysis successfully converted cellulose into glucose with the yield ranging between 70-90% over five days of being hydrolyzed (Kim et al., 2011, Chung et al., 2005, Wyman et al., 1992)

Although the glucan conversions are reasonable for the lower enzyme loading, xylose yields and conversion were found lower for both 72 h and 168 h of hydrolysis as presented in figure 3.4. After 72 h-hydrolysis, the maximum xylose yield of 40 mg g^{-1} was achieved via treatment M (60 °C, 75 min, 0. 625 g g^{-1}) corresponding to a 28.9% conversion. Our results were comparable with Zhou et al. (2012) who tested dilute acid pretreatment of switchgrass and reported a xylose yield 0-36.7 mg/g raw biomass. However, yields of xylose obtained in our research were lower than several other studies on switchgrass. For example, Keshwani and Cheng (2010) used 2% NaOH pretreatment (10 min, 150 °C) and obtained a xylose yield

of 0.127 g g^{-1} raw biomass after hydrolyzing with Cellulase (Celluclast 1.5L) from *Trichoderma reesei* (E.C.3.2.1.4) supplemented with cellobiase (Novozyme 188) Similarly, Bals et al. (2010) reported xylose yields in the range of 0.201 and 0.208 g g^{-1} switchgrass using AFEX pretreatment, perhaps due to a combination of enzymes such as Accelerase, the b-glucosidase, Multifect Xylanasae and Multifect Pectinase that were introduced during the hydrolysis stage.

Lower conversion of glucan after 72 h of hydrolysis in this study was probably due to two reasons. Firstly it is believed that in our research, part of the acid soluble lignin that solubilized during the hydrolysis was bound to glucose resulting in an ASL-glucose complex as suggested by Xiang et al. (2004). Secondly it was hypothesized that, even after pretreatment, the glucan was still bound to the remaining AIL in the switchgrass matrix resulting in lower conversion of glucan

Moreover, at 168 h hydrolysis, the glucan conversion rate increased which is good, but left a lower xylan conversion than 72 h.

In our research, the xylose yields were rather lower. It is believed that Cellic Ctec2 alone was not able to effectively convert xylan into xylose despite allowing for longer hydrolysis time (figure 3.4) perhaps due to further degradation of xylose (figure 3.5) (Schell et al., 2003, Xiang et al., 2004, Yat et al., 2008, Kumar and Wyman, 2008, Kumar et al., 2011, Qing et al., 2010, Shi et al., 2011, Chiaramonti et al., 2012).

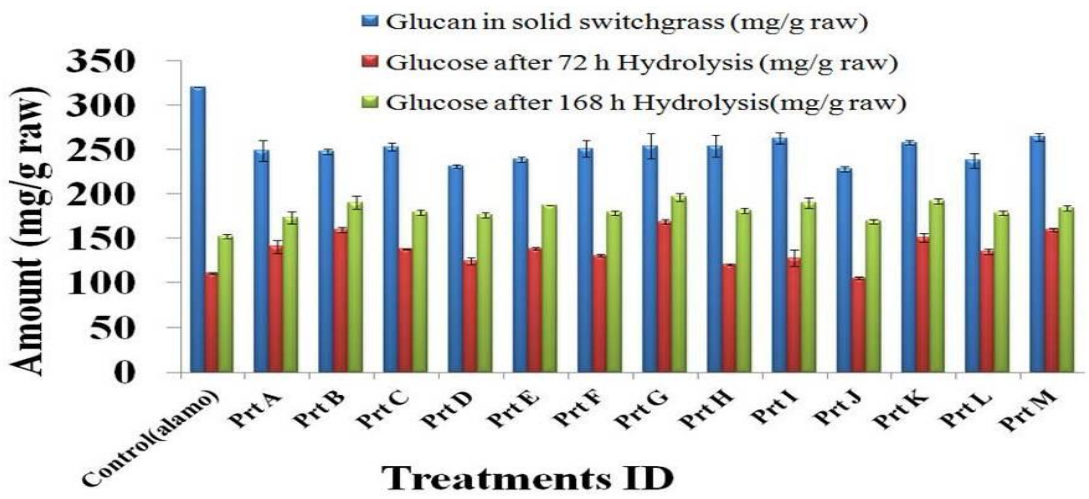

Figure 3.3. Amount of glucan solid biomass, and glucose in hydrolysis after 72 h and 168 h.

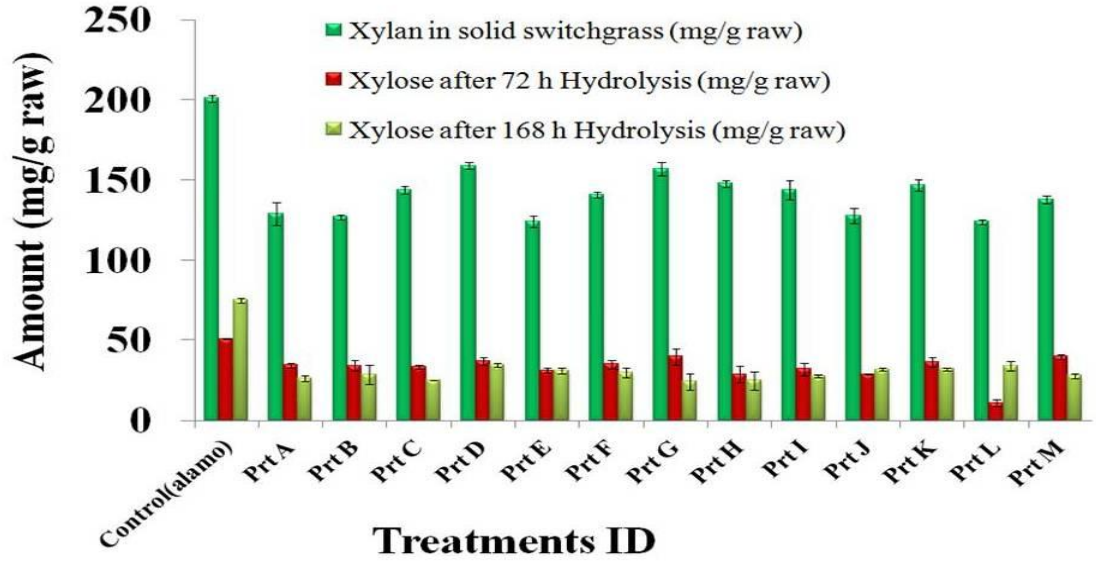

Figure 3.4. Amount of xylan in solid biomass, and xylose in hydrolysis after 72 h and 168 h.

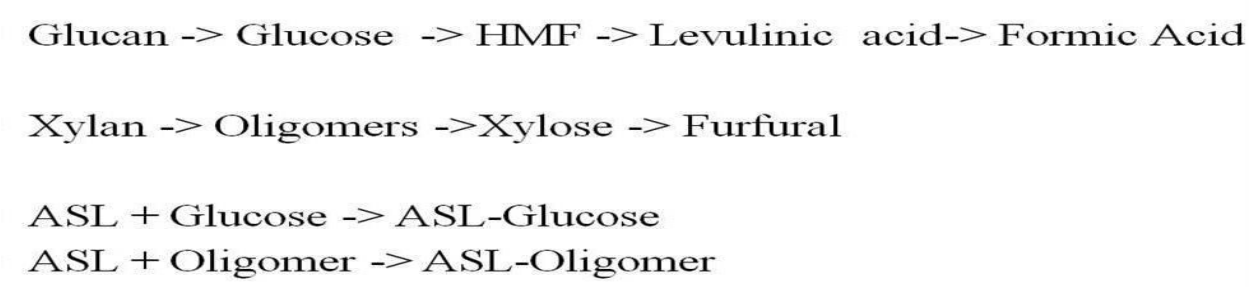

Figure 3.5. Degradation process

3.4.4 Reusability of Niobium oxide solid catalyst

A total of four separate batches of switchgrass were pretreated using the same catalyst. As shown in figure 3.6, a significant **($p < 0.05$)** reduction in ASL lignin was observed even after the fourth reuse. However, the extent of delignification decreased from 37.6% (batch 1) to 21.3% (batch 4) for ASL while, similar trend was also observed for AIL. Upon inspection, it was found that due to small particle size, the catalyst was continuously being lost during filtration and separation procedures. It was observed only 59% of the original mass (0.25 $g\ g^{-1}$) of catalyst remained after the fourth use. Nonetheless, the amount of available glucose was consistent while xylose showed a slight increase perhaps due to loss of the catalyst. Upon hydrolysis, all four batches of pretreated switchgrass exhibited consistent glucan conversions of 59.94 – 72.50% with 40% (g enzyme / g dry biomass) loading. Surprisingly, lower catalyst loading affected less on delignification but gave a higher glucan conversion. Thus, it is suggested that lowering catalyst loading is somehow important factor to consider that might happen to improve the conversion rate for both 40 % and 57.26% enzyme loading. In

addition, as expected, when a 57.26% (g enzyme / g dry biomass) loading were employed, glucose conversions up to 81.28 %.

