

## ABSTRACT

ANSANAY, YANE OKTOVINA. Sulfonic Acid Solid Catalytic Pretreatment and Hydrolysis of Biomass. (Under the direction of Dr Praveen Kolar.)

One of the challenges in the production of bioethanol from lignocellulosic biomass is disruption of the complex structure of the biomass to obtain monomeric sugars via pretreatment. Chemical pretreatments that utilize homogeneous chemicals such as  $H_2SO_4$  are attractive due to the higher reaction rates and mass transfer efficiencies. However acid pretreatment requires special downstream processing in the form of neutralization, which involves costly and inefficient separation from homogeneous reaction mixtures, resulting in a sulfate waste. Therefore, “green agents” such as solid acid catalysts can address some of these challenges by facilitating use of mild operating conditions with higher selectivity, thereby allowing easy separation from products and catalyst reusability. Hence for the present research, it was hypothesized that solid acid catalysts can pretreat and hydrolyze biomasses. Therefore, in this research, supported sulfonic acid catalysts were evaluated as pretreatment and hydrolysis agents for Switchgrass, Gamagrass, Miscanthus x giganteus, and Triticale hay. The objectives were to (1) synthesize, evaluate, and compare sulfonic acid catalysts for pretreatment of switchgrass, (2) evaluate p-toluenesulfonic acid catalyst for direct hydrolysis of switchgrass, and (3) test the efficiency of magnetic p-toluenesulfonic acid catalysts for pretreatment of four types of lignocellulosic biomasses, viz, switchgrass, miscanthus x giganteus, gamagrass, and triticale hay.

For the first objective, three supported sulfonic acid catalysts were synthesized using activated carbon as support and sulfuric acid, p-toluene sulfonic acid, and methane sulfonic acid as precursors to obtain sulfonic acid catalyst (AC-SA), methane sulfonic acid catalyst (AC-MAS) and p-toluene sulfonic acid catalyst (AC-pTSA). The catalysts were evaluated in

batch experiments using three temperatures (30, 60 and 90 °C) and two reaction times (90 and 120 minutes) and switchgrass as feedstock (0.25 g/g raw switchgrass). A proc mixed model was employed to analyze the data along with slice effects test. Results suggested that glucose yields produced after enzymatic hydrolysis ranged between 31.5 and 61.5%, with the maximum yield obtained from switchgrass treated with AC-pTSA at 90 °C for 120 min. Further, results from characterization of catalysts via Boehm titration, BET surface area, TGA, and FTIR analyzers indicated that sulfonation improved the total acidity and lowered pore volume. In addition, the catalyst was reused three times with no significant difference in glucose yields ( $p > 0.05$ ).

For the second objective, AC-pTSA was employed as a catalyst for direct hydrolysis of biomass. For this part of the research, baseline experiments were performed using pure feedstocks including cellulose, starch, and cellobiose. Subsequently switchgrass was used as a feedstock for hydrolysis. In addition, effects of conventional pretreatments such as ultrasonication, NaOH, and H<sub>2</sub>SO<sub>4</sub> were also employed prior to catalytic hydrolysis of switchgrass. Results indicated that for model biomasses, i.e., starch and cellobiose, catalytic hydrolysis resulted in glucose yields of  $190.07 \pm 2.02 \text{ mg g}^{-1}$  and  $237.1 \pm 0.86 \text{ mg g}^{-1}$ , respectively. However, for cellulose, the catalyst exhibited poor activity perhaps, due to strong hydrogen bonding and higher crystallinity resulting in low solubility in the liquid. For raw switchgrass, a glucose yield of  $72.67 \pm 1.03 \text{ mg g}^{-1}$  (conversion of  $23.25 \pm 0.33 \%$ ) was obtained. In addition, ultrasonication prior to catalytic hydrolysis yielded  $16.91 \pm 0.05 \%$  of glucose. Interestingly, chemical pretreatments (NaOH and H<sub>2</sub>SO<sub>4</sub>) of switchgrass actually inhibited the subsequent catalytic hydrolysis and the glucose yields were in the range of  $0.26 - 2.48 \text{ mg g}^{-1}$ .

Finally, to enhance the separation of the catalyst from biomass (after pretreatment), the catalyst was magnetized via chemical impregnation. The catalyst was tested for pretreatment of four types of biomasses viz., Switchgrass, Gamagrass, Miscanthus x giganteus and Triticale hay at 90 °C for 2 h and followed by enzymatic hydrolysis using Ctec2. Data analysis via Proc Glimmix suggested that the glucose yields of magnetic catalysts were similar to regular catalyst, with a maximum yield of  $65.07 \pm 1.63$  % (for Switchgrass). In addition, results from reusability studies using magnetic catalysts indicated that there was a slight reduction in catalytic activity during the second run.

Overall, results from this research suggest that sulfonic acid catalysts have high potential to replace conventional acids for pretreatment and with further improvement in catalytic activity, may possibly be used for direct hydrolysis, making the biomass to alcohol processes more efficient and environment friendly.

Sulfonic Acid Solid Catalytic Pretreatment and Hydrolysis of Biomass

by  
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A dissertaion submitted to the graduate faculty of  
North Carolina State University  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

Biological & Agricultural Engineering

Raleigh, North Carolina

2015

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## **DEDICATION**

I dedicated my dissertation work to my beloved parents Bapa Glenn Demas Ansanay and Mama Betty Enneke Manori and my big family.

## **BIOGRAPHY**

Yane Oktovina Ansanay, was born on the 4<sup>th</sup> of January 1986, in Jayapura, Indonesia. She was born to father Glenn Ansanay and mother Betty Manori. She grew up in the beautiful city of Jayapura in Papua until she finished her high school. She got a scholarship from Papua Government to pursue her bachelor's degree in Physics from 2004 to 2008. In August 2010, she was able to continue her master's degree at North Carolina State University in the Department of Biological and Agricultural Engineering under the Fulbright scholarship program and completed her master's program in December 2012. She started her Doctoral of Philosophy in January 2013 in Biological and Agricultural Engineering under the direction of Dr. Praveen Kolar. She is planning to complete her Doctoral degree in December 2015 and go back to her hometown Jayapura and pursue her career there.

## ACKNOWLEDGEMENTS

I am honored by the opportunity to study in the Biological and Agricultural Engineering Department at North Carolina State University. During this exciting moment, I would like to express my gratitude for people and organization that helped me through these years of studying.

First of all, I would like to express my most precious gratitude to my beloved parents, Bapa Glenn Ansanay and Mama Betty Manori for brought me to this world and for their constant prayers, faithful love, and support all the way till I finally completed all levels of formal education. I would also love to thank my grandpas and grandmas, my beloved brothers (K'Paul, Kelly and Paskalino) and my beloved sisters (K'Marice, April, Enekke), my beautiful nieces (Revalina and Aprilia), aunts (Vera, Sarah, Voni, Ania, Vero, Darsih and others aunties), uncles and cousins for endless support and caring for me all the time.

I would like to take this moment, to express another most precious gratitude toward my Advisor Dr. Praveen Kolar. I would not have reached this level without his advising and encouragements. I am so thankful for open minded professor like him, who gave me space and time to work according to my own schedule. I am so thankful for his quick responses and discussions that we had.

I would also like to take this opportunity to thank Dr. Ratna Sharma for her advice and also her kindness so I am allowed to use many of her instruments easily. I also wanted to thank her for the opportunity of teaching some sessions in her class BAE 425/525 Spring 2015 and working together to prepare materials and procedures for two summer camps 2014 and 2015. I also wanted to thank Dr. Jay Cheng for feedback on the progress of my research and also for

allowing me to use some of his instruments in the lab. I also wanted to thank Dr. Sunkyu Park for his feedback on my research progress and especially I wanted to thank him for the opportunity to learn about WINGEM simulation. I also wanted to express my gratitude toward Dr. Consuello Arellano for her guidance and help with statistical analysis. I also wanted to thank her for her kindness and for the stimulating discussions we had over lunches. I would like to also express my gratitude to professors and staff at BAE NCSU Dr. Willits, Dr. Evans, Dr. Shah, Dr. Chinn, Ms. Heather, Ms. Betsy, Ms. Tiwa, and Sherry.

I would like to thank Dr. Evgeny Danilov from Department of Chemistry, North Carolina State University for helping me run samples using FTIR. I also would like to express my gratitude to Dr. Joel Pawlak, from Department of Forest Biomaterials, North Carolina State University for allowing me to learn about TGA and run some samples using the instrument. I would like to also express my gratitude toward Dr. Mike Boyette (Biological and Agricultural Engineering Department), North Carolina State University for allowing me to use Ultrasonicator for my experiment.

I would also like to thank Rachel and Hiroshi from 270A Weaver lab for all the help. I would also like to express my gratitude toward Lalitendu Das and Rachel Slivka for their helping hands in the lab and to all my friends from BAE Department, especially thanks to Veronica, Woochul, Jess, Yu, Zhimin, Yiyang, Han, NCSU friends, all Indonesian fellows at NC, Fulbright fellows, friends from NET Church and especially to Nugroho's family.

In this chance, I also like to thank my former teacher Prof Yohanes Surya and Dr. Topo Suprihadi for their encouragements for me to pursue my study in the US. I would also like to express my gratitude towards Dinas Pendidikan dan Pengajaran Propinsi Papua for their generosity.

Last but not least, I would also like to thank a very wonderful friend of mine, my darling fiancé Korinus Waimbo in United Kingdom, his faithful love, prayers and support along the way. I wanted to thank him for always available whenever I need to discuss about many issues. I also wanted to thank him for teaching me about modeling and how to solve computer issues.

I would also like to take this opportunity to thank some people that we have talked to and shared my thought frequently over internet, my beloved sister April in Japan, my lovely friends Bapa Pdt Daud Sekeluarga, Ibu Pendeta Grace, Anike, Zakaria, Yosef and George in Papua. Thank you so much for all your care and support. Last but not least, I would also like to acknowledge friends from The Pioneers of Papua community.

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# Chapter 1

## Introduction

Pretreatment of lignocellulosic biomass is one of the key steps in producing sugars and further converting them into biofuels. Although the methods for treating lignocellulosic biomass have been developed and used for decades, however, various environmental issues and high costs of unit operations are motivating researchers to develop newer and environment friendly pretreatment technologies.

The present research focused on synthesis and testing of sulfonic solid acid catalysts for pretreatment and hydrolysis of biomass. The objectives were to (1) synthesize, evaluate, and compare sulfonic acid catalysts for pretreatment of switchgrass, (2) evaluate p-toluenesulfonic acid catalyst for direct hydrolysis of switchgrass, and (3) test the efficiency of magnetic p-toluenesulfonic acid catalysts for pretreatment on four types of lignocellulosic biomasses, viz, switchgrass, miscanthus x giganteus, gamagrass, and triticale hay.

This dissertation consists of six chapters. Chapter 2 is expected to provide the reader with an overview of the problem faced by biomass to fuels processes. The chapter also provides a short summary of the various pretreatment techniques and the problems associated with them. The opportunities/problems identified in Chapter 2 were used to formulate the research questions that were investigated in this dissertation through Chapters 3-5.

Chapter 3 dealt with synthesis and testing of activated carbon supported sulfonic acid catalysts for pretreatment of switchgrass. The catalysts were synthesized via impregnation of sulfonic acids on activated carbon surface. The catalysts were characterized via standard

methods to determine the surface chemistry of the catalysts. Batch experiments were performed to evaluate the efficacy of the sulfonic acid catalysts as pretreatment agents.

In Chapter 4, sulfonic acid catalyst was employed to directly hydrolyze various feedstocks namely, cellulose, starch, cellobiose, and switchgrass into sugars. In addition, effects of conventional pretreatments such as NaOH and H<sub>2</sub>SO<sub>4</sub> on hydrolysis of switchgrass using sulfonic acid catalyst were also tested.

In Chapter 5, the catalysts were chemically modified to impart magnetic properties to facilitate easy recovery of the spent catalysts. Two types of magnetic catalysts were synthesized, which were then systematically tested for pretreatment of four types of biomass including, switchgrass, *mischantus x giganteus*, gamagrass and triticale hay. In addition, reusability of the magnetic catalysts was also studied.

The overall conclusions and the future directions of the present research are summarized in Chapter 6. The dissertation concludes with the appendices consisting of statistical analysis of the raw data collected as a part of this research.

It may be noted that chapters 3-5 are expected to be submitted to refereed journals and hence complied in manuscript formats.

## Chapter 2

### Literature Review

#### 2.1 Biomass for energy and environment

The concern for energy supply has risen for years worldwide. The primary reason is due to the limited resources of crude oil, coal, and natural gas. Meanwhile, the consumption of these fossil-based resources has also contributed to an increase in carbon dioxide emissions in the atmosphere (Kumar et al., 2009; Guo et al., 2012). Therefore, in order to provide alternative, better, inexpensive, and sustainable energy sources that can counter the ill effects of fossil fuel consumption, intensive research is being conducted across the world. (Rodríguez et al., 2011; Nel and Cooper, 2009; Shafiee and Topal, 2009). At present, several types of alternative energy resources including solar, wind, geothermal, nuclear, hydropower, and biomass are being studied. Among aforementioned options, biomass is the most promising source due to the accessible of this source across the globe (Zhou et al., 2011).

Biomass to bio-renewable energy was suggested as a source of alternative fuel due to the fact that biomass is obtained from organic materials such as grass, algae, wood, agricultural crops and residues which consume CO<sub>2</sub> from atmosphere, water, and sunlight to grow (Zhou et al., 2011). As a result, it is considered that biomass is sustainable, carbon negative and also environmental friendly source of energy for the production of fuel, (Agbor et al., 2011; Wang et al., 2007; Bransby et al., 1998). From the US perspective, the departments of Energy and Agriculture have reported that at 1.3 billion

tons of biomass could be available in the USA for the purpose of biofuel production (Perlack et al., 2005). Meanwhile, International Energy Agency reported that the amount of world oil energy consumption in 2007 was  $148.26 \times 10^{18}$  J which is approximately same as the amount of energy can be produced from less than 10 % of yearly global biomass growth (International energy agency, 2009). In addition, a study reported that world biofuel production from cellulose (originally from biomass) is expected to increase by 6.7% per year to meet the target of  $2.7 \times 10^6$  barrels of oil equivalent daily in 2030 (Energy information administration, 2011). For the proposed target, it is predicted that the world production of crop to biofuel can partially replace the need of gasoline up to 32% (Balat and Balat, 2009).

## 2.2 Lignocellulosic biomass for second generation of bioenergy

Biomass to produce energy and fuels may come from many different sources such as agricultural crops including corn, sugar cane and cassava. According on the report published by Jessen 2006, starch based ethanol in the US will peak between 12 to 15 billion gallons per year, which accounts for about 10% from total projected of 140 billion gallons annually. However, as food crops, use of corn, sugar cane, and cassava as ethanol feedstock will create a conflict and competition between food and fuel (Elobeid et al., 2007). In order to minimize the competition between food and fuel, alternative sources of carbohydrate-rich biomass (raw lignocellulosic feedstock) such as wood, agricultural residues, and non-edible grasses must be considered for the production of ethanol and other fuels (Agbor et al., 2011; Kumar et al., 2009; Lynd et al., 2002; Bobleter 1994).

### 2.2.1 Raw Lignocellulosic feedstock

Raw lignocellulosic biomasses are categorized into two classes called woody and non-woody biomass. The sources of raw lignocellulosic biomass can come from energy crops, agricultural residues, and forestall residues (García et al., 2014). In this study, non-woody biomasses are used. Energy crops such as miscanthus, and others perennial grasses like switchgrass and gamagrass are under many investigation studies focusing on their uses for the production of bioenergy. Furthermore, in addition to energy crops, current trend of utilizing lignocellulosic biomass to produce biofuel is also coming from agricultural residues such as triticale hay, cotton stalks, corn stover, rice straw and wheat straw (Mussatto and Teixeira, 2010; García et al., 2014).

### 2.2.2 Structure of lignocellulosic biomass

Unlike sugarcane or corn plants, the general structure of lignocellulosic biomass is complicated and consisted of three major polymers namely, cellulose, hemicelluloses, and lignin (Kumar et al., 2009; Agbor et al., 2011) as presented from Fig. 2.1.

These polymers are associated with each other as presented in Fig. 2.1. The composition of each component varies depending on the type, source of biomass, season, and location where the lignocellulosic biomass is grown and procured.

Cellulose and hemicellulose are two main components of carbohydrate, in which plants store their energy. In addition, lignocellulosic biomasses are also composed of lignin, small portion of pectin, protein, extractives and ash (Jørgensen et al., 2007; Chandra et al., 2007; Zhou et al., 2011).

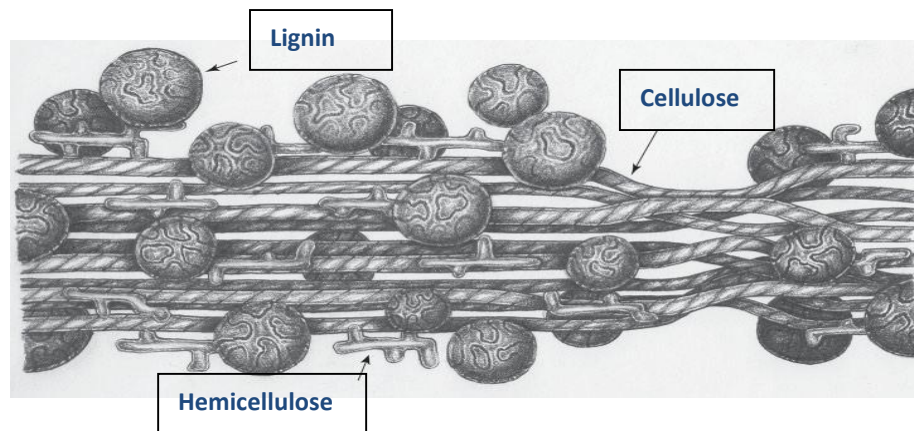


Figure 2.1 General structure of lignocellulosic material (adapted from Stokke 2014).

### 2.2.3 Types of lignocellulosic biomass

There are many different varieties of lignocellulosic biomasses that have been studied for the purpose of bioenergy production. As a part of this dissertation, four types of lignocellulosic biomasses were evaluated including Alamo Switchgrass, Gamagrass, Miscanthus and Triticale hay, which are briefly summarized below:

#### 2.2.3.1 Alamo Switchgrass

Switchgrass (*Panicum virgatum*), is a perennial, warm season prairie grass that has been selected as a model biomass feedstock by The US Department of Energy (Shen et al., 2009). Switchgrass has several advantages over other biomass feedstocks including high tolerance to weather conditions and easily adaptability to the poor soils

(Hu et al., 2011), and requiring minimal agronomical inputs. Most importantly switchgrass is a native to North America and grows well in two thirds of the eastern US (Mann et al., 2009; Hu et al., 2011). Therefore switchgrass is under investigation as a potential feedstock by several researchers including Yang et al., 2009; Kumar et al., 2011; Xu and Cheng, 2011; and others.

#### 2.2.3.2 Gamagrass

Another perennial warm-season C4 grass native to the US, namely, Gamagrass (*Tripsacum dactyloides*) has the potential to be utilized as biomass feedstock. This plant grows mostly in the southeastern region of the US. According to the report from USDA-NRCS (2007), Gamagrass yields are comparable with those of switchgrass (Eubanks et al., 2013; Keyser 2015). Similar to switchgrass, Gamagrass is also very adaptable to varieties of soils and climates (Ge et al., 2012; Lemus and Parrish, 2009). In addition, the plant possesses high carbohydrate content that makes Gamagrass a suitable candidate for bioenergy/biofuel production.

#### 2.2.3.3 Miscanthus

Miscanthus was popular crop in Japan as a forage and ornamental crop (Jessup 2009). This high-yield perennial grass native to Asia was introduced to Europe only in 1930 (Lasorella et al., 2011). Many varieties of Miscanthus have been developed that have been growing across the globe including America (Villaverde et al. 2009). Miscanthus has been reported for its efficiency in using soil nutrients, (William and Douglas, 2011). For biofuel production, *Miscanthus x giganteus* was suggested as highly productive, with high carbohydrate content, sterile, C<sub>4</sub> perennial grass

feedstock (Anderson et al., 2011). As a result several research groups have been studying *Miscanthus x giganteus* as a feedstock for sugar production (Panneerselvam et al., 2013; Yang et al., 2015; Yu et al., 2013, and others).

#### 2.2.3.4 Triticale hay

Triticale is a cereal grain crop that has been tested as first generation of biofuel due to high amount of starch content (Mcgoverin et al., 2011). However, due to competition with food, idea of utilizing Triticale as a feedstock did not take off. Instead triticale hay residue was suggested to be used for the purpose of bioenergy conversion. Chen et al (2007) has tested triticale hay for enzymatic hydrolysis and achieved maximum saccharification of 43.6%. Although the method ensiling triticale before enzymatic hydrolysis has been used by Chen et al 2007 and also suggested by Shrestha et al (2010), therefore, as this residue contain high carbohydrate source, other pretreatment methods should be included to treat prior hydrolysis (Inman et al., 2010). Fu et al (2010) reported that Ionic liquid of [C<sub>2</sub>mim]OA was found powerful to extract more than 50% lignin from triticale straw at 150 °C (for 1.5 h) which also resulted in complete cellulose hydrolysis.

### 2.3 Conversion of lignocellulosic biomass to fermentable sugars

As reported from many previous studies, lignocellulosic biomass contains high amount of carbohydrate; however accessing these carbohydrates is not easy (Mosier et al., 2005; Agbor et al., 2011). Proper techniques have to be employed to reach the carbohydrates within the biomass matrix. In other words the structure of the biomass has to be modified

to expose the carbohydrates. Two methods that have been used extensively are presented below.

### 2.3.1 Conventional Methods

In this approach, in order to produce fermentable sugar, lignocellulosic biomass has to be treated to modify the structure and then further continue with enzymatic hydrolysis in which enzyme is added to convert the long chain of carbohydrate into smaller sugar polymers. One such critical treatment to modify the biomass is pretreatment that is described below:

#### 2.3.1.1 Pretreatment

Unlike starch conversion, where enzymes are added directly facilitate processes of liquefaction and saccharification, lignocellulosic biomass, needs an additional step of pretreatment (before addition of enzymes) as the structure consists of complicated polymers. In addition, pretreatment may also facilitate other functions such as removal of lignin, and reduce the length of carbohydrate polymer as shown in Fig 2.2(Jørgensen et al., 2007; Kumar et al., 2009; Keshwani and Cheng, 2009; Henriks and Zeeman, 2009). Additionally, pretreatment even increases surface area of the lignocellulosic biomass and reduce cellulose crystallinity (Li et al., 2010; Park et al., 2010).

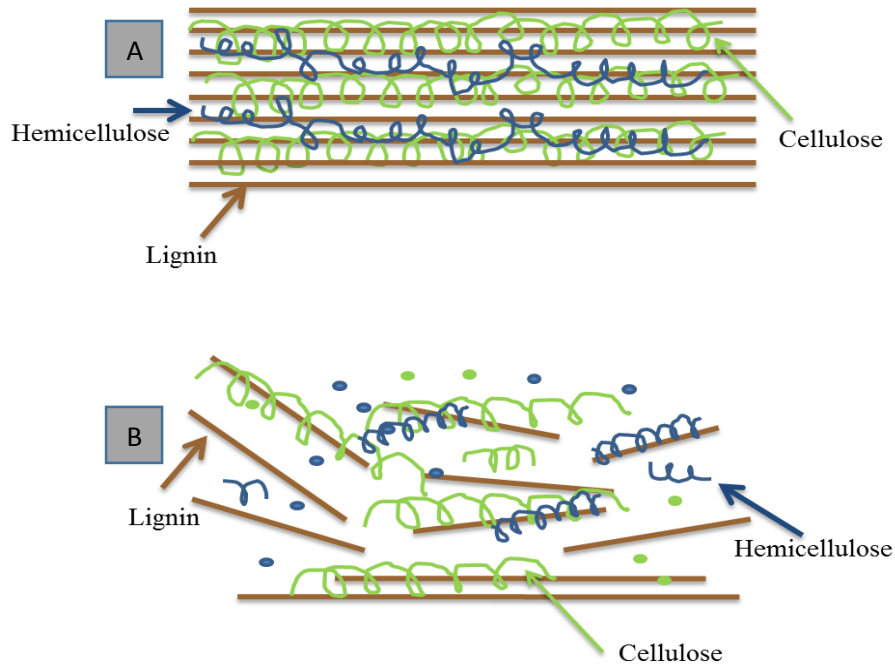


Figure 2.2 2D Schematic lignocellulosic before (A) and after (B) Pretreatment

Pretreatment of lignocellulosic biomass is also expected to minimize generation of undesirable co-products and still retain a majority of carbohydrate in the biomass matrix. Therefore, to satisfy the requirements of ideal pretreatment, several techniques have been studied and being studied. In general, the pretreatment methods may be divided into four categories namely, biological, physical, chemical, and physico-chemical.

#### A. Biological pretreatment

Biological pretreatment is widely known as safer and environmentally friendly method for treating lignocellulosic biomass (Kumar et al., 2009). Another advantage of this particular treatment is low energy requirement. As the name implies, in this technique, microorganisms play crucial role as treating agents. For example, brown, white and soft rot fungi are employed to degrade lignin and hemicellulose (Galbe and Zacchi, 2007). The mechanism of brown fungi is to attack cellulose, while both soft and white fungi are used to degrade lignin and attack cellulose at the same time via enzymes such as polyphenol oxidase, lignin peroxidases, and lactase (Lee et al., 2007; Agbor et al., 2011).

Although this technique is somewhat inexpensive and easy to conduct, the biological pretreatment rate is too slow for use in industrial settings as the time required for biological pretreatment is 10-14 days even under intensive observation (Agbor et al., 2011; Kumar et al., 2009). In addition, biological pretreatment requires large foot prints and hence may not be ideal for industrial production of alcohol from biomass at the present time.

#### B. Physical pretreatment

Typical physical treatment techniques include chipping, milling, and grinding. Physical treatment was widely known as mechanical treatment as this method mainly disrupts the structure of lignocellulosic biomass. The primary purpose of chipping is to avoid the mass transfer limitations and reduce heat, while milling

and grinding is particularly effective for the size reduction (Schell and Hardwood, 1994; Agbor et al., 2011).

In this physical treatment, biomass is resized into smaller size and as a result crystallinity and degree of polymerization (DP) of biomass is also reduced (Sun and Cheng, 2002). After biomass been harvested, the logs are reduced to a size between 10-50 mm, followed by chipping to a size of 10-30 mm. Milling and grinding is employed to reduce the size further to 0.2-2 mm (Sun and Cheng, 2002). For smaller particle sizes, it was reported that vibratory ball milling was found to be better in reducing cellulose crystallinity of spruce and aspen chips compared to ordinary ball milling. In addition, vibratory ball milling was also reported to improve the digestibility of both aforementioned biomasses (Kumar et al., 2009). However, further size reduction to a the size less than 40 mesh (0.4 mm) did not always in improving the sugar conversion of the biomass (Chang et al., 1997).

Other physical treatment methods such as, applying gamma rays to effectively cleave  $\beta$ -1, 4 glyosidic bonds, to create high surface area and reduce crystallinity of cellulose was also suggested (Takacs et al., 2000). However it may be noted that conventional physical treatments are very expensive and may increase the cost of bioenergy significantly higher than that of fossil fuel. Hence alternate approaches such as chemical treatments become necessary.

## C. Chemical pretreatment

In order to increase cellulose or hemicellulose susceptibility, chemical pretreatment is highly recommended. Chemicals used for treating biomass including alkali, acids, organic solvents, and ionic liquids. Although the target of each chemical is different, overall, chemical pretreatment is an effective method to remove lignin, partly solubilize hemicellulose, and reduce crystallinity.

### 1. Acid pretreatment

Acid pretreatment has been used for treating lignocellulosic biomass for decades. Typically, solutions containing less than 4 wt% of sulfuric acid, hydrochloric acid, and phosphoric acid have been used to convert biomass into sugar (Nguyen 2000; McMillan 1994; Torget et al., 1990). Acid, as an agent, has proven to effectively break down the hydrogen bonds leading to the swelling of intra-crystalline cellulose and also attack the intermolecular and intramolecular structure between cellulose, hemicellulose and lignin (Chang et al., 1981). Although the hydrogen bonding was broken, however, the fact is only small portion of cellulose is hydrolyzed, while in general, higher portion of hemicellulose was solubilized into the liquid medium and only small portion of lignin was removed (Mosier et al., 2005). Acid pretreatment can be prepared from either concentrated or dilute acid solutions. Concentrated acid is not preferable or recommended due to high corrosiveness, the need for special reactors to pretreat the biomass, and extensive neutralization needed after pretreatment process (Agbor et al., 2011). Hence dilute acid pretreatment

is widely used from small scale laboratory to industrial production of glucose from lignocellulosic biomass. Chung et al (2005) reported the use of dilute sulfuric acid 1.2% (w/w) to treat switchgrass at 180 °C that resulted in cellulose conversion of 90% after 72 h enzymatic hydrolysis. Another dilute sulfuric acid study was performed by Rajan and Carrier (2014) to treat wheat straw at 140 °C for 30 min followed by enzymatic hydrolysis and achieved glucose yield of 89%. Therefore, dilute acid pretreatment has effectively proven to improve the formation of glucose. However dilute acid also resulted in degradation of monomers, along with corrosion of the chamber/batch used and recycle of the liquid chemical (Mosier et al., 2005). Therefore, additional step has to be included in the downstream unit after dilute acid treatment.