Moreover, from figure 3.7 it is clear that, higher enzyme loading also contributed higher glucan conversion up to 81.28 % which are comparable to the results obtained from other studies (Kim et al., 2011, Chung et al., 2005). However, the same trend was found where the xylan did not get converted supporting our premise that Cellic Ctec2 was not equipped to hydrolyze hemicelluloses, additionally the xylan remained still in strong intact with lignin portion.

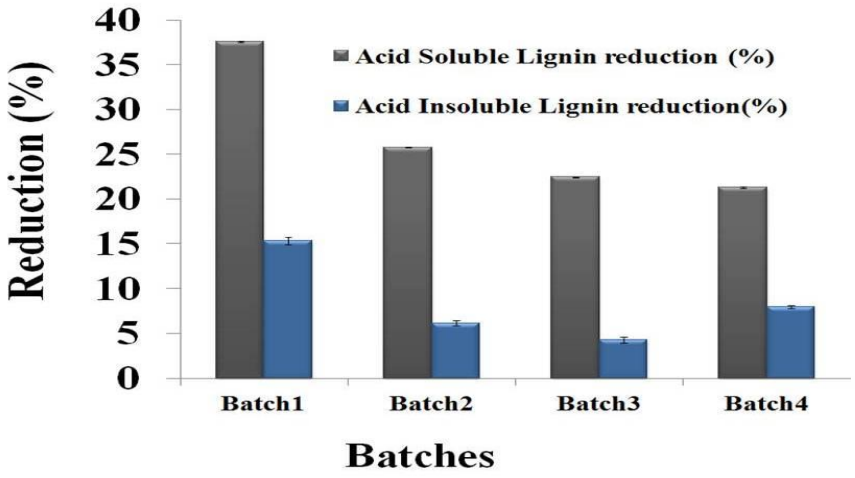

Figure 3.6. Effect of reusability of niobium oxide on delignification of switchgrass.

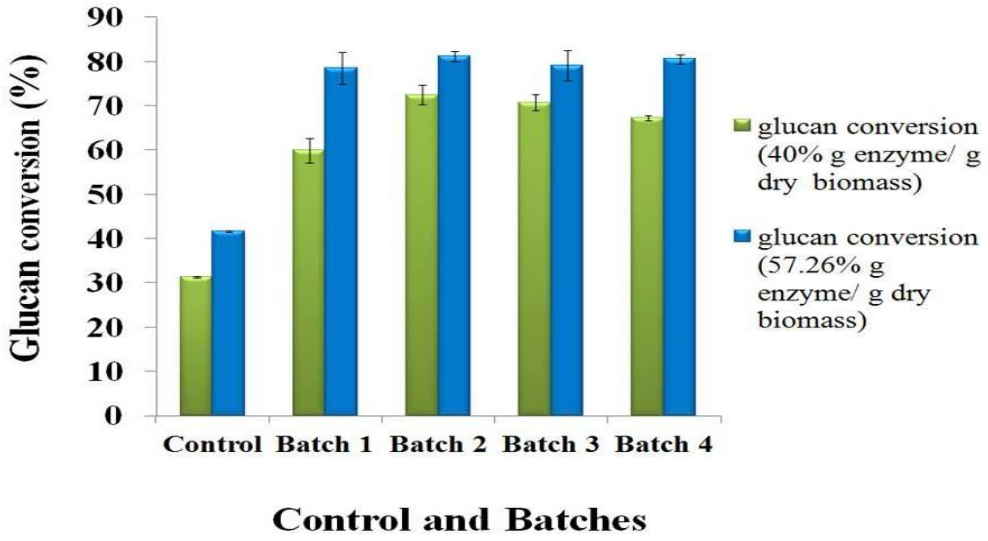

Figure 3.7. Comparison of glucan conversion between control and 4 bacthes

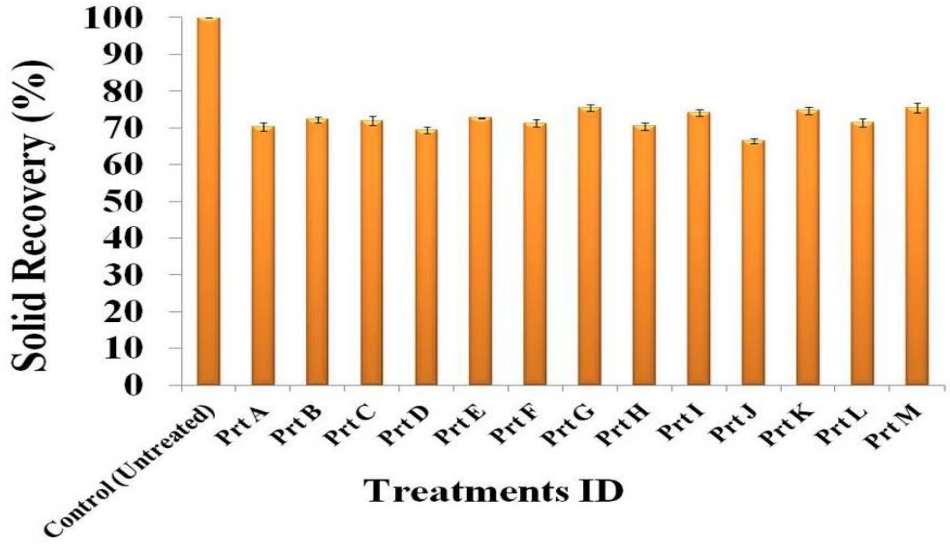

Figure 3.8. Solid Recovery

3.4.5 Solid Recovery

Figure 3.8 shows the solid recovery obtained for the control (untreated) and all treatments.

In our research, an average solid recovery of 71.93 ±0.94 %was obtained, with the minimum and maximum of recovery at 66.35 ±0.61 % and 75.43 ±1.39 %. No significant differences ($P>0.05$) were found between the treatments. In comparison, the solid recovery obtained in this study was higher compared to other pretreatments tested previously. For example, the recovery for dilute acid SO_2, liquid hot water, soaking in aqueous ammonia, and lime reported were 60.4, 62.4, 60.1, 62.1 and 65.2 % respectively (Wyman et al., 2011). The recovery recorded through this study indicated that, the major component of switchgrass such as lignin and carbohydrate might collapse during the treatment. A study of 0.5%NaOH reported that the maximum solid recovery obtained was about 57.7 % (Xu et al., 2010) which is lower compare to our study. Overall, as higher catalyst loading increased, the solid recovery was found decreased.

3.4.6 Optimization of experimental conditions

Efficiency and economics are two important considerations for pretreatment of lignocellulosic biomass (Agbor et al., 2011, Karunanithy and Muthukumarappan, 2011). Hence, out of 13 different treatment combinations evaluated, treatment G (60 °C, 120 min, 0.25 g g^{-1}) and B (30 °C, 120 min, 0.625 g g^{-1}) have been selected as two best conditions based on higher conversion of sugar after 72 h hydrolysis. The reason of selecting 72 h instead of 168 h was based on fact that the conversion obtained after 168 h was only 19%

higher than 72 h. However, based on the analysis performed by JMP package, the optimal conditions suggested to maximize glucan in pretreated and glucose release after 72 h hydrolysis are presented in table 3.2 below.