## 2. Alkali pretreatment

Alkali pretreatment is performed using chemicals such as NaOH, KOH, and Ca(OH)<sub>2</sub>. These chemicals are widely known as swelling agents for both crystalline and amorphous cellulose that can effectively destroy the linkage between lignin and carbohydrate, thus opening up an adequate access of enzymes to carbohydrates (Agbor et al., 2011; Hendriks and Zeeman, 2009). The alkali pretreatment may also improve the internal surface area of the biomass, reduce crystallinity, and decrease degree of polymerization (DP). Wu et al (2011) reported more than 90% cellulose conversion into glucose after 24 h saccharification of sweet sorghum treated using 1M or higher concentration of NaOH. In addition, overall glucan conversion of 70% was

achieved after corn stover treated using condition of 0.08 g NaOH/g corn stover followed by 120 hours of enzymatic hydrolysis (Chen et al., 2013). However, alkali treatment is very effective for biomasses with less lignin contents, while the efficiency was found less for the biomasses with higher lignin contents (Agbor et al., 2011).

### 3. Organic solvent pretreatment

Organosolv pretreatment is known for its advantage of removing lignin, although not as efficiently as organosolv pulping (Agbor et al., 2011). The process involves an organic or aqueous solvent mixture with inorganic acid catalyst such as HCL or H<sub>2</sub>SO<sub>4</sub> to dissolve lignin by breaking down the internal lignin and loosening hemicellulose structure (Kumar et al., 2009; Alvira et al., 2010). Many organosolv solvents that were already used include, methanol, ethanol, acetone, ethylene glycol and tetrahydrofurfuryl alcohol. Organosolv pretreatment using 60-80% aqueous methanol containing 0.2% HCl to treat pinewood was reported to remove 75% of original lignin when treated at 170 °C for 45 minutes (Zhao et al., 2009). Another study was performed using mixed softwood as feedstock and aqueous ethanol organosolation extraction method reported that lignin residue range was observed from 6.4% to 27.4% (w/w) (Pan et al., 2005). As lignin was the primary target of organic solvent pretreatment, Papatheofanous et al (1995) suggested to combine organosolv pretreatment with dilute acid pretreatment, so both hemicellulose and majority of lignin can be removed prior enzymatic

hydrolysis. Although, organosolv pretreatment seems promising for the conversion of biomass into fermentable sugars, however, these solvents are expensive commercially and the solvent in the system needs an extra treatment to separate and recycle after use, therefore making this treatment economically not feasible (Sun and Cheng, 2002; Alvira et al., 2010).

#### 4. Ionic liquid pretreatment

Ionic liquids (ILs) consist of organic salts with low melting temperatures below 100 °C. In addition to being considered environmentally friendly, ILs possess high polarity, non-volatility, high thermal stability, and both anions and cations (Agbor et al., 2011; Alvira et al., 2010; Zavrel et al., 2009). In most cases, to fractionate biomass, imidazonium salts were used. It was suggested that during the reaction, ILs solutions will compete with lignocellulosic biomass for hydrogen bonding, therefore disrupting three dimensional network of the biomass structure (Moultrop et al., 2005). Wang et al., 2011 reported that IL solution [AMIM][Cl] could extract up to 62% cellulose from wood chips under mild conditions, and cellulose was recovered via precipitation by the addition of dimethyl sulfoxide/water (Wang et al., 2011). Another study using ionic liquid by Li et al, 2010 reported that 69.2% of total lignin from switchgrass feedstock was removed after treated with [C2mim][OAc] at 160 °C for 3 hours reaction time. However the details mechanisms of these ILs solutions are still under investigation (Agbor et al., 2011).

#### D. Solid Acid Catalyst pretreatment

As pretreatment agent, solid acid catalyst has attracted much attention lately. Some of the advantages of using a solid acid catalyst include moderately high activity, reusability, easy separation, less corrosive, and generates less waste effluents (Jiang et al., 2012; Guo et al., 2012; Hara 2010; Ansanay et al., 2014). In addition, if the precursor material is magnetized, the spent catalyst could be easily separated by applying a simple magnetic force (Lai et al., 2011; Guo et al., 2013; Peña et al., 2014). Peña et al (2014) has reported maximum glucose yield of 90% achieved after corn stover was treated using propyl-sulfonic nanoparticle at 180 °C and followed by enzymatic hydrolysis using 2 ml of Accelerase enzyme. Another study by Qian (2013) reported that rice straw was treated using sulfated zirconia catalyst at 150 °C for 3 h then followed by enzymatic hydrolysis was able to produce monosaccharides yield of 450 g kg<sup>-1</sup>. In addition, a maximum glucose yield of 81.28% achieved from our previous work when switchgrass was treated using niobium oxide solid catalyst at 60 °C and 120 minutes followed by the addition of 0.476 ml of Cellic ctec2 enzyme (Ansanay et al., 2014). Therefore, solid acid catalyst offers an environmental friendly approach to convert biomass to alcohol on a commercial scale. Considering aforementioned benefits, this research focused on synthesizing highly active solid acid catalysts to pretreat a variety of biomass feedstocks for subsequent sugar production.

### 2.3.1.2 Enzymatic Hydrolysis

Enzymes have been used to improve the production of sugars from biomass feedstocks for several decades. Enzymatic hydrolysis, the process of breaking down longer carbohydrate chains into simple sugars via enzymes is one of the critical steps in biomass to energy processes. Because biomass consists of cellulose and hemicellulose, at least two classes of enzymes are needed. These two types of enzymes used in general are *cellulase* to convert cellulose into six carbon sugars and *xylanase* for typical five carbon sugars production. In general, cellulolytic enzyme is a mixture of three individual enzymes namely, *exoglucanases* or *cellobiohydrolases (CBH)*, *endoglucanases*, and  *$\beta$  glucosidase*. The operational mechanisms of these three enzymes are: (1) At first *cellobiohydrolases (CBH)* or *exoglucanase* will attack along the cellulose chain, therefore cleaving off the polymer cellobiose units from the ends, after which (2) *endoglucanase* randomly attack  $\beta$ -1,4 glycosidic bonds in the middle part of cellulose chain and hydrolyze them, and (3) lastly  *$\beta$  glucosidase* is the enzyme responsible to produce glucose from cellobiose units released (Jørgensen et al., 2007; Guo et al., 2012). On the other hand, hemicellulose is more complicated as this polymer is a mixture of five and six carbon sugar and also other groups. Therefore for hemicellulolytic systems, the general mechanisms include (1) hydrolysis of internal bonds of xylan chain via *endo-1,4-  $\beta$ -D xylanases*, (2) release of xylobiose units by *exoxylanase* and (3) attack of *xylooligosaccharides* from non-reducing end and free the xylose by *1,4-  $\beta$ -D xylodidases* (Jørgensen et al., 2007; Keshwani and Cheng, 2009). It may be noted that while providing the enzymes to attack biomass during enzymatic hydrolysis, it is also

important to provide the system with acceptable conditions under which enzymes are able to work optimally, such as pH at approximately (4.8 ~ 5) and temperature between 48-50 °C.

## 2.3.2 Direct hydrolysis

### 2.3.2.1 Concentrated Acid Hydrolysis

Concentrated acids have been used to hydrolyze carbohydrate from lignocellulosic biomass since early 19<sup>th</sup> century, but the process become commercially available only in the early 20<sup>th</sup> century (Guo et al., 2012). When concentrated acid is applied as an agent for hydrolysis, it is believed that acid enters into the structure of cellulose, therefore leads to cellulose swelling and further disrupting its inter and intra-molecular chain of hydrogen bonding resulting in breaking of glycosidic bonds (Binder and Raines, 2010). In one of their studies, Saeman et al (1945) reported that at low temperature of 50 C and at atmospheric pressure, concentrated sulfuric acid higher than 50% concentration can swell cellulose, while further increase the concentration greater than 62% (or same as 39% HCl), cellulose changed from swollen phase into soluble forms such as hydrolyzed cellulose, cellulose dextrin, oligosaccharides and D-glucose (Guo et al., 2012). Even in the United States, several studies have investigated application of sulfuric acid and successfully reported conversions of cellulose and hemicellulose between 80 and 90% (Farone and Cuzens, 1998; Wright and Power 1987).

Although concentrated acid is powerful agent for hydrolyzing carbohydrate from biomass, problems related to the downstream processing and neutralization and corrosion of the chamber system have made direct hydrolysis process less attractive from economics and environmental perspective (Kumar et al., 2009).

#### 2.3.2.2 Solid Acid Hydrolysis

In order to minimize the use of enzymes, reduce the downstream unit operations, minimize use of hazardous liquid acids, and reduce potential chemical pollution, solid acid catalysts have been proposed for converting carbohydrate into simple sugars (Lai et al., 2011; Guo et al., 2012; Zhou et al., 2011). In addition, as a solid material, the catalyst could be recycled several times, which make this process economically attractive. A solid acid catalyst is defined as a material that can either donate proton (Bronsted acid) or accept electron (Lewis acid). However, B-acid catalysts are more applicable for biomass to sugar production (Abbadi et al., 1998; Dhepe et al., 2005) due to the catalytic function that is derived from its acidic center located at the surface (Guo et al., 2012). Hence there has been a growing interest in employing solid acid catalysts for direct hydrolysis. For example, model biomass compounds including cellulose, starch, and cellobiose have been utilized for the glucose production via solid acid catalyst hydrolysis (Takagaki et al., 2008; Kitano et al., 2009; Lai et al., 2011). Takagaki et al (2008) reported the use  $\text{HNbMoO}_6$  and Amberlyst-15 solid acid catalysts for starch hydrolysis at  $100\text{ }^\circ\text{C}$  for 15 h that produced 21 and 3.4 % glucose yields respectively. While Kitano et al (2009) reported a maximum of cellobiose conversion to glucose of more than 70% after 9 h

of reaction at 90 °C using carbon based solid catalyst. Similarly to enhance the hydrolysis of cellulose, Hu et al (2014) has treated cellulose with ionic liquid prior hydrolysis and achieved the glucose yield of 55% using 120 °C for 24 h hydrolyzing period.

Despite the enormous potential of solid acid catalyst to convert model biomass to monomer sugar (glucose), there are very limited reports on convert lignocellulosic biomass into sugars via solid acid catalysts (Li and Qian, 2011; Yamaguchi and Hara, 2010). Therefore, the overall goal of this research is to evaluate solid acid catalysts for pretreatment and hydrolysis of lignocellulosic biomasses. Specifically the focus is to:

- (1) Synthesize, evaluate, and compare sulfonic acid catalysts derived from sulfuric acid, methanesulfonic acid, and p-toluenesulfonic acid for pretreatment of switchgrass
- (2) Evaluate p-toluenesulfonic acid catalyst for direct hydrolysis of switchgrass, and
- (3) Test the efficacy of magnetic p-toluenesulfonic acid catalysts for pretreatment on four types of lignocellulosic biomasses, viz, switchgrass, miscanthus x giganteus, gamagrass, and triticale hay.

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## Chapter 3

### **Activated carbon-supported sulfonic acid pretreatment of switchgrass for production of fermentable sugars**

#### Abstract

Solid acid catalysts have recently received considerable attention as pretreatment agents for conversion of carbohydrates into monomeric sugars. In the present research, activated carbon-supported sulfonic acid catalysts were synthesized and tested as pretreatment agents for pretreatment of switchgrass into glucose. The catalysts were synthesized by impregnating sulfuric acid, methanesulfonic acid, and p-toluenesulfonic acid on activated carbon supports. Characterization of catalysts suggested increasing in surface acidities, while surface area, and pore volumes decreased substantially as a result sulfonation. Batch experiments were performed in 125-mL beakers to investigate the effects of temperature (30, 60, 90 °C), reaction time (90 and 120 min) on the yields of glucose. Enzymatic hydrolysis of pretreated switchgrass using Ctec2 yielded up to 61.5% glucose. Durability tests indicated that sulfonic solid acid catalysts were able to maintain activity even after three cycles. From the results obtained, solid acid catalysts appear to serve as effective pretreatment agents and can potentially reduce the use of conventional liquid acids and bases in biomass into biofuel production.

**Keywords:** Sulfonic solid catalysts, Pretreatment, Switchgrass, Ctec2.

### 3.1 Introduction

Depletion of fossil fuels, global warming caused by CO<sub>2</sub> emissions due to their combustion and geopolitical tensions have resulted in significant interest in exploration of alternative renewable resources (Guo and Fang, 2013; Huang and Fu, 2013). Many types of energy systems including wind, solar, geothermal, hydropower, and biomass are being extensively evaluated. However, at the present time, it appears that energy from biomass is more promising when compared to the rest of aforementioned alternative resources (Mousdale, 2008). Biomass can be grown and produced everywhere on earth and the major component of biomass is organic carbon (Huang and Fu, 2013; Galbe and Zacchi, 2007). Hence, lignocellulosic biomass has attracted research attention as a major biomass source. Specifically, perennial grasses such as switchgrass and miscanthus have attracted the attention of bioenergy researchers due to their faster rate of growth even on non-arable lands with limited agronomic inputs (McLaughlin, 1992; Schmer et al., 2008; Somerville et al., 2010). For example, switchgrass consists of two major carbohydrate components including cellulose and hemicellulose which account for up to 54-70% of the composition, followed by lignin (10-27 %) (Ansanay et al., 2014).

Conversion of switchgrass to alcohols involves pretreatment of switchgrass, followed by hydrolysis and fermentation (Kumar et al., 2011; Yang et al., 2009). Pretreatment allows for disruption of the switchgrass structure (Kumar et al., 2009; Huang and Fu, 2013) by breaking down lignin that binds to cellulose and hemicelluloses, reducing the crystalline structure of cellulose, and increasing available surface area that facilitates enzymatic reactions with cellulose and hemicelluloses (Mosier et al., 2005 and Wyman et al., 2005). Hence several pretreatment methods, viz., acid (Wyman et al., 1992, Chung et al., 2005, Dien et al., 2006,

Wyman et al., 2011), base (Chang et al., 1997, Wang et al., 2008), ammonia (Alizadeh et al., 2005, Kurakake et al., 2001), hot water (Wyman et al., 2005) and ozone (Vidal and Molinier., 1998; Panneerselvam et al., 2013) have been explored extensively for the last two decades (Kumar et al., 2009; Hendriks and Zeeman, 2009; Mosier et al., 2005; Qian 2013). However, the most commonly used pretreatment method employs dilute acid with the temperature ranging from 140 to 215 °C (Agbor et al., 2011). Sulfuric acid works well as pretreatment agent by solubilizing hemicellulose and depolymerizing lignin (Wyman et al. 2011). However, sulfuric acid is highly corrosive and requires specialized equipment to pretreat biomass (Kumar et al., 2009). In addition, the spent liquor needs additional downstream treatment before safe disposal, thereby adding costs to the overall process.

Hence research on biomass pretreatment is now moving towards recyclable and solid acid catalysts (Guo and Fang, 2013; Qian 2013). Solid acid catalysts are simple to synthesize and could be reused several times with minimal loss in activity (Zhou et al., 2011; Guo et al., 2012). Wang et al. (2012) reported use of silica catalyst (160 °C for 12 h) for conversion of cellulose into glucose. In addition the catalyst was reused three times with slight variation in activity. Similarly, Kitano et al. (2009) was able to reuse carbon-based solid acid catalyst five times to produce glucose (25-30 % conversion) from cellobiose.

Considering the importance of switchgrass as a bioenergy crop in the US, we are also interested in developing solid acid catalysts for pretreatment of switchgrass. However at the present time there is limited information on the efficacy of solid acid catalysts for pretreatment of switchgrass. Therefore, the overall goal of this research was to synthesize solid acid catalysts capable of pretreating switchgrass for subsequent hydrolysis. Specifically our objectives were to (1) synthesize activated carbon-supported sulfonic acid catalysts using

sulfuric acid, *p*-toluene sulfonic acid, and methane sulfonic acid as precursors, (2) test the effects of pretreatment time and temperature on glucose yield, and (3) test the reusability of catalysts in pretreatment reactions.

## 3.2 Materials and Methods

### 3.2.1 Switchgrass Preparation

*Alamo* switchgrass used in this study was harvested in mid July 2011 from North Carolina State University Field Laboratory in Reedy Creek Road Field Raleigh, NC. Switchgrass was dried in the field for to 3 days and baled with a conventional square hay baler. Switchgrass samples were then grounded to pass 2-mm sieve and transferred into air-tight plastic bags and stored at room temperature until they were used.

### 3.2.2 Catalyst Preparation

Three solid acid catalysts were synthesized using activated carbon and *p*-Toluenesulfonic acid (pTSA), Methanesulfonic acid (MSA), and Sulfuric acid (SA) as precursors. Briefly, 50 g of activated carbon (C270C, Fisher Scientific) was impregnated with 100 mL of MSA and SA for 6 h. For pTSA catalyst, the support (activated carbon) was impregnated with a solution of pTSA in water (67 g dissolved in 100 mL water). This was followed by washing with DI water (1 h) and re-soaking in DI water overnight. Subsequently the catalysts were dried at 105 °C (2 h), calcined at 250 °C (2 h), and stored until further use.

### 3.2.3 Catalytic Pretreatment of switchgrass

1.5 g of catalyst and 6 g of switchgrass were mixed with 90 ml of deionized water in a heated conical flask at atmospheric pressure used 350 rpm (Fig 3.1). Temperatures of 30, 60, and 90 °C and pretreatment times of 90 and 120 min were selected. All experiments were performed in triplicates using a factorial experimental design. After pretreatment, catalyst was manually separated and biomass was vacuum filtration. Subsequently, the catalyst was dried at 105 °C for 2 h and stored for subsequent reuse, while the recovered switchgrass was stored at 4 °C for subsequent enzymatic hydrolysis.

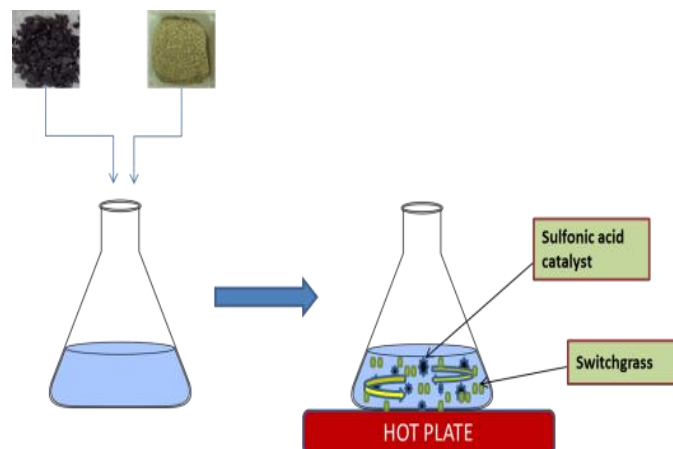


Fig 3.1. Overview of sulfonic solid acid pretreatment of switchgrass

### 3.2.4 Reusability of the catalyst

Durability of the catalysts was assessed by reusing them under all conditions. After first use, catalyst was separated and dried for 2 h at 105 °C and prepared for the next batch of pretreatment. Treated switchgrass samples were separated and stored for enzymatic hydrolysis, composition analysis, and BET surface area.

### 3.2.5 Enzymatic Hydrolysis

All pretreated switchgrass samples were hydrolyzed by mixing (150 rpm for 72 h) 1 g of switchgrass (dry basis) with 0.167 mL of *Cellic®Ctec2* (Novozymes North America, Franklinton, NC) ((3.5% w/w (g enzyme protein g<sup>-1</sup> dry biomass)) (activity ≈ 119 FPU ml<sup>-1</sup>) (Reye et al., 2011) and 40 µg ml<sup>-1</sup> of tetracycline hydrochloride (to minimize any bacterial growth during hydrolysis). In addition, 0.05M sodium citrate buffer was added to bring the total hydrolysate volume to 20 mL corresponding with 5% solid loading.

### 3.2.6 Composition Analysis

Composition of raw and pretreated switchgrass were determined using standard National Renewable Energy Laboratory (NREL) procedures (Sluiter 2005a, b, 2008). The samples were analyzed for acid insoluble lignin (AIL), acid soluble lignin (ASL), moisture, and carbohydrate contents (glucan, xylan, arabinan). AIL and ASL were determined via two-step acid hydrolysis in which switchgrass was hydrolyzed in 72% sulfuric acid at 30 °C for 1 h, followed by 1 h hydrolysis in 4% sulfuric acid at 121 °C. The clear acid hydrolysate was separated from solid residues via filtration through crucible and stored at 4 °C for further analysis to determine ASL and total carbohydrate content via UV-Vis spectrophotometer that was set to 205 nm. The retained solid residues were placed in an oven at 105 °C before

placing in a furnace at 550 °C for determining AIL. Total sugars including 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 set to 65 °C with an eluant (5 mM Sulfuric Acid) flow of 0.6 mL min<sup>-1</sup>. The data was quantified based on comparison with glucose, xylose, and arabinose standards analyzed by the HPLC.

### 3.3 Catalyst Characterization

#### 3.3.1 BET Surface Area Analysis

BET Surface area analyzer Micromeritics Gemini VII 2390 was used for the surface area analysis. At approximately 0.5 g of catalyst samples were degased at 150 °C (2 h) followed by nitrogen adsorption to determine the specific surface area, pore volume, pore size, and isotherms.

#### 3.3.2 Determination of surface functional groups

Boehm titration method was used to quantify the surface functional group of catalysts.

Briefly, 0.5 g of catalyst was mixed with solutions of 0.05M NaHCO<sub>3</sub>, 0.05 M Na<sub>2</sub>CO<sub>3</sub> and 0.05 M NaOH at 125 rpm for 24 h at room temperature as described by Evangelin et al. (2012). After separating the catalysts from the solutions, 10 mL of each solution was titrated with 0.05 M HCl using Methyl red as indicator. As suggested by Evangelin et al., 2012 and Mukherjee et al., 2011, it was assumed that NaOH neutralized carboxylic, lactonic, and

phenolic groups,  $\text{NaHCO}_3$  neutralized carboxylic groups, and  $\text{Na}_2\text{CO}_3$  neutralized both carboxylic and lactones groups.

### 3.3.3 Thermogravimetric analysis

The catalyst samples were also analyzed using a Thermogravimetric analyzer (TGA, Q500, TA Instruments, New Castle, DE). Approximately 18-35 mg of catalyst sample was placed on a platinum pan and heated from 0 to 600 °C at a rate of 30 °C  $\text{min}^{-1}$  under nitrogen atmosphere.

### 3.3.4 Fourier Transform Infra-Red (FTIR) analysis

Attenuated total reflection (ATR-FTIR) was used to analyze the presence of sulfonic groups on the surface of activated carbon catalysts. ATR-FTIR was carried out using wavenumber range of 4000 – 500  $\text{cm}^{-1}$ .

## 3.4 Experimental design and Statistical Analysis

All experiments in this study were performed in triplicates and all catalysts were reused for three times. Four treatment variables (catalyst type, pretreatment temperature, pretreatment time, and catalyst durability) were tested in this research. While catalyst (AC-SA, AC-pTSA, and AC-MSA), temperature (30, 60 and 90 °C), and catalyst durability (Run 1, Run 2, Run 3) had 3 levels, the pretreatment time (90 and 120 min) had 2 levels. A Proc mixed model was used to analyze the data and slice effects test was adapted to observe main and interaction effects for all treatment combinations using SAS 9.3 (Cary, NC) within 95% confidence limits.

## 3.5 Results and Discussion

### 3.5.1 Catalyst Characterization

Data obtained from BET surface area analyzer for raw activated carbon (control) and three sulfonated activated carbon catalysts are presented in table 3.1. It appeared that after sulfonation, specific surface area and pore volume of all three sulfonated catalysts decreased slightly (when compared with control) despite constant pore diameter at around 20Å. The surface area decreased probably due to the oxidation reaction between carbon and sulfonic acid molecules. As a result, the surface of pores within activated carbon was potentially occupied by sulfonic groups (-SO<sub>3</sub>H), thereby slightly reducing the available pore volume, which was also corroborated via adsorption isotherm plots (Figure 3.2). Our results are similar to the data presented by Liu et al. (2010), who investigated sulfonation of activated carbon and reported that surface area, pore volume and pore diameter were reduced as a result of sulfonation. It appears that impregnation agents used in this research were able to react with carbon surface and yield an acidic surface capable of pretreating switchgrass as presented in table 3.1.

The spectra obtained from FTIR also indicated that sulfonic groups were present for all three sulfonated activated carbon catalysts (Fig 3.3). After sulfonation, the amount of energy transmitted was reduced possibly, due to the absorption of sulfonic group. Therefore the dips for stretching vibrations of undissociated sulfonic groups and -SO<sub>3</sub>-species were assigned to 1398.2 and 1350 cm<sup>-1</sup> as suggested by Peña et al., 2014 and Givan et al., 2002.

Table 3.1. Physio-chemical characterization of the sulfonic acid catalyst used for pretreatment of switchgrass

<b>Characteristic</b>	<b>Raw AC</b>	<b>AC-SA</b>	<b>AC-MSA</b>	<b>AC-pTSA</b>
Surface area (m <sup>2</sup> /g)	781.337 ± 30.94	734.553 ± 29.06	668.954 ± 25.99	317.809 ± 7.12
Pore volume (cm <sup>3</sup> /g)	0.405 ± 0.02	0.37 ± 0.03	0.338 ± 0.02	0.145 ± 0.00
Pore size (Å)	20.749 ± 0.83	20.246 ± 0.8	20.232 ± 0.79	20.605 ± 0.46
Carboxylic (mmol/g)	0.025 ± 0.01	0.2 ± 0.00	0.2 ± 0.00	0.175 ± 0.002
Lactone (mmol/g)	0.025 ± 0.00	0.09 ± 0.005	0.05 ± 0.00	0.125 ± 0.00
Phenolic (mmol/g)	0.05 ± 0.00	0.075 ± 0.007	0.05 ± 0.00	0.125 ± 0.01
Total surface acidity (mmol/g)	0.1 ± 0.03	0.365 ± 0.03	0.3 ± 0.00	0.425 ± 0.02

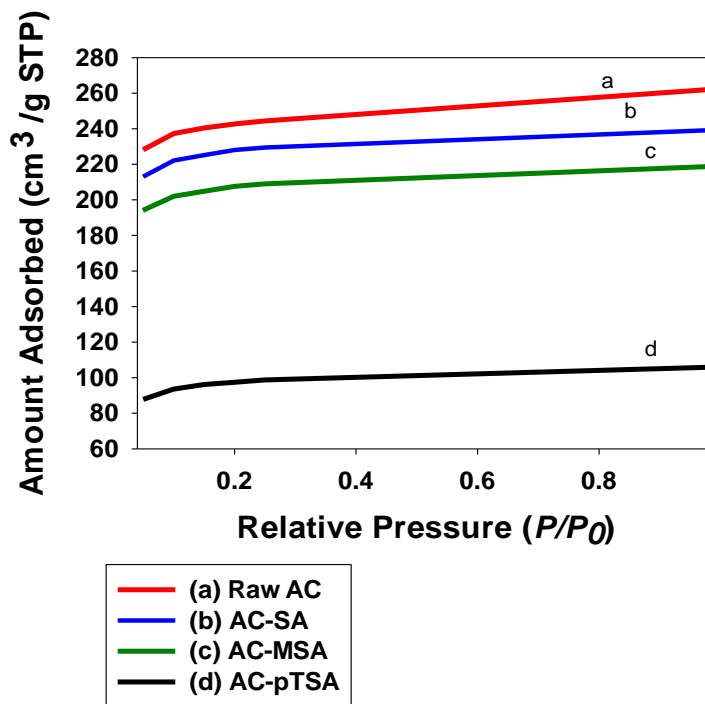


Fig. 3.2 Adsorption isotherm of raw AC (control) and Sulfonated activated carbon catalysts.

Thermal stabilities of the raw and sulfonated catalysts were examined by thermogravimetric analysis (TGA) under a nitrogen gas atmosphere. As shown in Fig 3.4, initial degradation of all samples was due to the presence of moisture or water adsorbed on the surface of activated carbon. As seen from Fig 3.4, the degradation profile of raw activated carbon (Fig 3.4a) shows only one degradation peak that was observed before 100 °C and stayed considerably stable up to 600 °C which was due to the evaporation of water molecules. Meanwhile, for sulfonated catalysts, at least two weight losses regimes were observed. Overall, the first degradation, which was assumed to occur due to evaporation of water adsorbed at the surface of catalysts. However, evaporation profiles in Fig 4b-d, suggested the amount of water present in

sulfonated catalysts were lower compared to the raw activated carbon, and therefore weight loss percentages for sulfonated acid catalysts were found lower compared to raw activated carbon. The second weight loss shown in Fig 4b-d indicated the decomposition of sulfonic group ( $\text{SO}_3\text{H}$ ) beyond temperatures 250-300 °C.