Table 3.2. Treatment condition suggested (generated from JMP)

Goal / Conditions	For maximizing glucan in solid biomass	For maximizing glucose during hydrolysis (72 h)
Temperature (°C)	49.025	35.425
Reaction time (min)	76.272	149.58
Catalyst loading (g g^{-1} raw biomass)	0.614	0.403

3.5 Conclusion

Niobium oxide catalyst was evaluated as a pretreatment agent for delignifiying switchgrass. Batch experiments indicated that niobium oxide was able to reduce lignin concentrations up to 60%. Hydrolysis of niobium oxide treated switchgrass using Cellic®Ctec2 yielded 0.196 g g^{-1} glucose and 0.04 g g^{-1} xylose. The catalyst was reused four times without significant loss of activity. Such catalysts are expected to lower pretreatment costs and eventually reduce the costs of biomass to ethanol processes and make ethanol production green and environmental friendly.

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CHAPTER 4: MECHANISM OF DELIGNIFICATION OF SWITCHGRASS DURING NIOBIUM OXIDE PRETREATMENT

4.1 Abstract

The purpose of this chapter is to investigate the role of niobium oxide as a pretreatment agent for switchgrass. Alamo switchgrass was pretreated with 0.25 g g^{-1} of niobium oxide at 60 °C for 20-120 min in a 150-mL batch reactor. Subsequently, switchgrass samples were characterized using scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and Brunauer-Emmett- Teller (BET) surface area. Results indicated that maximum delignification for acid soluble lignin and acid insoluble lignin was 37.54 % and 16.48% respectively after 120 minutes of pretreatment. Analysis of the switchgrass samples using SEM indicated that niobium oxide pretreatment was able to disrupt the external structure of switchgrass. The structural disruption was also supported by the BET analysis which revealed that the specific surface area of switchgrass was increased by 31% as a result of pretreatment. In addition, XPS analysis of the pretreated surface indicated an increase in surface oxygen content which suggested that niobium oxide was able to delignify switchgrass via selective oxidation.

Keyword: Niobium Oxide Pretreatment, Switchgrass, Reaction Time, Lignin, Surface Area.

4.2 Introduction

Due to an increasing problem of CO_2 emissions and shortage of crude oil supply, there is a surge in renewable energy research from lignocellulosic biomass. Switchgrass is one such lignocellulosic biomass that can serve as a feedstock for fuel alcohol production without competing with food and feed. Hence several research groups throughout the world are focusing on ethanol production from switchgrass. Typically, conversion of switchgrass to ethanol involves pretreatment of the biomass matrix, followed by hydrolysis and fermentation. Among the three aforementioned steps, pretreatment is one of the most challenging and expensive. Several researches such as (Wyman et al., 1992, Chung et al., 2005, Dien et al., 2006, Wyman et al., 2011, Zhou et al., 2011, Chang et al., 1997, Wang et al., 2008 and Xu et al., 2008, Alizade et al., 2005, Kurakate et al., 2001, and Vidal and Molinier., 1998) have used acids, bases, ammonia, steam, ozone, and others for pretreating switchgrass. Most of these were found to be effective; however, these chemicals are harsh, environmentally unfriendly, and cannot be recycled. Hence, newer, cheaper, safer, and greener pretreatment processes have to be developed for efficient conversion of biomass to ethanol.

We have been testing solid catalysts as pretreatment agents for switchgrass. In one of our recent work, we employed solid niobium oxide catalyst to pretreat switchgrass. Our results indicated that niobium oxide was able to significantly delignify switchgrass with moderate effect on sugars. However, the mechanism of niobium oxide pretreatment is not clear. Hence

in this work we investigated the role of niobium oxide in delignification of switchgrass and sugar production.

In this particular study, niobium oxide pretreatment, classified as chemical pretreatment, has been applied with the aim to alter the structure of switchgrass. Specifically, in this research, the goal was to observe the effect of retention time selected to several outputs such as pH, lignin content, sugar availability and sugar conversion after hydrolysis stage. The catalyst loading and temperature of the pretreatment were set at 0.25 g/g raw biomass and 60^0C, respectively, while the retention times were maintained at 20, 40, 60, 80, 100 and 120 minutes. The results were analyzed using SAS 9.2 software.

4.3 Materials and Methods

4.3.1 Biomass preparation

Alamo switchgrass used in this study was obtained from Reedy Creek Road Field Laboratory located on the west edge of Raleigh, NC. The switchgrass was harvested in mid July 2011, field cured, and baled with a conventional square hay baler. A subsample was collected, ground to pass through a 2 mm sieve, and stored at room temperature in tightly sealed plastic bags until analyzed for composition and subsequent pretreatment with niobium solid acid.

4.3.2 Niobium oxide pretreatment

Niobium (V) oxide (Nb_2O_5) obtained from Fisher Scientific Co was used as an acid catalyst for pretreatment of switchgrass. Typically, 6 g of switchgrass was mixed with 90 mL deoinized (DI) water (15% loading) in a 120- mL serum bottle (reactor). Based on our previous research, a catalyst loading of 0.25 g/g raw biomass and a pretreatment temperature of 60^0C were employed. After adding a predetermined amount of catalyst to the switchgrass slurry, the serum bottles were sealed and contents of the reactors were stirred (400 rpm) on a temperature-controlled hot plate for a predetermined time. Subsequently, the switchgrass was separated from the slurry via filtration and washing with 100 mL with DI water to remove any catalyst particles. The recovered switchgrass was hydrolyzed while the catalyst was stored for subsequent reuse.

4.3.3 Enzymatic Hydrolysis

Enzymatic hydrolysis of the pretreated switchgrass was performed in 50-ml centrifuge tubes placed in automated shaking water bath (50 °C). A 20-mL slurry (pH =5) consisting of 1 g (db) of pretreated switchgrass (equivalent to 5% solid loading), *Cellic®Ctec2* (Novozymes North America, Franklinton, NC) (equivalent to 57. 26 % (g enzyme/ g dry biomass) with a density of 1.203 g/ ml along with 0.05 M sodium citrate buffer was agitated at 150 rpm for 72 hours. In addition, 40 µg/ml of tetracycline hydrochloride was added to the tubes to minimize bacterial growth during hydrolysis. After hydrolysis, the hydrolyzates were

centrifuged and the supernatant was stored for a short time period at -4^{0}C and -80^{0}C for a long time for sugar analysis.

4.3.4 Experimental design and statistical analysis

All the treatments in this study were conducted in triplicate. Since there are 6 retention times selected with triplicate, so a total of 18 samples were evaluated through the study. Data obtained from untreated samples was considered as reference data for comparison purposes. A simple linear regression model procedure with tukey adjustment from SAS 9.2 software (SAS Institute Inc., Cary, NC) was used for all the analysis. Additionally, excel solver to analyze initial rate degradation constants of lignin and carbohydrate assuming first kinetic model was used in this study.

4.3.5 Analytical method

Moisture, lignin, and sugar contents of the raw and pretreated switchgrass were determined using National Renewable Energy Laboratory (NREL) procedures (Sluiter et al., 2005a, b, 2008). Carbohydrate content and monomeric sugars such as glucose, xylose and arabinose for hydrolysate samples were determined using high-performance liquid chromatography (HPLC) (Dionex UltiMate 3000, Dionex Corporation, Sunnyvale, CA, USA) equipped with a refractive index detector and an Aminex HPX-87H column. Separations were performed at 65 °C using a 0.6 mL min^{-1} of 5 mM H_2SO_4. The products were quantified by comparing with standards of

glucose, cellobiose, xylose and arabinose (0.625-10 g L^{-1}). The yields of monomeric sugars were calculated based on per gram of raw biomass.

4.3.6 B.E.T Analysis

B.E.T analysis is a technique used to determine the specific surface area of materials. Switchgrass samples prepared for this particular test were dried in the vacuum oven at 40 ^{0}C for 24 h. A minimum of 0.5 g of sample was required for the B.E.T to successfully determine the surface area. The sample was transferred into a test tube for degassing at 150 ^{0}C for 2 h. The adsorbate, nitrogen gas in this case, is injected into the sample cell with a calibrated piston. Subsequently, the samples were analyzed using a BET surface area analyzer.

4.3.7 X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) has been used to analyze surface changes of untreated and pretreated switchgrass. Samples for this analysis were prepared by drying the samples in a vacuum oven for 24 h at 40 ^{0}C until the moisture content was less than 5%. X-Ray photoelectron spectroscopy data were collected using a Riber LAS-3000 with MgKα excitation (1254eV). Energy calibration was established by referencing to adventitious Carbon (C1s line at 285.0 eV binding energy). The takeoff angle, incidence angle, and x-ray source to analyzer were maintained at ~75°, ~20°, ~55°, respectively while the base pressure in the analysis chamber was maintained around 10^{-10} Torr .