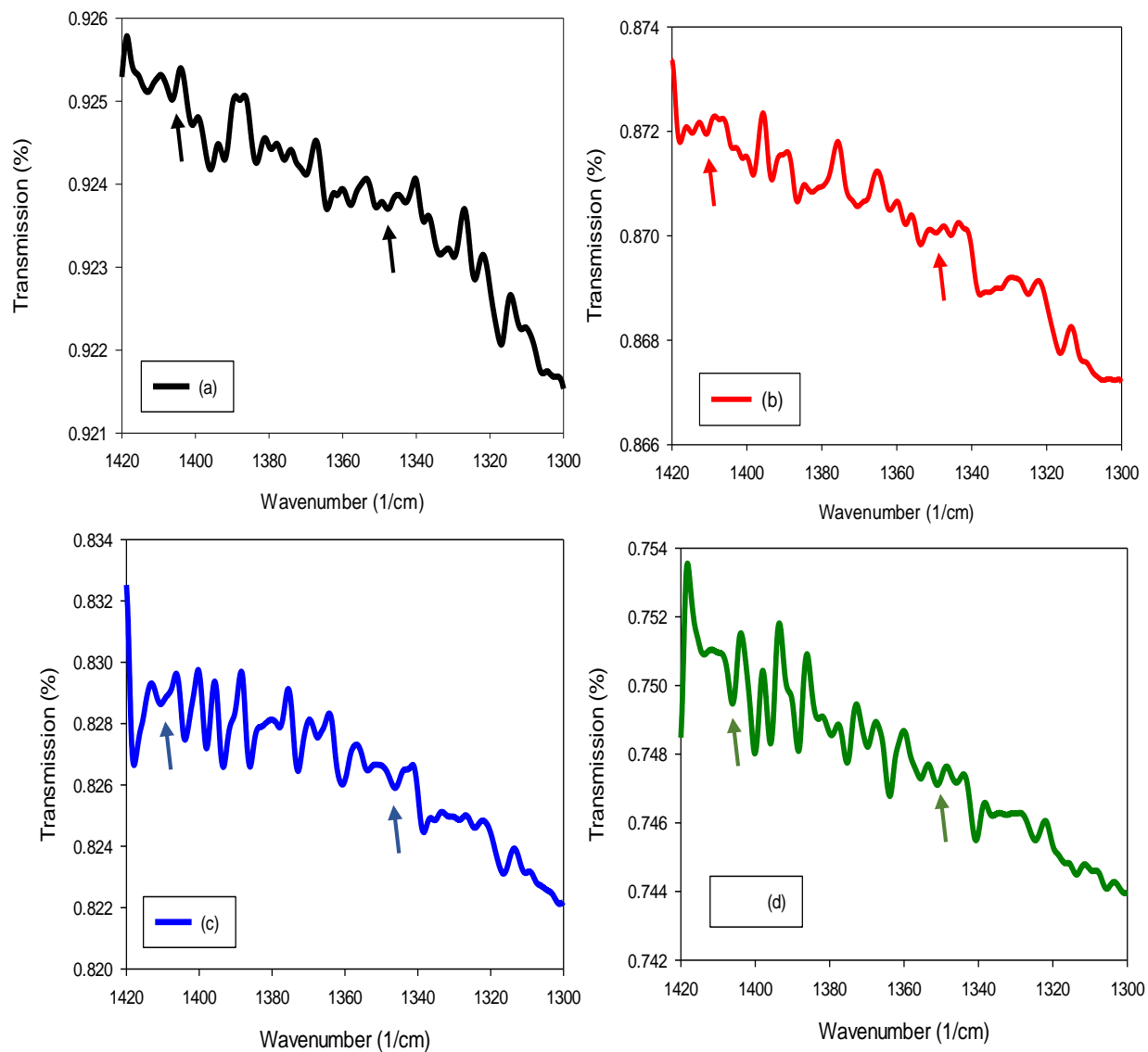


Fig 3.3 FTIR Spectra of a) Raw AC, b) AC-SA, c) AC-pTSA and d) AC-MAS Catalysts

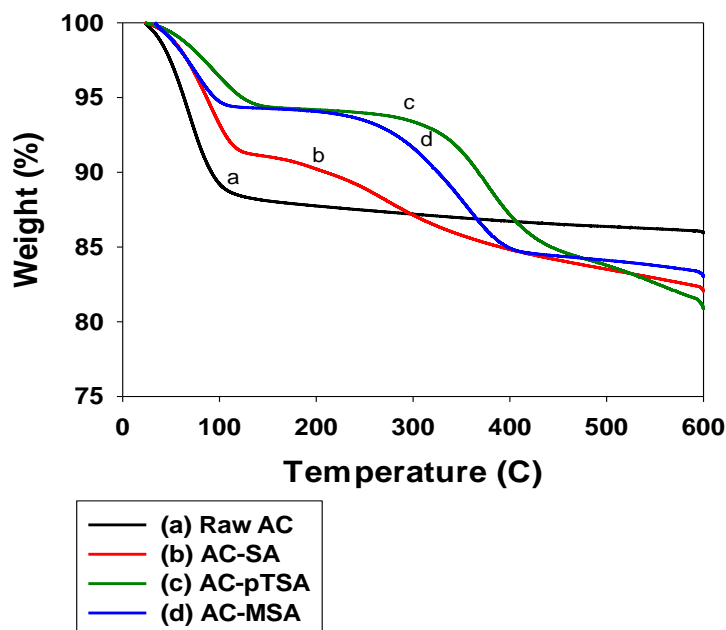


Fig 3.4 TG analysis of Raw and Sulfonated Acid Catalysts

### 3.5.2 Effect of pretreatment temperature on glucose production

Effect of temperature on glucose yield for all catalysts is presented in Figs 3.5 (a-f.)

Temperature was found to have a significant effect on glucose yields ( $p < 0.05$ ) obtained from switchgrass treated using three different catalysts AC-SA, AC-pTSA, and AC-MSA. For the temperature range tested (30-90 °C), glucose yield ranged from 31.5 – 61.5% for AC-pTSA, 37.3- 56.8% for AC-MSA, and 45.4-59.3% for AC-SA. Interestingly at lower temperatures (30 and 60 °C) AC-SA provided with the highest yields when compared to AC-pTSA and AC-MSA for both 90 and 120 min pretreatment times. However at 90 °C, glucose yields appeared to be somewhat similar ( $p = 0.05$ ). These data suggest that AC-pTSA and AC-

MSA are activated at higher temperatures and likely enhance the rate of reaction between switchgrass and sulfonic acid groups.

Our results are consistent with those reported by Peña et al. (2014), who tested Propyl-Sulfonic acid as a solid catalyst to treat corn stover at much high temperatures of 160, 180 and 200 °C and observed glucose yields between 59% (160 °C) and 90% (180 °C). In our research we also observed a glucose yield of up to 61.5% at substantially lower pretreatment temperature of 90 °C when AC-pTSA was used. In a different study by Qian (2013), sulfated zirconia (SA-J1) was employed (3 h at 150 °C) to pretreat rice straw resulting in a maximum monosacharides yield of 450 g Kg<sup>-1</sup> (or approximately 76% of holocellulose in rice straw).

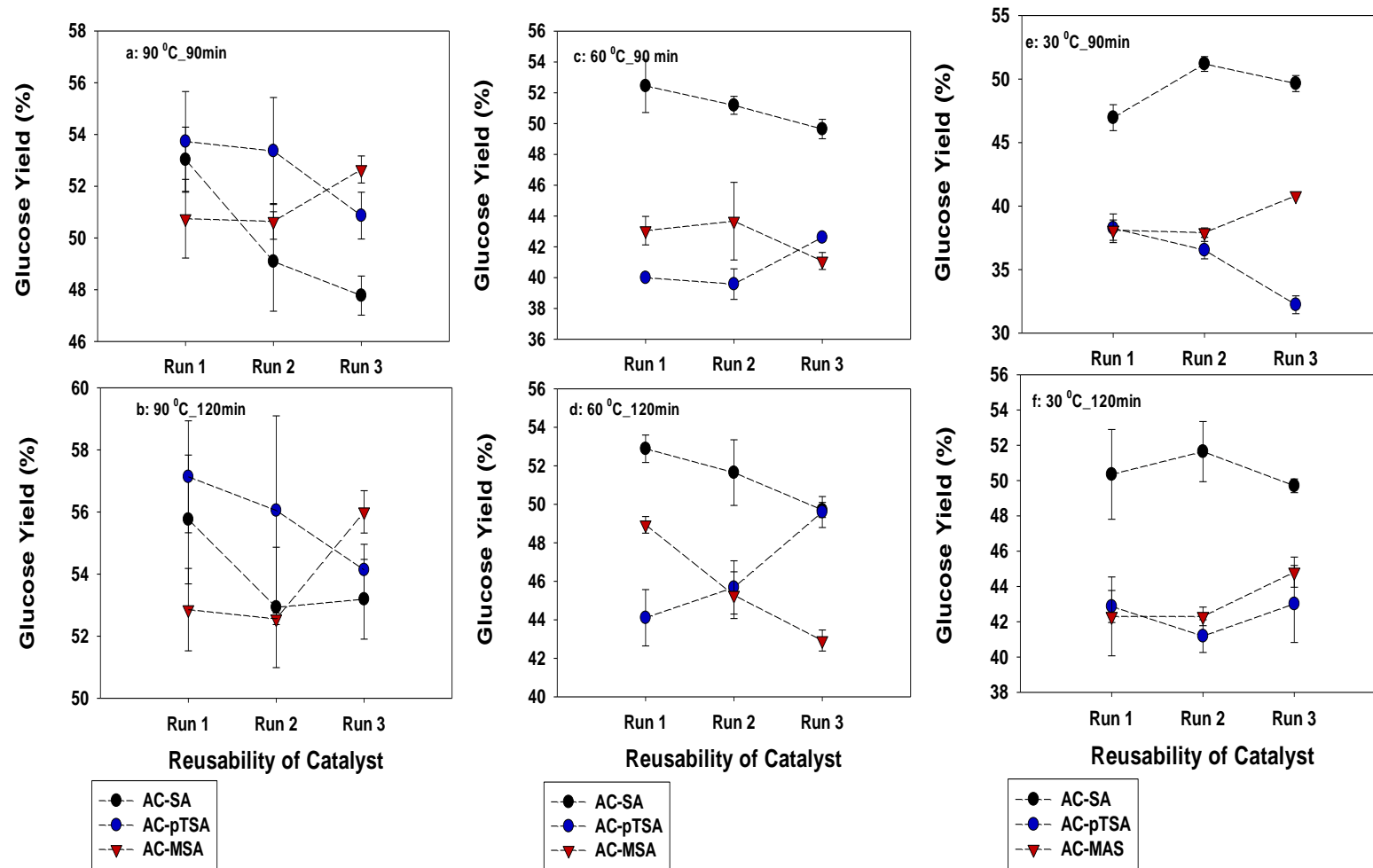


Fig 3.5 Gluyield (Glucose Yields in percentage) for three different solid acid catalysts for three temperatures of 30, 60 and 90 °C at two different reaction times of 90 and 120 minutes

Thus, it appears that catalytic activity of sulfonic group can significantly alter the structure of lignocellulosic biomass and possibly facilitate favorable enzymatic interaction to convert long chain carbohydrates to simple sugars.

### 3.5.3 Effect of pretreatment time on glucose production

Results suggested that reaction time has a significant effect ( $p < 0.05$ ) on glucose production. Overall, longer reaction time allowed for better glucose yields were produced. At lowest temperature tested (30 °C), the increase in glucose yields observed after 90 to 120 min of pretreatment were between 0.06 and 10.77 % for AC-SA, AC-MSA and AC-pTSA. Our results are similar to Qian (2013), who also reported monosaccharides yield increased by 12.5% when reaction time was increased from 1 to 3 h, when sulfated zirconia (SA-J1) was used as a catalyst at 150 °C. As suggested by Guo and Fang (2013) and Qian (2013), longer reaction time between catalyst and switchgrass may have created additional porosity in the switchgrass matrix which may have facilitated favorable adsorption by enzymes on switchgrass surface during hydrolysis. However, as the temperature increased to 60 and 90 °C, the increase in glucose yield was impacted due to secondary reactions between glucose and the catalysts as was also observed by Qian (2013).

### 3.5.4 Effect of the reusability of catalyst on glucose production

Experimental data suggested that the catalysts were able to maintain activity even after they were reused three times. Overall, catalyst durability was found not significantly different between the catalysts uses ( $p= 0.18$ ). For the case of 90 °C, when AC-SA was employed as a

catalyst, the change in conversion was not significant ( $p > 0.05$ ) for 120 minutes (yield of  $53.9 \pm 1.01\%$ ), while for 90 minutes pretreatment, change in conversion was significant ( $p < 0.05$ ) corresponding to yield of  $49.9 \pm 1.05\%$ . Meanwhile, both AC-pTSA and AC-MSA exhibited similar results in which yields were not significant ( $p > 0.05$ ) for reaction time of 90 minutes, while 120 minutes have impacted significantly ( $p < 0.05$ ). One reason for different yields observed between uses was possibly due to repeated agitation of carbon particles that resulted in breakdown of structure and may have enhanced the effective surface area and hence the activity of some of the catalysts.

### 3.5.5 Effect of sulfonic solid acid pretreatment on delignification

Figure 3.6 presents plots for delignification by all treatment conditions. Analysis revealed that reaction time and temperature did not individually affect delignification of switchgrass ( $p=0.1762$  and  $p=0.9735$ ). However, the interaction effect of combination between catalyst, temperature, time and reuse was significant ( $p < 0.05$ ) suggesting that delignification varied with each temperature and reaction time. In addition, catalyst type and the number of times the catalyst was reused had a significant effect on delignification of switchgrass ( $p < 0.05$ ).

Despite exhibiting no clear trend, it was observed that activated carbon treated with sulfuric acid provided the least delignification (5.1 – 15.2%), when compared to AC- pTSA (14.0-24.8%) and AC-MSA (14.3– 22.2 %). It is interesting to note that despite minimal delignification, switchgrass pretreated with AC-SA provided highest glucose yields when compared to AC-pTSA and AC-MSA. It is theorized that AC-SA might disrupt the structure of lignin and make the cellulose and hemicellulose portions of switchgrass more susceptible to

enzymatic hydrolysis. Similar results were reported by Li et al 2012, in which dilute acid was used to treat wood chips and only 2.7% lignin was removed. In addition, freeze-dried switchgrass was treated with dilute sulfuric acid resulting in maximum lignin removal of 9.51% (Yang et al., 2009). Therefore, from our results it may be theorized that sulfonic solid catalyst behaved somewhat similar to dilute acid for pretreatment of switchgrass.

### 3.6 Conclusion

Activated carbon-supported sulfonic acids were evaluated as solid acid catalysts for pretreatment of switchgrass for subsequent enzymatic conversion of glucan into glucose. Results indicated that solid acid catalysts were effective for pretreatment of switchgrass. Temperature and reaction time were found to significantly influence the pretreatment process. In addition the catalysts were successfully reused three times with minimal loss of activity. Our results suggest that solid acid catalysts may potentially reduce the use of acids for treatment and make the biomass to alcohol operations more effective and environmental friendly.

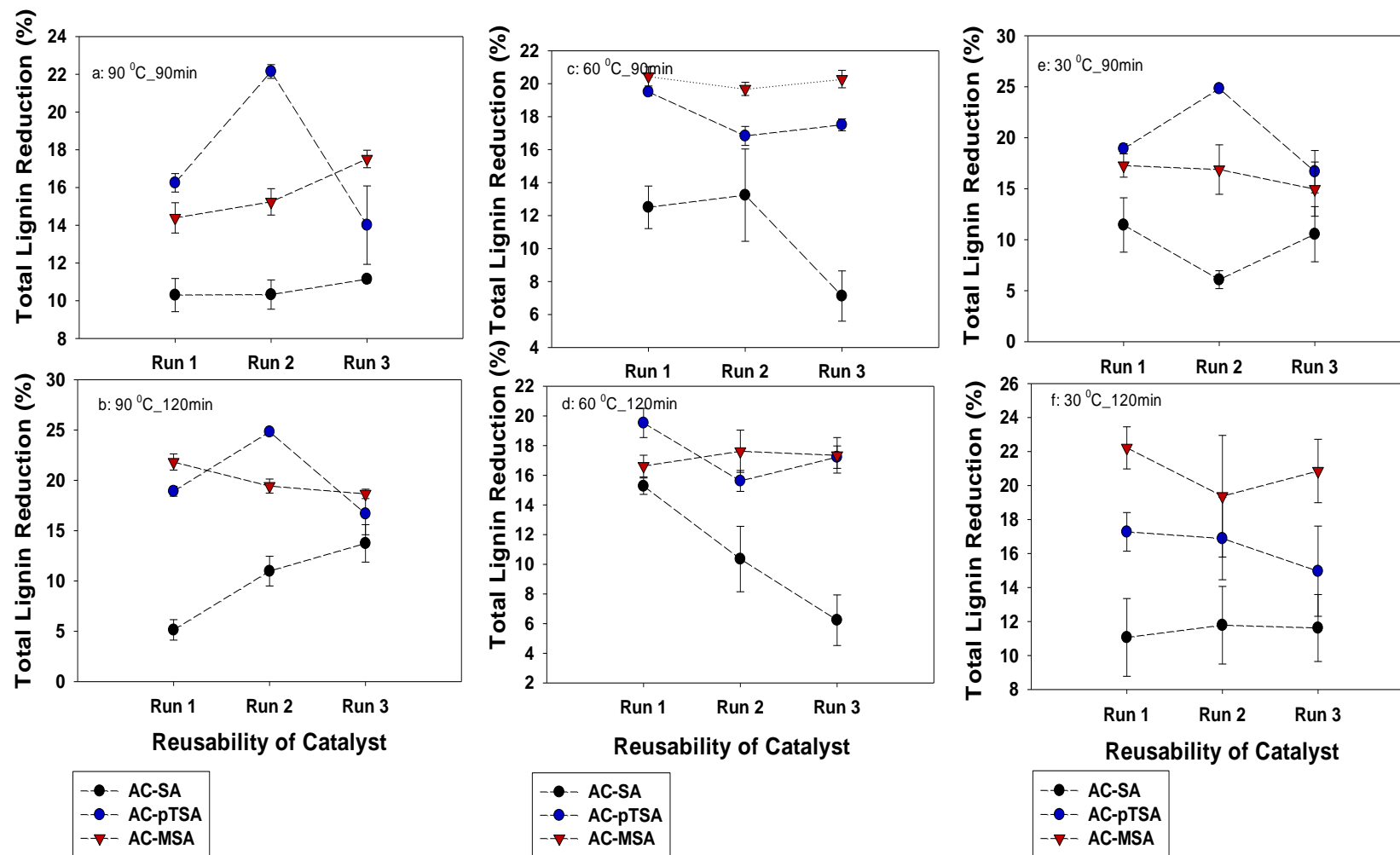


Fig 3.6 TLR (Total reduction lignin in percentage) for three different solid acid catalysts for three temperatures of 30, 60 and 90 °C at two different reaction times of 90 to 120 min.

### 3.7 Acknowledgments

I would like to thank Dr. Evgeny Danilov from Department of Chemistry, North Carolina State University for helping me run samples using FTIR and I also would like to express my gratitude to Dr. Joel Pawlak, from Department of Forest Biomaterials, North Carolina State University for allowed me to learn about TGA and run some samples using this instrument.

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## Chapter 4

### Pretreatment and hydrolysis of switchgrass into sugars using activated carbon supported sulfonic acid catalyst

#### Abstract

Activated carbon-supported sulfonic acid catalyst was utilized for hydrolysis of cellulose, starch cellobiose, and switchgrass. After sulfonation, total acidity was improved 5 times when compared with raw activated carbon. Model biomasses including cellulose, starch, and cellobiose were hydrolyzed at 90 °C for 6 and 24 h., Switchgrass was hydrolyzed at two different temperatures of 75 and 90 °C for 6, 12, 18 and 24 h. The effects of physical (ultrasonication) and chemicals (NaOH and H<sub>2</sub>SO<sub>4</sub>) treatments prior to hydrolysis of switchgrass were also studied. For model biomasses, highest glucose was produced from cellobiose corresponding to 237.1 ± 0.86 mg g<sup>-1</sup> (yield of 23.7 ± 0.08%) after 24 h, while switchgrass provided with a maximum glucose yield of 23.25 ± 0.33% corresponding to 72.67 ± 1.03 mg g<sup>-1</sup> after 18 h at 90 °C. Furthermore, pretreatments included in this study did not significantly affect the improvement of sugars production. From the overall results obtained, it appears that sulfonic acid catalyst has the potential for direct hydrolysis of lignocellulosic biomass due to the high acidity although biomass need to be first dissolved in the solution to enhance the solid catalytic reaction.

**Keywords:** Activated carbon-supported sulfonic acid catalyst, Hydrolysis, Treatment prior hydrolysis, Model Biomass and Switchgrass.

## 4.1 Introduction

The world energy demand has significantly increased for the past few decades due to rapid increase of population and advancements in transportation industry (Agbor et al., 2011). At the same time, continual use of fossil energy has resulted in environmental issues such as increased amounts of gas emissions (Kumar et al., 2009). In order to fulfill the need for energy in many different sectors while limiting the ill effects on the environment, it is crucial to develop alternative paths to produce sustainable and clean energy. Energy from lignocellulosic biomass can provide many benefits due to the abundant availability and low cost of feedstock (Lynd et al., 2002; Huang and Fu, 2013). Typically, carbohydrates in biomass are used as a source for biofuel production such as bioethanol and biobutanol (Alvira et al., 2010; Mosier et al., 2005). Therefore, processes to convert carbohydrates into alcohols have to be developed so that bioethanol from lignocellulosic biomass could be effectively commercialized. Only then the biomass to alcohol industry can compete with the petroleum industry.

In general, to convert biomass into alcohols, several unit operations need to be employed (Kumar et al., 2009; Agbor et al., 2011). The first operation includes pretreatment of biomass to prepare the biomass matrix for subsequent hydrolysis to synthesize sugars (Chiaromonti et al., 2012). The final step includes fermentation of sugars into alcohols or other chemicals depending on the market requirement. Biomass pretreatment involves physical, chemical, or biological processes that help the cellulases and hemicellulases access cellulose and hemicellulose portions of biomass matrix, although of late, chemical pretreatments using acids, bases, ammonia, ozone, and others are being widely studied.

Despite their efficacy, the overall pretreatment phase can cost at least 20% from the total production expenses (Chiaramonti et al., 2012; Brodeur et al., 2011; Yang and Wyman, 2008). In addition, chemical pretreatment agents such as acids and bases are corrosive and hence need special containers for storage and pretreatment (Kumar et al., 2009). Further, after the pretreatment the spent chemicals need to be treated, which may add additional steps and cost to the overall economics (Chiaramonti et al., 2012). Considering these issues with liquid pretreatments, it was recently proposed to use solid acids/bases for pretreatment (Peña et al., 2014; Ansanay et al., 2014; Tan and Lee, 2015).

After biomass pretreatment, another significantly expensive unit operation in biomass conversion is hydrolysis of biomass. Typically cellulolytic and hemicelluolytic enzymes are used to hydrolyze the biomass into sugars (Jørgensen et al., 2007; Keshwani and Cheng, 2009). Although the process is effective and provides with conversions over 90%, the high cost of enzymes makes biomass to alcohol processes very expensive (Chiaramonti et al., 2012; Jiang et al., 2012). In addition, the spent enzymes cannot be recycled easily. Hence researchers are looking at alternate options to hydrolyze lignocellulosic biomass (Guo et al., 2012; Zhou et al., 2011).

One approach to hydrolysis of biomass is to use solid acid catalysts (Qian 2013; Li and Qian, 2011). Solid catalysts can be synthesized easily and recycled with no significant loss of activity (Guo and Fang, 2013; Hu et al., 2014; Wang et al., 2012a; Peña et al., 2014). Recently, Hu et al (2014) and Wang et al (2012a) have explored hydrolysis of pure cellulose and cellobiose into sugars. Wang et al (2012a) reported the use of silica catalyst under

hydrothermal conditions at 160 °C for 12 h reaction to hydrolyze cellulose and observed 73.3 % cellulose conversion corresponding with 50.1% glucose yield. Meanwhile, SUCRA-SO<sub>3</sub>H was able to hydrolyze IL-pretreated cellulose for 24 h at 120 °C and observed glucose yield of 55%, while starch was observed to achieve higher glucose yield of 92% hydrolyzing and cellobiose conversion reached 100% with the same agent of SUCRA-SO<sub>3</sub>H (Hu et al., 2014).

While many studies have been conducted on model biomass components, to our knowledge solid acid catalysts have not been investigated extensively for direct hydrolysis of biomass. Based on extensive literature and our limited preliminary data, it was hypothesized that biomass could be directly hydrolyzed using solid acid catalysts. Hence, in this research the overall goal is to explore hydrolysis of biomass into sugars using solid sulfonic acid catalysts. Specifically the focus were on studying the efficacy of activated carbon-supported sulfonic acid catalyst for (1) direct hydrolysis of switchgrass into fermentable sugars and (2) hydrolysis of switchgrass pretreated with ultrasound, sodium hydroxide, and sulfuric acid.

## 4.2 Materials and Methods

### 4.2.1 Feedstocks

Switchgrass, maize starch, cellulose, and cellobiose were used as feedstocks in this research. Switchgrass was harvested in July 2011 from NCSU field labs and field dried. The stock was ground to pass size of 2-mm sieve and stored in the lab until further use. The switchgrass consisted of glucan (28.14%), xylan (13.47%), ASL (3.21%) and AIL (22.35%). Maize starch (CAS 9005-25-8), Lab Grade A cellulose (CAS 9004-34-6), and D(+) cellobiose (CAS 528-50-7) were procured from ACROS Organics and Fisher Scientific, respectively.

#### 4.2.2 Catalyst preparation

Catalyst used in this study was prepared by impregnating 60 g of activated carbon (size between 1-2 mm) with pToluene sulfonic acid solution. p-Toluene sulfonic acid solution was prepared by mixing 67 g of pToluene sulfonic acid with 100 ml of deionized water. The activated carbon was soaked in the acid solution for 48 h, separated by vacuum filtration, followed by drying for 2 h at 105 °C and calcination for 2 h at 250 °C.

#### 4.2.3 Catalyst characterization

To quantify the total acidic sites on the surface of the catalyst, Boehm titration method was employed. Typically, 0.5 g of catalyst was equilibrated with three base solutions of 0.05M NaHCO<sub>3</sub>, 0.05 M Na<sub>2</sub>CO<sub>3</sub> and 0.05 M NaOH at 125 rpm for 24 h at room temperature as described by Evangelin et al. (2012). After separating the catalysts from the solutions, 10 mL of each solution was titrated with 0.05 M HCl using Methyl red as indicator. It was assumed that carboxylic, lactonic, and phenolic groups were neutralized by NaOH, while NaHCO<sub>3</sub> neutralized carboxylic groups, and Na<sub>2</sub>CO<sub>3</sub> neutralized both carboxylic and lactones groups (Evangelin et al., 2012; Mukherjee et al., 2011).

#### 4.2.4 Pretreatment and hydrolysis

The feedstocks were converted into sugars according to the conditions summarized in table 4.1. All experiments were performed in triplicates in batch reactors in which catalyst and biomass was mixed (1:1) with 50 mL water.

## Batch experiments

In the first phase, hydrolysis experiments were performed using pure feedstocks such as cellulose, cellobiose, and starch to obtain baseline data. The feedstocks were hydrolyzed using activated carbon-supported sulfonic acid catalyst for 6 and 24 h at 90 °C. In the second phase, switchgrass was hydrolyzed directly using the catalyst. However, the catalyst particles were dispersed into switchgrass particles using an ultrasonication system for 1 min to ensure uniform mixing of batch reactor contents. Direct hydrolysis was performed at 75 °C and 90 °C for four reaction times of 6, 12, 18 and 24 h. After hydrolysis, catalyst was separated manually from the mixing slurry followed by the separation of wet samples via vacuum filtration. pHs of the liquid hydrolysates were measured and glucose contents were determined. In the third phase, switchgrass samples were treated prior to hydrolysis via ultrasonication (physical), NaOH (chemical), and H<sub>2</sub>SO<sub>4</sub> (chemical).

Physical pretreatment was conducted using Hielscher UIP 1000hd corresponding to ultrasonication for 5, 15, and 25 min at 100% amplitude. Ultrasonication was selected due to the effectiveness of this method to promote lignocellulosic dissolution suggested by Guo et al (2012). Chemical pretreatments included pretreatment in an autoclave with 2% NaOH and 1% H<sub>2</sub>SO<sub>4</sub> for 1 h at 121 °C as suggested by Wang et al (2010), Zhou et al (2012) and Shi et al (2011). After each pretreatment the biomass was separated via vacuum filtration, washed and prepared for sulfonic acid catalytic hydrolysis.