4.3.8 Scanning electron microscopy (SEM)

Samples used for scanning electron microscopy generally have to be vacuum compatible, which in this case meant that they must first be dried. Around 2 g of each sample were dried under vacuum at 400C for up to 48 hours. The samples then dried again to 0% moisture in a liquid nitrogen drying system. The samples were then coated with 10nm of Au/Pd (60/40) and inserted into a Hitachi S-3200N SEM at the Analytical Instrumentation Facility at NC State University. Images were collected using an Everhart-Thornley secondary electron detector at 5kV.

4.4 Results and discussions

4.4.1 Delignification of switchgrass

Reaction time, together with temperature and catalyst loading, has been observed to influence the severity of the biomass and consequently improve the effectiveness of hydrolysis. As the pretreatment reaction times increased (20-120 min), the color or the appearance (from the visual observation) of the treated switchgrass was found not significantly different with the control. In fact, there was a previous study stated in which, after pretreatment, including dilute acid pretreatment, as reaction time increased the biomass tended to be dark-brown in appearance (Foston and Ragauskus, 2010, Donohoe et al., 2011) which is different from results obtained through this

study. As presented in figure 4.1, the study was also observed control background labeled as time 0 which contained untreated switchgrass placed into deionized water in a chamber. Interestingly, as shown in figure 4.1, the pH dropped significantly (0-20 min) and remained stable between 20 up to 120 minutes. Through this observation, it seems that at time 0 min, the system purely represented the pH of deionized water alone which was about 6.36. However, after 20 min of reaction, the pH was dropped to a range of 5.86-5.89. Even though, the dropping was observed, but in general, it was also significantly higher and nearly neutral than dilute sulfuric acid that is found normally turn the pH to be (< 2.5) (Schell et al., 2003). Based on the pH range of liquid hydrolyzate in this study, it is suggested that our treatment could be categorized as weak acid treatment. Moreover, the initial lignin degradation rates (0-20 min) for acid soluble and acid insoluble lignin were estimated to be 0.0209 mg/ (g raw-min), while acid insoluble lignin was measured at 0.1196 mg/ (g raw-min). The delignification levels were summarized in figure 4.2, suggest that the highest delignification of acid soluble lignin was found to be 37.54 %, while the highest removal of acid insoluble lignin was achieved corresponding to 16.48 %. Interestingly, both highest delignifications happened at 120 min of the reaction time. As a comparison, a previous study reported that dilute acid pretreatment was effective to remove only a minor part of acid soluble/ acid insoluble lignin (Nlewem and Thrash, 2010). Li et al, 2010 reported during dilute acid treatment acid soluble lignin and insoluble lignin of *Alamo* switchgrass were decreased by 13.7 and 8.7 %

respectively, which is lower compared to our results, however Wyman's group reported acid insoluble lignin of *Dacotah* switchgrass in their study was decreased to 16% from the original amount (Wyman et al., 2011). As our results show mild pH range constant above 5, than it is clear to conclude that this pH range can also minimize the hydrolysis to monosaccharides, and therefore the formation of degradation products during the pretreatment (Kumar et al., 2011, Mosier et al., 2005, Hendriks and Zeeman, 2009). Thus, in this study, it is believed that, neutralization of the treated switchgrass is not necessarily to be conducted after treatment.

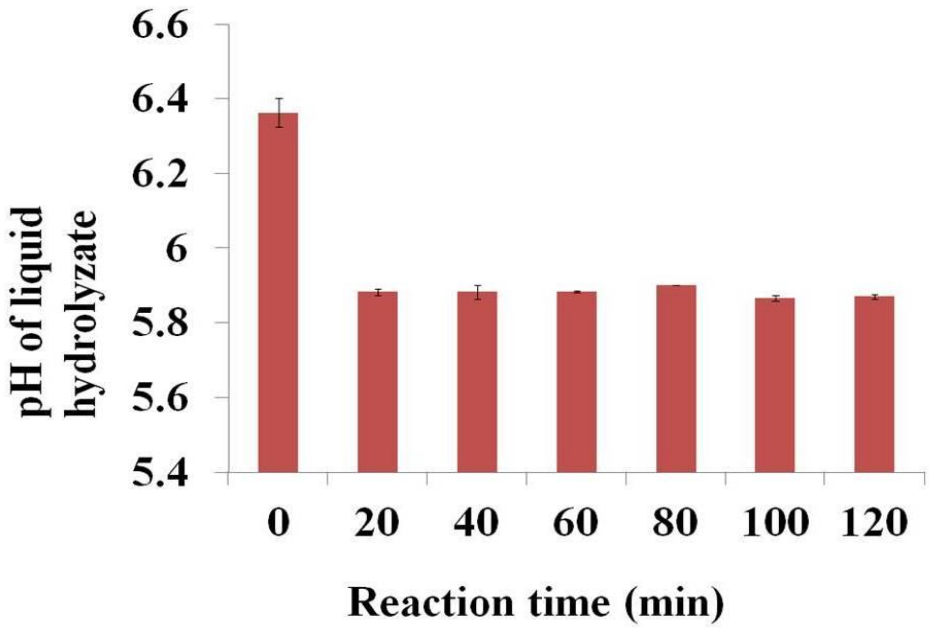

Figure 4.1. pH for the different reaction time.

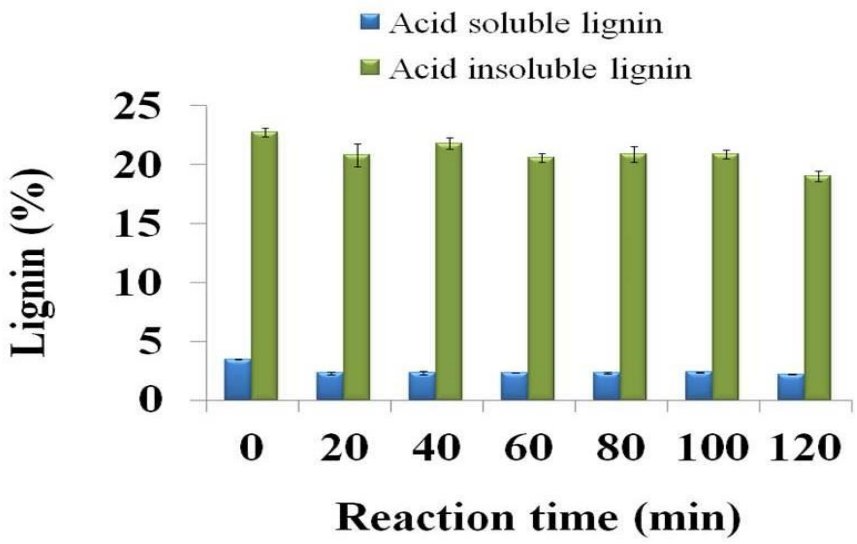

Figure 4.2. Delignification of lignin

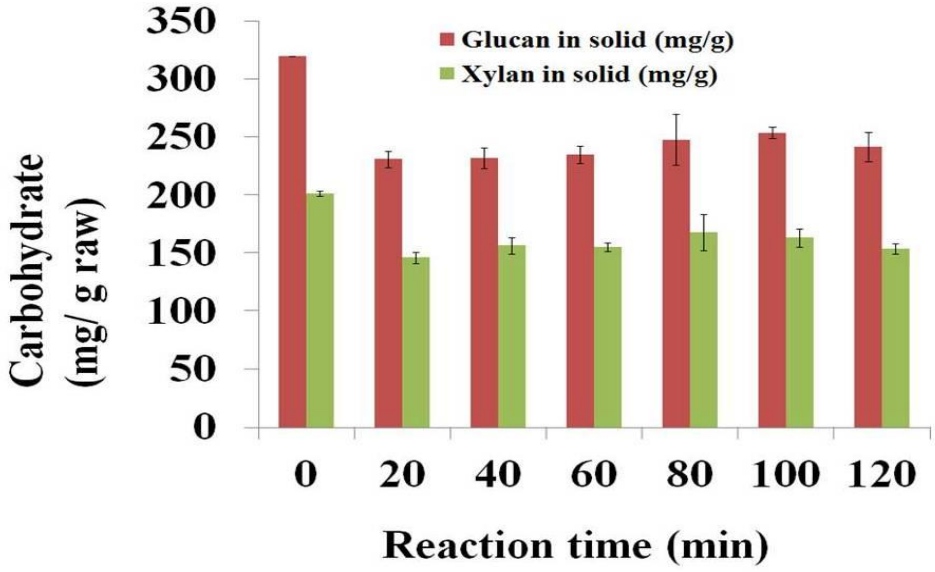

Figure 4.3. Glucan and Xylan in the solid switchgrass presented as mg/ g raw biomass.