Table 4.1 Pretreatment and hydrolysis conditions employed for converting biomass into sugars

Feedstock	Prior Treatment	Hydrolysis Reaction time (h)	Temperature (C)
Cellobiose	No	6 and 24	90
Starch	No	6 and 24	90
Cellulose	No	6 and 24	90
Switchgrass	No	6, 12, 18, 24	75 and 90
Switchgrass	Ultrasound -100% Amplitude, 5 min	6 and 24	90
Swithgrass	Ultrasound -100% Amplitude , 15 min	6 and 24	90
Swithgrass	Ultrasound -100% Amplitude , 25 min	6 and 24	90
Switchgrass	NaOH 2% (w/v) Autoclave 1h, 121 °C	6 and 24	90
Swithgrass	H <sub>2</sub> SO <sub>4</sub> 1% (w/v) Autoclave 1h, 121 °C	6 and 24	90

#### 4.2.5 Soluble sugar analysis

Monomeric soluble sugars in the liquid were analyzed using a 2950 YSI biochemistry analyzer capable of determining concentrations of soluble sugars such as glucose and xylose. Typically, 1 ml of each sample was prepared in an eppendorf tube and exposed to the enzyme immobilized sensor to obtain concentrations of glucose and xylose in g/L.

#### 4.2.6 Statistical analysis

All experiments were conducted in triplicates. Data were analyzed using Proc Glimmix with confidence limits of 95% using SAS 9.3 (Cary, NC) to understand the effects of catalyst and pretreatment on glucose yield.

### 4.3 Results and Discussions

#### 4.3.1 Catalyst total acidity characterization

The surface acidity data for the catalyst obtained from Boehm Titration are presented in table 4.2. Compared to raw activated carbon (total acidity =  $0.1 \pm 0.03 \text{ mmol g}^{-1}$ ) the total acidity of sulfonic acid catalyst ( $0.51 \pm 0.01 \text{ mmol g}^{-1}$ ) synthesized in this research was about 5 times higher, suggesting that treatment of the catalyst resulted in impregnation of sulfonic acid groups on the surface.

Table 4.2 Catalyst acidity function

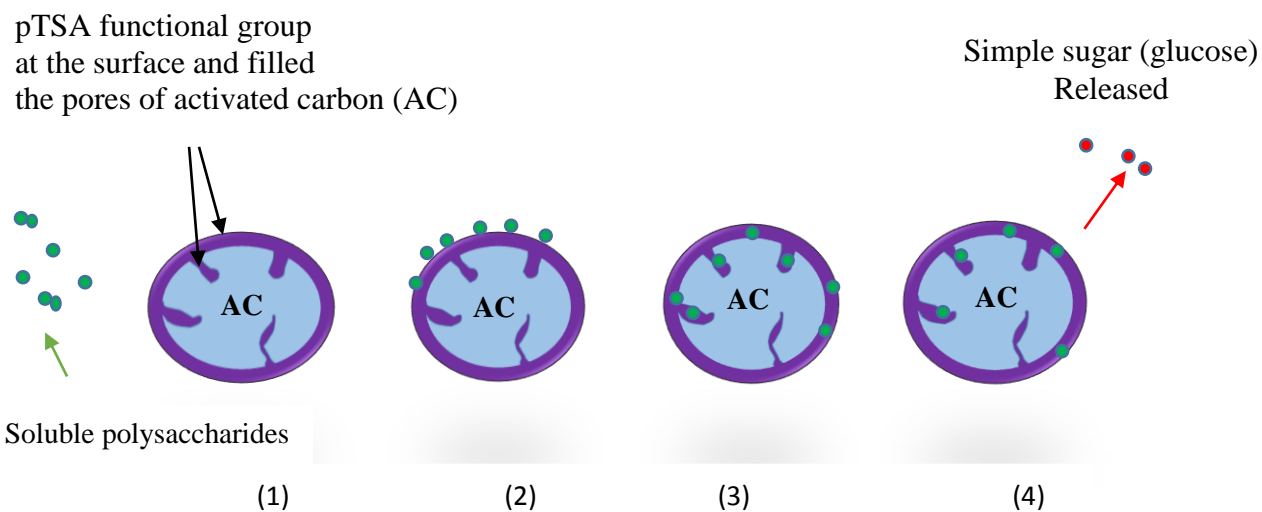
Acidic function ( $\text{mmol g}^{-1}$ )	AC-pTsOH
Carboxyl	$0.375 \pm 0.003$
Lactone	$0.005 \pm 0.003$
Phenolic	$0.085 \pm 0.005$
Total surface acidity	$0.51 \pm 0.01$

#### 4.3.2 Hydrolysis of pure feedstocks

The data obtained from hydrolysis ( $90 \text{ }^\circ\text{C}$ ) of cellulose, maize starch, and cellobiose are presented in Figure 4.1. When hydrolysis was performed without the catalyst glucose yields for starch and cellobiose were minimal ( $0 \text{ mg g}^{-1}$  for starch and  $0.99 - 1.64 \text{ mg g}^{-1}$  for cellobiose). However, as expected, when catalyst was added to the system, glucose yield from starch and cellobiose increased gradually as hydrolyzing time increased. The rate of

production of glucose from starch was initially faster up to 6 h and increased rather slowly thereafter to reach a maximum at 24 h. Starch is believed to consist of amylose and amylopectin. Amylopectin, being a highly branched structure, therefore making the hydrogen bonding of this polymer prone to easy disassociation especially when higher temperature was employed. Meanwhile amylose, a tightly packed linear polymer, probably possessed strong intermolecular hydrogen bonding that was difficult to be accessed by water. It was theorized that during the first 6 h of hydrolyzing time, most of amylopectin dissolved into the water solution, resulting in a higher rate. However as the amount of dissolved starch available in the slurry reduced, the rate of production of glucose was also slower than the first 6 h (Green et al., 1975).

It is also observed that the rate of glucose produced from cellobiose was higher compared to the starch. This is perhaps due to the simple structure of cellobiose that consists only of two glucose molecules linked by a  $\beta$ , 1-4 glycosidic bond. Therefore the catalyst was able to break the hydrogen bond relatively easily. In general, sulfonic solid acid hydrolysis steps are presented in Scheme 4.1 We theorize that solubility of the feedstock is the key for successful hydrolysis. Furthermore, for hydrolysis to take place, soluble polysaccharide has to be in close contact with solid catalyst and followed by adsorption and diffusion into surface or internal pores of solid catalyst. At this stage, the hydrogen bonding of the dissolved polysaccharide is broken into simple sugars such as glucose as described by Guo et al (2012).



Scheme 4.1. Schematic mechanism of sulfonic solid acid hydrolysis.

Therefore, in our research, the production of glucose for cellobiose ( $237.1 \pm 0.86 \text{ mg g}^{-1}$  (conversion of  $23.7 \pm 0.08\%$ )) was higher than that of starch ( $190.07 \pm 2.02 \text{ mg g}^{-1}$  feedstock (conversion of  $19 \pm 0.2\%$ )) after 24 h hydrolysis.

These results are similar to those of Takagaki et al. (2008) who also hydrolyzed starch at  $100^\circ\text{C}$  for 15 h using  $\text{HNbMoO}_6$  and Amberlyst-15 as solid acid catalysts. The authors reported a 21% glucose yield for  $\text{HNbMoO}_6$  and that 3.4% glucose yield when Amberlyst-15 was employed (Takagaki et al, 2008).

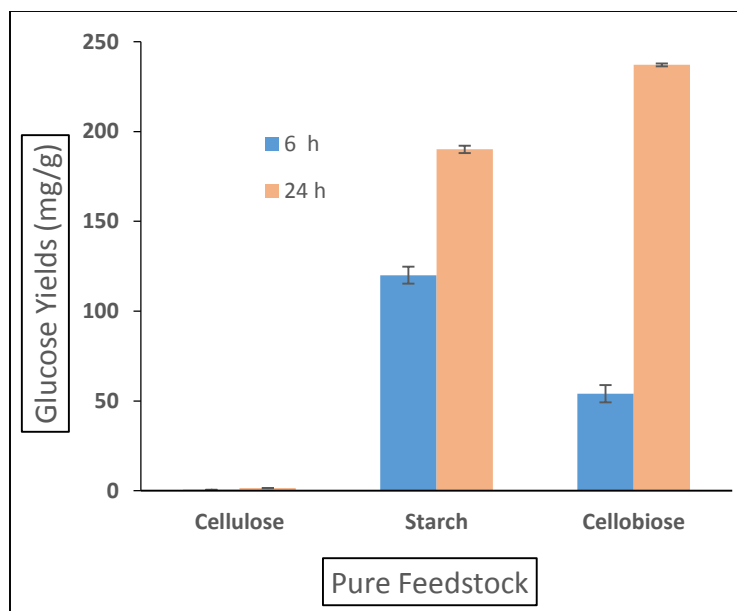


Fig 4.1 Hydrolysis of pure feedstocks using activated carbon-supported sulfonic acid catalyst.

In addition, rates of glucose formation from hydrolysis of cellobiose were found to be ranging between  $50 \pm 4.4$  and  $54.89 \pm 0.19 \mu\text{mol h}^{-1}$ . The rates obtained in our research are comparable to carbon-based solid acid catalysts ( $87 \mu\text{mol h}^{-1}$ ) reported by Kitano et al. (2009). But our rates are higher than silica-supported nafion ( $4.7 \mu\text{mol h}^{-1}$ ), Amberlyst 15 ( $27.5 \mu\text{mol h}^{-1}$ ), Nafion NR-50 ( $25.9 \mu\text{mol h}^{-1}$ ), niobic acid ( $5.1 \mu\text{mol h}^{-1}$ ), and H-mordenite ( $0.6 \mu\text{mol h}^{-1}$ ) (Kitano et al., 2009). Therefore, it appears that sulfonic solid acid catalyst prepared in our research is effective in converting cellobiose into glucose.

Interestingly, the catalyst seemed to be ineffective when cellulose was used as feedstock. A maximum glucose yield of  $1.54 \pm 0.04 \text{ mg g}^{-1}$  was obtained at  $90^\circ\text{C}$  for 6 and 24 h. It appears that in addition to surface acidity of the catalyst (Zhou et al., 2011), solubility of the

feedstock also plays a role in conversion. In our research the pH of the solution for all feedstocks tested were between 2.19 -2.39 (4.07 – 6.45 mmol L<sup>-1</sup>). However the solubility of cellobiose was the highest followed by starch and cellulose (Soest 1994) corroborating that critical role of biomass solubility. Recently Hu et al (2014) also observed similar trends with respect to cellulose hydrolysis via solid acid catalysts. The authors reported glucose yields of 4 and 3% from cellulose at 120 °C (24 h) using SUCRA-SO<sub>3</sub>H and SUCRO-SO<sub>3</sub>H solid acid catalysts, respectively (Hu et al., 2014). As cellulose is equipped with extensive hydrogen bonding and highly crystalline structure, several authors including Guo and Fang (2013), Wang et al. (2012b), and Zhu et al. (2006) suggested ionic liquid treatment to enhance hydrolysis activity of the catalyst.

#### 4.3.3 Ultrasonication-assisted direct hydrolysis

When switchgrass was hydrolyzed without the addition of catalyst at 75 and 90 °C at four hydrolyzing times of 6, 12, 18 and 24 h, glucose yields were between 5.7 – 12.9 mg g<sup>-1</sup>. However, with the presence of catalyst data presented in Fig 4.2 shown hydrolysis performed at higher temperature resulted in significantly higher glucose yields than 75 °C ( $p < 0.05$ ). The yields from four different hydrolyzing times at 75 °C were found not significantly different ( $p > 0.05$ ). In addition, yields produced at 90 °C with 18 and 24 hydrolyzing times were also found not significantly different ( $p > 0.05$ ).

As expected, glucose yield increased with hydrolysis time and a maximum glucose yield of  $72.67 \pm 1.03$  mg g<sup>-1</sup> (yield of  $23.25 \pm 0.33\%$ ) was achieved after 18 h of hydrolysis at 90 °C. Our results are similar to those of Li and Qian (2011), who studied the use of Amberlyst 15Dry for hydrolysis of rice straw. In their research, a maximum monosaccharides of 148.7

g kg<sup>-1</sup> rice straw yield was achieved at 150 °C after 3 h with the ratio of 10% solid content. In addition, 75 g kg<sup>-1</sup> of glucose was reported after 3 h of reaction and stayed relatively stable up to 6 hours (Li and Qian, 2011). In a different study by Hu et al. (2014), SUCRA-SO<sub>3</sub>H and SUCRO-SO<sub>3</sub>H to hydrolyze carbohydrate from rice straw treated with ionic liquid (C<sub>4</sub>mim. OAc) and reported glucose yields of 19.5 % and 16.5 % respectively.

In a different study, liquid p-Toluene sulfonic acid (0.100 mol H<sup>+</sup>/L) was used to hydrolyze 0.1 g corn stover at 150 °C for 2.5 h. The authors, Amarasekara and Wiredu (2012), reported a maximum glucose yield of 35 μmol and 38 μmol at 160 °C. In our research we also obtained 53.49 ± 1.05 μmol (at 75 °C after 6 h) and a maximum of 403.76 ± 5.74 μmol at 90 °C after 18 h.

Extrapolating these results and comparing with ours, the authors, Amarasekara and Wiredu (2012) would have obtained 350 – 380 μmol of glucose, which appears similar to our results.

Similarly, Yamaguchi and Hara (2010) employed carbon-based solid acid catalyst bearing SO<sub>3</sub>H, COOH and OH groups for hydrolyzing of Japanese cedar, bagasse and rice straw at 100 °C for 2 h using a catalyst loadings of 2:2.5-3:4. The authors reported glucose yields of 14.3 - 42.5 mg g<sup>-1</sup>, which is similar to the results obtained in our research. Therefore, activated carbon-supported pToluene sulfonic acid catalyst has the potential to directly convert lignocellulosic biomass into sugars.

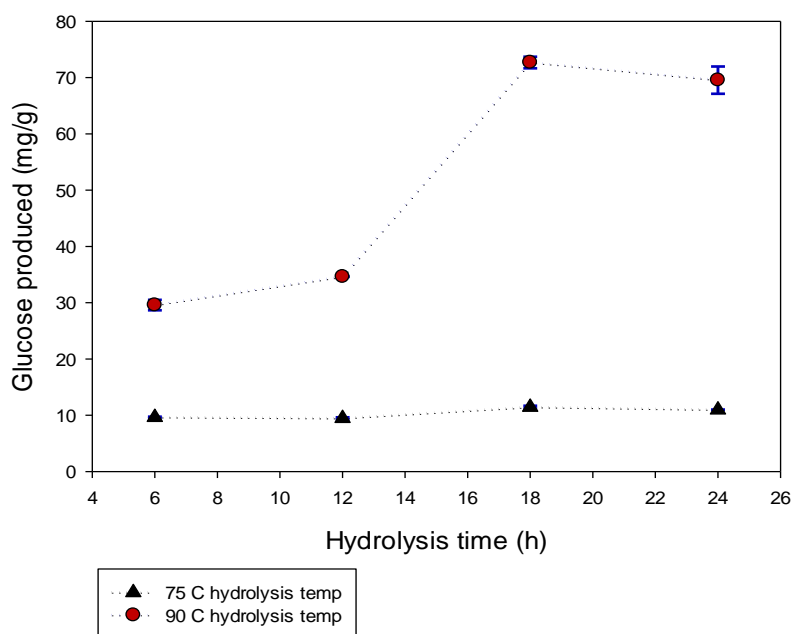


Fig 4.2 Sulfonic solid acid catalyst hydrolysis of switchgrass for 6 and 24 h at 75 and 90 °C.