4.4.2 Carbohydrate composition

All samples after pretreatment were analyzed in order to investigate the availability of polysaccharides remaining in the solid biomass that can be hydrolyzed into fermentable sugar. Typically, liquid of acid soluble lignin prepared based on NREL protocol was analyzed using HPLC to detect the carbohydrate content of switchgrass as described from section 4.3.5. After 20 min of retention time, it can be seen from figure 4.3 that the glucan and xylan were dropped significantly to a level of 72.15% and 72.58% respectively from the original amount present in control (0 min). However, when initial rate method was applied, it was observed that degradation rates for both glucan and xylan were measured to be 0.0163 mg/ (g raw-min) and 0.0160 mg/ (g raw-min), respectivelly. Interestingly, these two were similar, suggesting that niobium oxide pretreatment degrades glucan and xylan similarly. Moreover, for the durations (20 -120) min, levels of glucan and xylan were observed remained nearly stable as can be seen from figure 4.3.

At any of these pretreatment conditions, it was observed that the average of glucan plus xylan available in the pretreated biomass were about 240.21 mg g^{-1} and 157.11 mg g^{-1} respectively. By applying theoretical ethanol formula, in average, there is 69.3 gallons of ethanol per dry ton of feedstock can be produced from the amount of carbohydrate (glucan+ xylan) left it the solid biomass after treatment. In addition, glucose and xylose were also released during pretreatment were about 26.88-36.18 mg g^{-1} raw and 25.53-34.77 mg g^{-1} raw respectively.

These data provide evidence that, in overall, the mechanism of Niobium pretreatment happened to decrease lignin, but also was found to affect partially glucan and xylan.

In fact, the results and mechanism of acid pretreatment obtained in our study was different compared to others acid treatments ((1wt% (140^0C), 0.5wt% (160^0C), 1.2% (160^0C)) of H_2SO_4 that were found removing the xylan almost completely and fairly ineffective at the removal of the majority of lignin (Wyman et al., 2011, Kim et al., 2011, Li et al., 2010). Based on a previous study reported the low pH (**<3**) of the medium cause the precipitation in which can solubilized lignin and also catalyzes the degradation of hemicelluloses (Kumar et al., 2011). It seems that there is a straight correlation between the pH of the system with the amount of hemmicellulose removed. As discussed earlier in previous section, that pH in our study range constant above 5, thus, it was observed mild pH was not found effectively removing the hemmicellulose. Consequently, after being treated, higher percentage of xylan was remained in the solid biomass.

4.4.3 Sugar yields

Hydrolysis was conducted for 72 h as described from 4.3.3. All the pretreatments and control (untreated) switchgrass used the same amount of the enzyme of 57.26% (g enzyme/ g dry biomass). The amount of enzyme used accounted for 2. 38 % of the total volume of the hydrolyzate in each sample tube.

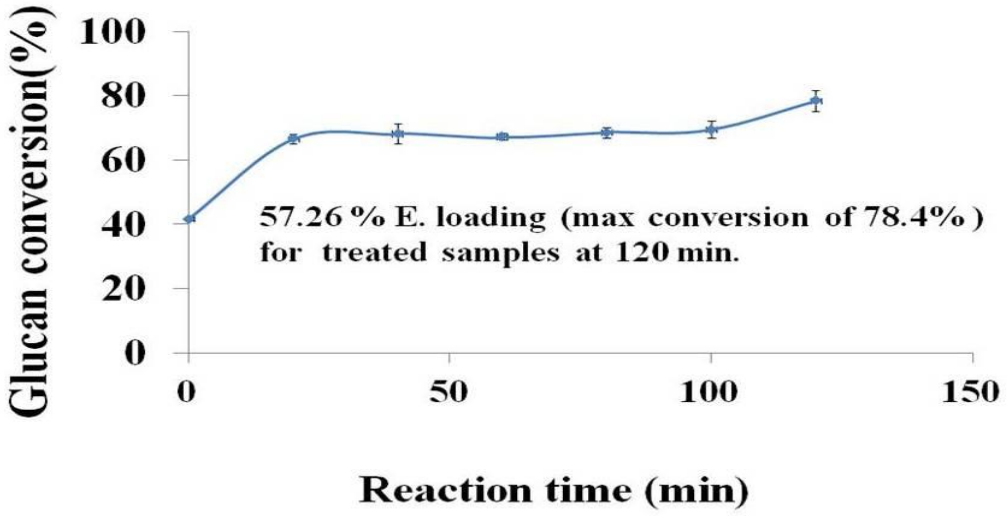

Figure 4.4. Glucan conversion

It was observed that higher glucose yields and the conversion rates also reflected digestibility of the pretreated switchgrass. The glucose conversion increased initially and remained constant between 20 and 100 min. However after 100 min, the glucan conversion increased significantly (**$p<0.05$**). This evidence indicates that there was a strong correlation between the removal rate of lignin and digestibility of the biomass. As shown from figure 4.2, the highest delignification for acid soluble and acid insoluble lignin was observed at 120 min. It was found samples pretreated for 120 min provided a glucose yield of 221.525 mg/ g raw biomass respectively, corresponding to 78.39 % perhaps due an optimal delignification. Additionally, the surface area of the 120-min pretreated samples increased from 0.6540 m^2/g to 0.8552

m^2/g (a 31 % increase) because of removal of lignin that might have resulted in increased porosity of the switchgrass matrix.

The glucan conversion in this study is comparable to several dilute acid pretreatment studies conducted previously. A glucose yield of 83 % was achieved when the study used Spezyme CP alone (type of enzyme); when the combination with Novozyme 188 added, the conversion increased up to 90% (Wyman et al., 2011, Li et al., 2010) reported total processing time over 72 h hydrolysis was able to reach 85% glucose yield. Chung et al., 2005 reported optimal conditions of 1.2% (w/w) at 180^0C of dilute sulfuric acid pretreatment achieved a cellulose conversion of 90%. As the enzyme used in this study reported was blended with aggressive cellulases, high level of β-glucosidase and hemicellulase, however, cellobiose was still detected in the hydrolysate liquid. As shown in figure 4.5, it confirmed that in this study, higher loading of 57.26% of *Cellic®Ctec2* still found not to efficiently enough to get higher glucan conversion. Thus, in order to convert the leftover of cellobiose, higher enzyme loading might be needed, however, enzyme dose need to be significantly reduced to make the conversion process more feasible (Wyman 2007). For that reason, optimization of pretreatment conditions is one of the most important stages in the development of an efficient and economic pretreatment method.

β-1, 4 glucanase β- glucosidase

Cellulose ----------→ Cellobiose ----------→Glucose

Figure 4.5. Kinetics of cellulose hydrolysis

4.4.4 Effect of Niobium solid acid pretreatment on structural change

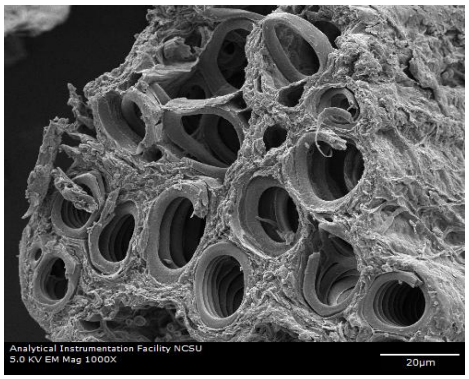

Figure 4.6. Control (Untreated Switchgrass)

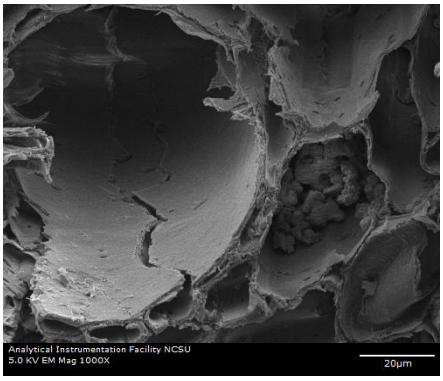

Figure 4.7. Pretreated Switchgrass (Treatment L)

Micrographs obtained from SEM revealed that pretreatment of switchgrass with niobium oxide significantly altered the surface structure. Figure 4.6 and 4.7 show sample micrographs of untreated and pretreated samples. As can be seen from figure 4.6, untreated switchgrass had a compact structure, well defined edges and was highly fibrilar. However, niobium oxide pretreatment significantly altered the fibrilar structure. The structure appeared to be disrupted and broken as a result of pretreatment. Specifically, as shown in figure 4.7 the rigid inner and outer parts of the biomass were disrupted and broken. Since the majority components of the inner part (primary and secondary wall) are cellulose and hemicelluloses, niobium oxide treatment was found to have broken down some component of the inner part which resulted in a moderate loss of sugars. Moreover, the lignin (outer surface) was also found to be disrupted, there was a partial relocation of fibril at the surface observed. Owing to its acidic nature, niobium oxide is expected to have oxidized the lignin present in switchgrass resulting in reduction of total lignin concentration. These results are consistent with earlier observation that high glucose yields are obtained from pretreated switchgrass than untreated switchgrass. As a comparison, (Donohoe et al., 2011, Marzialetti et al., 2010) reported a similar trend that took place throughout their study, in which, the structure of untreated switchgrass was found to be orderly with a smooth surface cell wall while samples after pretreatments were found to alerted surfaces.

Our observations were also consistent with the data obtained from XPS analysis. As expected, carbon was the biggest compound detected (87.68%) followed by oxygen (12.32%). When pretreated sample was analyzed, carbon content was found to be lower (about 84.19%); but oxygen was found to be higher (15.10%) than control. In other words, O/C ratio of on the surface of the pretreated sample was increased by 25% increased in O/C ratio as can be seen from figure 4.8. This additional oxygen was predicted to have come from the oxidation reaction of solid niobium oxide (Nb_2O_5) catalyst with the surface of switchgrass. In addition, an additional peak of nitrogen for 0.71% was also observed on the pretreated sample surface suggesting that the nitrogenous compounds were exposed as a result of lignin removal from the surface.

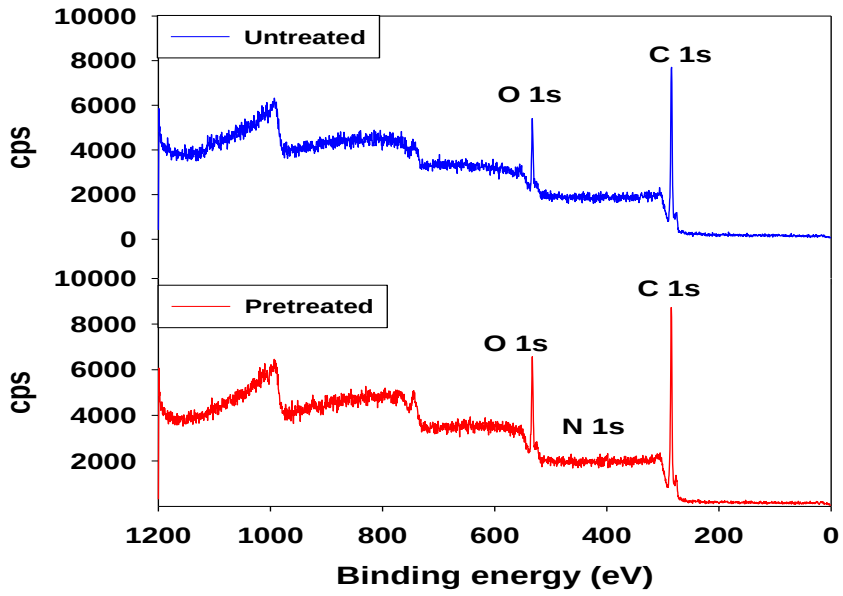

Figure 4.8. XPS result for untreated and pretreated switchgrass

4.5 Conclusions

Our results indicated that niobium oxide delignified switchgrass primarily via oxidative mechanism. Niobium oxide disrupts the structure of switchgrass and increases the specific surface area thereby allowing better enzyme adsorption on the surface. The maximum glucose yield was obtained after 120 min of pretreatment. In addition to increased internal surface area, decreased lignin content and the increased amount of oxygen, other characteristics such as crystalinity, degree of polymerization and other internal component rearrangement may have also played a role in improving the carbohydrate digestibility.

4.6 References

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Wyman, C. E., Balan, V., Dale, B.E., T.B., Elander, R.T., Falls, M., Hames, B., Holtzapple, M., Ladisch, M. R., Lee,Y. Y., Mosier, N.S., Pallapolu, V.R., Shi, J., Thomas, S.R., Warner, R.E. Comparative data on effects of leading pretreatments and enzyme loadings and formulations on sugar yields from different switchgrass sources. Bioresource Technol. 2011, 102, 11052-11062.

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CHAPTER 5: CONCLUSIONS AND PROPOSE FUTURE WORK

5.1 Conclusions

Lignocellulosic biomass is a promising feedstock to produce fermentable sugar for the production of ethanol. However, the complexity of lignocellulosic biomass is a major challenge that has to be met with to commercialize production of biofuels from lignocellulosic biomass. For example, pretreatment is invariably necessary to prepare the biomass for subsequent processing. In this research a novel solid acid catalyst, niobium oxide, was explored as a pretreatment agent for processing switchgrass. Our results indicate that:

1. Niobium oxide is a an effective catalyst for pretreatment of switchgrass

   In this study, solid niobium oxide acid catalyst was tested for pretreatment of switchgrass. In general, the results obtained through this study are comparable with other studies conducted previously, as described from chapter 3 and 4. Overall results of our study are summarized below.

   a. From the total (13 X 3) experiments generated using JMP statistical software, the highest delignification of acid soluble lignin and acid insoluble lignin corresponding to a reduction of 43.93% and 20.28 % were achieved from treatment E (60 °C, 30 min, 0. 25 g g^{-}) and C (90 °C, 30 min, 0. 625 g g^{-})

respectively. This removal rates also have been confirmed by the results obtained from SEM analysis that indicating about the structural changing after treatment.

b. All 39 samples were hydrolyzing using 40% (g enzyme/g dry biomass) for 72 h and 168 h. The glucan conversions for all samples were increased from 72 h to 168 h with corresponding to the maximum conversion of 59.9 % and 77.5%, respectively.

c. Higher glucose yields and conversion rates have reflected the digestibility of the pretreated switchgrass. After lignin was removed, internal surface area was found increased up to a 31 % than control. Data from XPS analysis also showed an increase in oxygen on the surface of the pretreated samples and a 25% increased in O/C ratio.

d. The catalyst from treatment G(60 °C, 120 min, 0. 25 g g^{-}), has been used up to four times, and still performed a high efficiency to reduce the lignin and altered the structure, thus increased the hydrolysis efficiency.

e. The second part of the study was trying to link the efficiency of the pretreatment as time increased. The highest delignification of acid soluble and acid insoluble lignin achieved at 120 minutes corresponding to 37.54 and 16.48 % of reduction.

f. It was observed at 120 minutes reaction time, the higher glucose yield and conversion rate were achieved.

2. Niobium oxide was found to have almost a neutral pH (5.86-5.89), thus neutralization after the treatment is not necessarily needed.

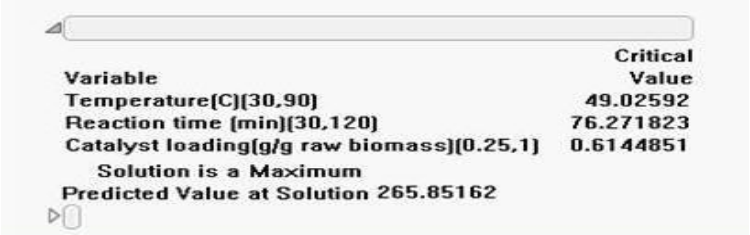
5.2 Future Work

Even, there are still a limited number of studies conducted to examine solid niobium oxide catalyst in the pretreatment of lignocellulosics biomass, however, through the data obtained in our study, it is suggested that niobium oxide catalyst should be included as one of the pretreatment methods as this catalyst found effectively removed the lignin to a certain number and resulted in higher glucan conversion compared to the untreated samples. However, there are also some aspects that are important to consider for the future uses, especially for the pretreatment of the solid phase. Filtration is one of the very critical steps as this process may enable the study to recover the right portion of solid biomass after the treatment conducted and separate the catalyst for the future uses. As the data from all the experiments obtained a lower conversion of xylan to xylose, it is suggested for the future study, additional tests are needed to be conducted in order to make sure if byproducts are also being generated during the process that might prevent the enzymes to work converting the hemicelluloses into a fermentable sugar.

APPENDICES

Appendix 1. Output from JMP Statistical Analysis

1. Pretreatment condition suggested to achieve maximum glucan left

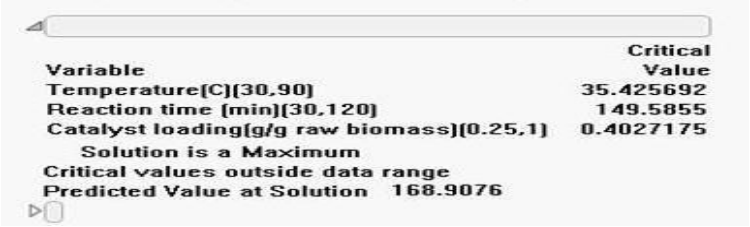


Variable	Critical Value
Temperature(C)(30,90)	49.02592
Reaction time (min)(30,120)	76.271823
Catalyst loading(g/g raw biomass)(0.25,1)	0.6144851

Solution is a Maximum

Predicted Value at Solution 265.85162

2. Pretreatment condition suggested to get maximum glucose after 72h hydrolysis



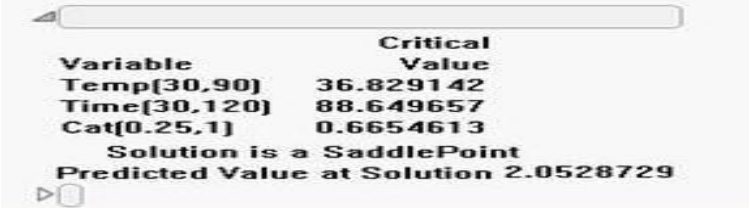
Variable	Critical Value
Temperature(C)(30,90)	35.425692
Reaction time (min)(30,120)	149.5855
Catalyst loading(g/g raw biomass)(0.25,1)	0.4027175

Solution is a Maximum

Critical values outside data range

Predicted Value at Solution 168.9076

3. Pretreatment condition suggested to get minimum Acid Soluble Lignin

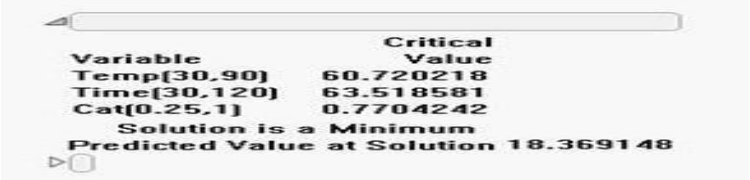


Variable	Critical Value
Temp[30,90]	36.829142
Time[30,120]	88.649657
Cat[0.25,1]	0.6654613

Solution is a SaddlePoint

Predicted Value at Solution 2.0528729

4. Pretreatment condition suggested to get minimum Acid Insoluble Lignin



Variable	Critical Value
Temp[30,90]	60.720218
Time[30,120]	63.518581
Cat[0.25,1]	0.7704242

Solution is a Minimum

Predicted Value at Solution 18.369148

Appendix 2. Table data for Chapter 3 and 4.

Table 1. Composition of untreated and treated of switchgrass (13 experiments)

ID	Glucan in solid switchgrass (mg/g raw)	Xylan in solid switchgrass (mg/g raw)	Acid Soluble Lignin (%)	Acid Insoluble Lignin (%)	Glu at 72 h hydrolysys (mg/g raw)	Glu at 168 h hydrolysys (mg/g raw)
Ctrl	320 [a,b] (0.271)	201 (2.182)	3.46 [c] (0.036)	22.778 (0.367)	111.11 (0.651)	152.57 (2.783)
A	249 (12.054)	129 (7.372)	2.026 (0.028)	19.099 (0.79)	140.96 (7.097)	173.54 (7.229)
B	248 (2.868)	127 (1.406)	2.095 (0.040)	19.601 (0.756)	160.10 (3.223)	190.72 (7.134)
C	253 (4.569)	144 (2.324)	2.053 (0.060)	18.157 (1.073)	138.22 (0.561)	179.12 (2.895)
D	231 (2.049)	159 (1.998)	1.974 (0.023)	20.995 (0.398)	124.94 (3.986)	176.51 (3.186)
E	239 (3.077)	124 (3.637)	1.94 (0.034)	19.936 (0.617)	138.58 (1.426)	187.33 (0.339)
F	251 (9.251)	141 (2.028)	2.194 (0.046)	19.94 (0.987)	131.09 (1.3020	179.26 (2.577)
G	254 (14.054)	157 (4.105)	2.159 (0.107)	19.273 (0.484)	169.16 (2.845)	196.88 (4.464)
H	254 (12.428)	148 (2.188)	2.04 (0.005)	18.6 (0.144)	120.86 (0.899)	181.61 (2.833)
I	263 (6.489)	144 (5.732)	2.19 (0.023)	19.66 (0.848)	127.99 (9.303)	190.19 (6.076)
J	228 (2.936)	128 (4.794)	2.24 (0.017)	20.74 (0.361)	105.60 (1.146)	169.65 (2.441)
K	258 (2.242)	147 (3.700)	2.08 (0.039)	18.7 (1.252)	151.22 (4.866)	192.15 (2.626)
L	238 (8.316)	124 (1.601)	2.013 (0.031)	19.85 (0.790)	135.63 (2.9)	178.99 (2.449)
M	264 (4.211)	138 (2.488)	2.14 (0.066)	18.9 (0.652)	160.44 (1.95)	184.26 (3.218)

[a] values are means (standard error) (n=3)
[b] values were calculated based on per gram of raw biomass
[c] values were calculated based on dry weight (%)
Glu = Glucose

Table 2. Composition of switchgrass (untreated and 4 batches)

Sample Id	ASL (%)	AIL (%)	Glucan (%)	Xylan (%)	Glu mg/g raw) LL	Glu mg/g raw) HL
Control	3.46 (0.064)	22.778 (0.637)	[a]32 (0.019)	[b]20.1 (0.01)	[c]111.11 (0.651)	148.08 (0.589)
Batch 1	2.159 (0.186)	19.273 (0.84)	25.4 (0.095)	15.7 (0.038)	169.166 (2.868)	221.525 (3.267)
Batch 2	2.567 (0.032)	21.37 (0.538)	25.8 (0.038)	17.3 (0.071)	207.824 (9.67)	232.979 (9.107)
Batch 3	2.602 (0.057)	21.8 (0.613)	25.6 (0.029)	17.6 (0.047)	201.327 (2.218)	224.972 (4.886)
Batch 4	2.723 (0.015)	20.96 (0.319)	25.7 (0.03)	18.5 (0.009)	192.159 (2.102)	230.023 (4.171)

[a] values are means (standard error) (n=3)
[b] values were calculated based on dry weight (%)
[c] values were calculated based on per gram of raw biomass
LL = lower loading: (40% enzyme)
HL = higher enzyme loading: (57.26% enzyme)
Glu = Glucose

Table 3. Composition of switchgrass (as pretreatment time increased)

Reaction time (min)	Glucan (mg/g raw)	Xylan (mg/g raw)	ASL (%)	AIL (%)	Glu (mg/g raw)	pH of liq hydrolysate
0 (control)	[c]320.08 [a](0.08)	201.22 (2.182)	[b]3.46 (0.036)	22.77 (0.367)	148.05 (0.589)	6.363 (0.038)
20	230.94 (6.932)	146.06 (4.904)	2.275 (0.09)	20.83 (0.986)	177.52 (3.824)	5.883 (0.008)
40	231.97 (9.284)	156.54 (7.22)	2.319 (0.167)	21.83 (0.504)	183.47 (8.011)	5.883 (0.018)
60	235.05 (7.535)	155.29 (3.403)	2.302 (0.021)	20.58 (0.373)	174.73 (2.701)	5.883 (0.003)
80	247.81 (21.82)	167.71 (15.738)	2.309 (0.045)	20.90 (0.694)	179.57 (4.224)	5.9 (0)
100	253.82 (4.87)	163.37 (7.9)	2.35 (0.011)	20.89 (0.394)	189.34 (6.954)	5.866 (0.006)
120	241.67 (12.47)	153.68 (4.266)	2.161 (0.04)	19.02 (0.442)	221.52 (3.267)	5.87 (0.006)

[a] values are means (standard error) (n=3)
[b] values were calculated based on dry weight (%)
[c] values were calculated based on per gram of raw biomass
Glu = Glucose

Appendix 3. Additional images information for Chapter 3 and 4.

Figure 1. Pretreatment conducts

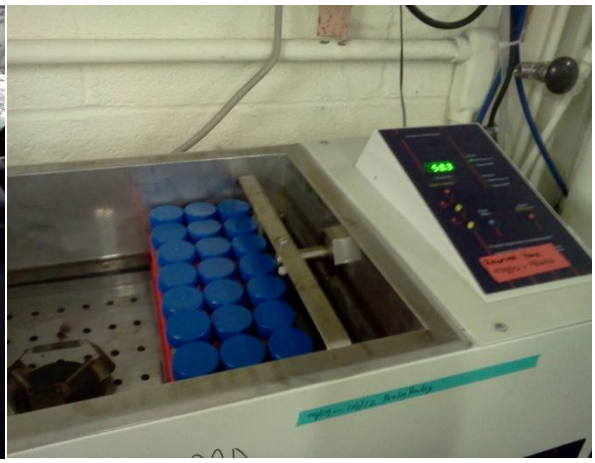


Figure 2. Enzymatic hydrolysis conducts

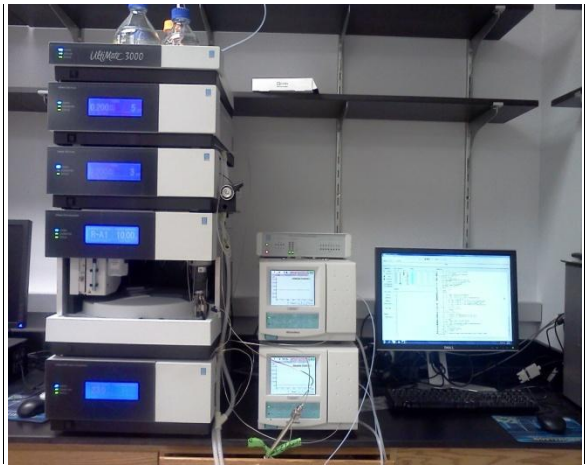


Figure 3. HPLC instrument

Figure 4. BET instrument

Figure 5. Scanning Electron Microscopy

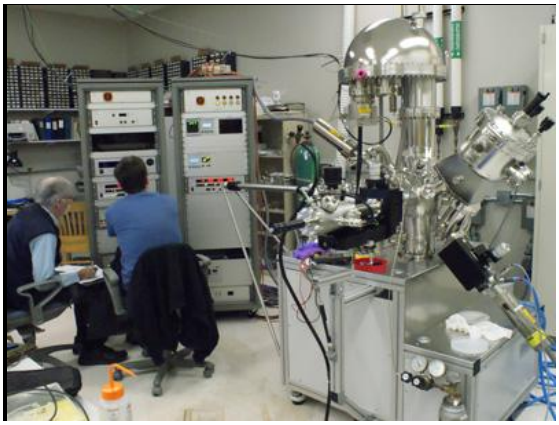
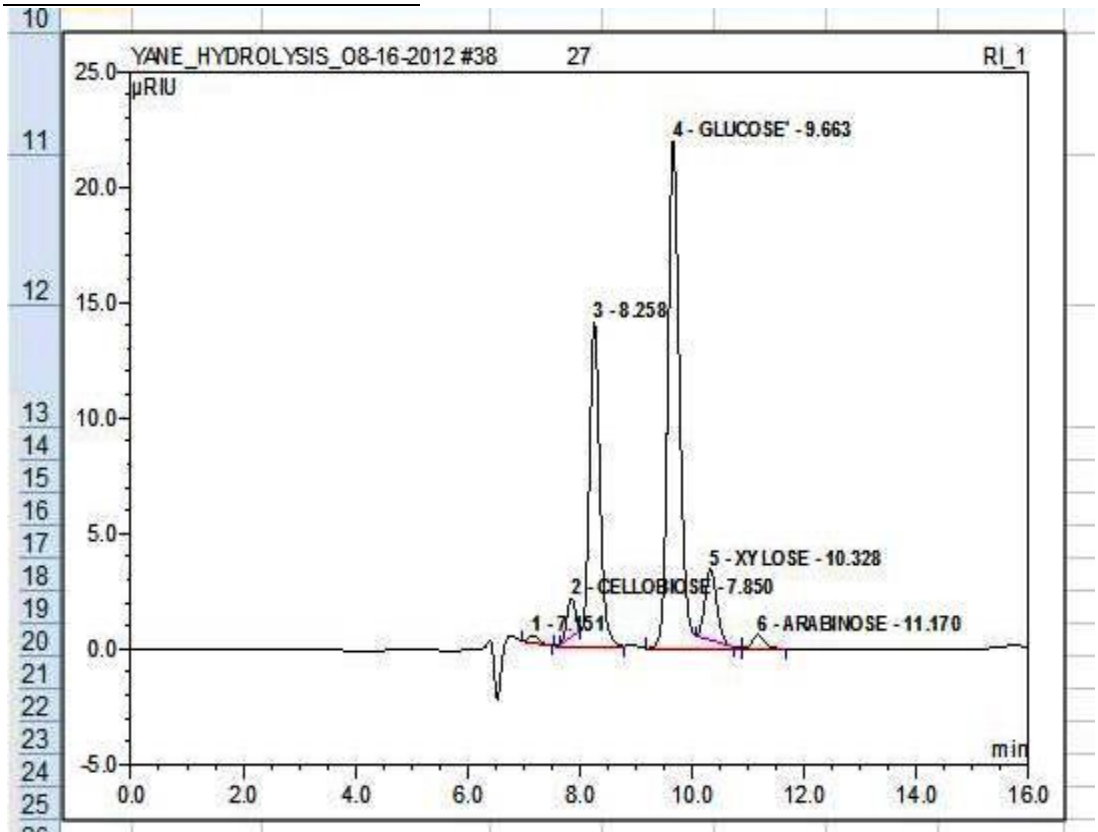


Figure 6. X-ray photoelectron spectroscopy

Appendix 4. Example Output from HPLC



No.	Ret.Time min	Peak Name	Height µRIU	Area µRIU*min	Rel.Area %	Amount	Type
1	7.15	n.a.	0.324	0.075	0.76	n.a.	BMB
2	7.85	CELLOBIOSE	1.639	0.283	2.85	0.152	Ru
3	8.26	n.a.	14.005	3.183	32.06	n.a.	BMB
4	9.66	GLUCOSE'	21.961	5.495	55.36	2.436	BM
5	10.33	XYLOSE	3.064	0.735	7.40	0.337	Rd
6	11.17	ARABINOSE	0.593	0.156	1.57	0.068	MB

Integration Summary

Appendix 5. Standard curve used to calculate amount of monomeric sugars released during enzymatic hydrolysis

Monomeric sugars released during enzymatic hydrolysis have been analyzed using 5 different standard concentrations from 0.625, 1.25, 2.5, 5 and 10 g/l of each glucose and xylose, arabinose and cellobiose.

1. **Standard curves**

Concentration	Area of glucose concentration	Area of xylose concentration	Area of arabinose concentration
0.625	1.7	1.58	1.589
1.25	3.343	3.138	3.166
2.5	6.123	5.774	5.84
5	13.231	12.574	12.819
10	24.522	23.3	23.753

2. **Monomeric sugars conversion**

Glucan conversion (%) $= \frac{Glucose\ released\ in\ hydrolysis}{Glucan\ available\ in\ solid\ x\ 1.111}$ **x 100**

Xylan conversion (%) $= \frac{Xylose\ released\ in\ hydrolysis}{Xylan\ available\ in\ solid\ x\ 1.136}$ **x 100**