#### 4.3.4 Effect of pretreatment on hydrolysis of switchgrass

As shown in Table 4.3, pretreatment using ultrasonication appeared to have significant effect on glucose yield. Without the presence of the catalyst, the yield of glucose in the separated liquid after 5, 15, and 25-min ultrasonication pretreatment was limited to 1.38-2.12 %. However, after the same pretreatment times, liquid separated from switchgrass-catalyst mixture yielded glucose in the range of 7.32-8.02%. The data suggested that catalyst was able to attack the cellulose portion of the switchgrass matrix facilitate hydrolysis.

In addition, as presented from Table 4.3, when hydrolysis of ultrasonicated switchgrass was performed the glucose yields increased. When switchgrass was hydrolyzed for 6 h, the glucose yields increased as pretreatment times increased. However, the data obtained after 24-h hydrolysis suggested that pretreatment times had no significant effect ( $p > 0.05$ ) on glucose yields suggesting that the all sites on switchgrass matrix were occupied by the catalyst.

Combined physical treatment and solid acid catalyst hydrolysis of lignocellulosic biomass was recently studied by Jiang et al. (2012). The authors employed microwave irradiation and 1:1 catalyst: corncob biomass ratio and hydrolysis was performed for 20-120 h at 110-140 °C. A maximum of 34.6% yield of glucose was observed after 60 h of hydrolysis at 130 °C and after 20 h hydrolysis at 140 °C (Jiang et al., 2012). While in our research, a maximum of  $16.91 \pm 0.05\%$  of glucose was obtained after 15 minutes of ultrasonication followed by 24 h of catalytic hydrolysis. Therefore, our data suggests ultrasonication did impact the structure of switchgrass to improve the interaction between switchgrass and sulfonic solid acid catalyst.

Table 4.3 Ultrasonication effect on switchgrass hydrolyzing using solid acid catalyst

Conditions	Ultrasounds	Glucose Yield (%)	Glucose (mg g <sup>-1</sup> )
Ultrasonication For 5 minutes	SG+Ult <sup>a</sup>	1.38 ± 0.002	4.30 ± 0.007
	SG+Cat+Ult <sup>b</sup>	7.32 ± 0.08	22.89 ± 0.25
	SG+Cat+Ult+6 h hydrolysis	12.95 ± 0.40	40.52 ± 1.27
	SG+Cat+Ult+24 h hydrolysis	16.81 ± 0.11	52.55 ± 0.36
Ultrasonication For 15 minutes	SG+Ult <sup>a</sup>	2.12 ± 0.01	6.63 ± 0.04
	SG+Cat+Ult <sup>b</sup>	7.65 ± 0.08	23.90 ± 0.25
	SG+Cat+Ult+6 h hydrolysis	15.11 ± 0.21	47.23 ± 0.65
	SG+Cat+Ult+24 h hydrolysis	16.91 ± 0.05	52.86 ± 0.16
Ultrasonication For 25 minutes	SG+Ult <sup>a</sup>	1.54 ± 0.08	4.80 ± 0.28
	SG+Cat+Ult <sup>b</sup>	8.02 ± 0.12	25.08 ± 0.38
	SG+Cat+Ult+6 h hydrolysis	16.76 ± 0.16	52.40 ± 0.49
	SG+Cat+Ult+24 h hydrolysis	16.80 ± 0.02	52.54 ± 0.06

<sup>a</sup> Switchgrass was mixed with water under ultrasonication effect, <sup>b</sup> switchgrass was mixed with water contained catalyst under ultrasonication effect,

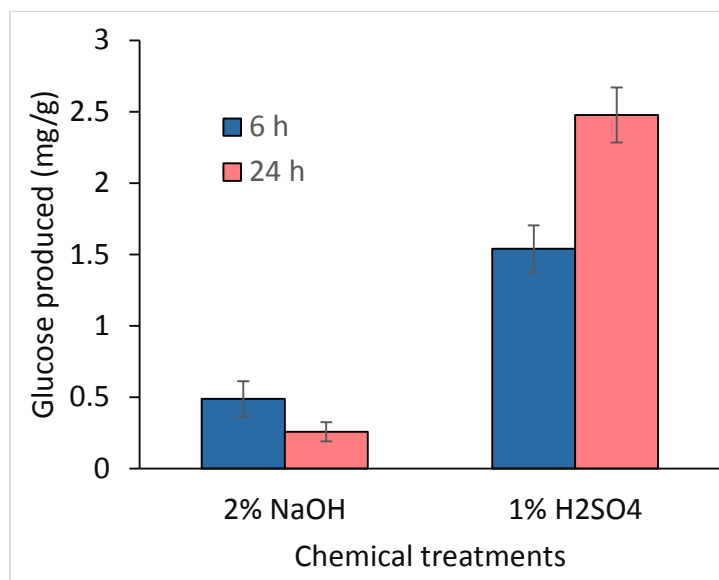


Fig 4.3 Sugar produced after 6 and 24 h sulfonic acid hydrolysis from switchgrass treated with chemical agents of NaOH and H<sub>2</sub>SO<sub>4</sub>

Data presented in Fig 4.3 suggest that chemical pretreatment altered the surface of the switchgrass and perhaps formed a barrier on the surface that was not accessible to sulfonic acid catalyst. However, addition studies, especially those investigating the surface structural changes to the biomass surface are suggested. In addition it is also theorized that chemical pretreatments might impact the solubility of cellulose in the biomass matrix, which may in turn inhibit catalytic hydrolysis. Additional studies on effects of using suitable solvents such as ionic liquids are recommended.

#### 4.4 Conclusion

An activated carbon-supported sulfonic solid acid catalyst was evaluated as a hydrolysis agent for conversion of cellulose, starch, cellobiose, and switchgrass into glucose.

Experiments were conducted to investigate the effects of temperatures, reaction times, and pretreatments on sulfonic solid acid hydrolysis. Increased hydrolyzing time using sulfonic acid catalyst significantly affected formation of glucose from model biomasses including cellobiose and starch. Furthermore, reaction time and temperature were found to have significant effects for the production of glucose from Switchgrass. Meanwhile, among three pretreatments agents used, ultrasonication with 100% amplitude as physical treatment was found to have improved the formation of glucose compared to 2% NaOH (w/v) and 1% H<sub>2</sub>SO<sub>4</sub> (w/v). Therefore, it is concluded that novel use of activated carbon-supported pToluene sulfonic acid catalyst can be potentially used to directly convert carbohydrate from lignocellulosic biomass into fermentable sugars.

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## Chapter 5

### Pretreatment of biomasses using magnetized sulfonic acid catalysts

#### Abstract

Three sulfonic solid acid catalysts, namely, regular, magnetic A and magnetic B were tested for pretreatment of four lignocellulosic biomass of switchgrass, gamagrass, miscanthus x giganteus and triticale hay at 90 °C for 2 h. A maximum total lignin reduction of  $17.73 \pm 0.63$  % was observed for triticale hay treated with magnetic A catalyst. Furthermore, maximum glucose yield after enzymatic hydrolysis was observed to be  $203.47 \pm 5.09$  mg g<sup>-1</sup> (conversion of  $65.07 \pm 1.63$  %) from Switchgrass treated with magnetic A catalyst.

Durability of magnetized catalysts were also tested and it was observed that magnetic A catalyst was consistent for gamagrass, miscanthus x giganteus and triticale hay , while magnetic B catalyst was found to maintain consistent yield for switchgrass feedstock.

**Keywords:** Magnetic catalysts, Lignocellulosic biomass, Pretreatment, Hydrolysis, Catalysts durability.

## 5.1 Introduction

Lignocellulosic biomass possesses distinctive advantages as one of the renewable sources of energy due to presence of high carbohydrate content. In addition to being inexpensive, lignocellulosic biomass offers sustainability and a high potential to reduce greenhouse gas emissions (Perlack et al., 2005; Zhou et al., 2011). However, one of the main challenges in converting biomass into energy involves disruption of the complex structure of the biomass to obtain monomeric sugars (Kumar et al., 2009; Agbor et al., 2011). Usually, physico-chemical pretreatment is required to ensure that biomass material becomes more accessible to enzymes either via removal of lignin or solubilization of hemicellulose (Mosier et al., 2005; Alvira et al., 2010). Several chemical pretreatments using acids, bases, organic solvents and ionic liquids were developed and studied extensively. Although, chemical pretreatment techniques are attractive due to the higher reaction efficiency and excellent mass transfer capabilities (Guo et al., 2012), use of these chemical agents lead to various environmental issues and also requires expensive unit operations (for neutralization) on the downstream side of the process (Peña et al., 2014). Therefore, reusable pretreatment agents that also minimize environmental impacts are required. One such option is to use solid acid catalyst as pretreatment agent for biomass (Hara 2010; Guo et al., 2012).

Utilizing solid acid catalysts can potentially address some of these aforementioned challenges associated with liquid pretreatments as solid acid catalysts allow for mild operating conditions and moderately high selectivity. In addition, solid acid catalysts allow for simple separation from products by vacuum filtration or magnetic separation (Lai et al., 2011; Peña et al., 2014; Guo et al., 2013). Further, the catalysts may be used repeatedly for

the reaction without neutralization, therefore minimizing energy consumption and waste (Zhou et al., 2011). Presently, little data is available on use of solid acid catalysts for pretreatment of real biomass streams although few studies have explored solid acid catalysts. For example, Peña et al (2014) reported glucose yield of 59% achieved from corn stover treated used Propyl-sulfonic (PS) acid-functionalized nanoparticle catalyst at 160 °C for 60 min followed by the addition of 2 ml of Accelerase enzyme along with 2.5 g wet corn stover for 24 h hydrolysis. The study also reported that as pretreatment temperature increased to 180 °C, the yield of glucose increased reached the maximum of 90%. In a different study, macroalgae cellulose residue was treated used Dowex (TM) Dr-G8 solid catalyst followed by enzymatic hydrolysis where two enzymes were employed (45 FPU/g of cellulase and 52 CBU/g of  $\beta$ -glucosidase) to produce glucose yield at around 94% even after 5 reuses (Tan and Lee, 2015). Therefore the present study was undertaken with two objectives in mind, which are to: (1) systematically evaluate activated carbon-supported sulfonic acid catalysts for pretreatment of Switchgrass, Gamagrass, Miscanthus and Triticale hay and (2) enhance the separation of spent catalysts via chemical encapsulation of magnetic particles on catalyst surface.

We hypothesize that (1) activated carbon-supported sulfonic acid catalysts can facilitate pretreatment of various biomass for subsequent enzymatic hydrolysis and (2) chemical encapsulation of magnetic particles on catalyst surface can facilitate simple separation of spent catalyst with no loss of catalytic activity.

## 5.2 Materials and Methods

### 5.2.1 Lignocellulosic feedstock

Switchgrass, Gamagrass, Miscanthus and Triticale hay were used as feedstocks in this research. Switchgrass was harvested in mid July 2011 from North Carolina State University Field Laboratory in Reedy Creek Road Raleigh, NC. The subsamples were field cured for 3 days. Gamagrass variety was harvested at the end of July 2012, and the postharvest samples were oven dried at 50 °C for 72 hours. Miscanthus giganteus was harvested from the Mountain Horticultural Crops Research and Extension Center (Mills River, NC) in December 2011 and oven dried at 45 °C for 72 h. These three biomass were ground to pass a 2mm sieve. Furthermore, triticale hay sample was collected from the field at Central Agricultural Research Center of Montana State University and ground to pass 1 mm sieve. All biomasses were placed in sealed plastic bags and stored until further use. The initial moisture contents were Switchgrass, Gamagrass, Miscanthus and Triticale hay 7.98, 6.54, 6.44 and 6.53%, respectively. In addition, the feedstocks were analyzed for their composition using standard methods (Sluiter et al., 2008) (Table 5.1).

Table 5.1 Initial composition analysis of four feedstock (dry basis)

Biomass/Composition	Glucan (%)	Xylan (%)	ASL (%)	AIL (%)
Alamo Switchgrass	28.14 ± 0.32	13.47 ± 0.28	3.21 ± 0.12	22.35 ± 0.6
Gamagrass	30.18 ± 0.64	12.88 ± 0.59	2.56 ± 0.04	22.17 ± 0.48
Miscanthus x giganteus	37.04 ± 0.21	11.79 ± 0.10	1.54 ± 0.06	21.92 ± 0.33
Triticale hay	27.97 ± 0.52	13.29 ± 0.54	3.29 ± 0.07	23.04 ± 0.46

## 5.2.2 Sulfonic Solid Acid Catalysts Preparation

### 5.2.2.1 Activated carbon-supported sulfonic acid catalyst (from chapter 4)

Catalyst used in this study was prepared by impregnating 60 g of activated carbon with pToluene sulfonic acid solution. pToluene sulfonic acid solution was prepared by mixing 67 g of pToluene sulfonic acid into 100 ml of deionized water. The activated carbon was soaked in the acid solution for 48 h, separated by filtration, followed by drying for 2 h at 105 °C and calcination for 2 h at 250 °C.

### 5.2.2.2 Magnetic Activated carbon sulfonic acid catalyst

Thirty grams of activated carbon (fine) was stirred in a 50 ml deionized water solution containing 12 g of iron (III) nitrate, similar to the procedure described by Guo et al. (2013). The pH of the solution was adjusted to 10 by adding 3M of sodium hydroxide solution. The mixture was stirred at 200 rpm at room temperature for 24 h, after which the solid was filtered and calcined at 400 °C under nitrogen flow for 3 h to obtain magnetically activated carbon. Subsequently, 20 g of magnetic carbon was mixed with an aqueous solution containing 20 mL deionized water, 13.5 g of - Toluene sulfonic acid, and 20 ml mercaptoacetic acid for 24 h at room temperature at 200 rpm. At the end of 24-h period, 3M sodium hydroxide solution was added until the pH of the slurry reached 7. At this stage, the solid was separated from the slurry and dried at 80 °C for 12 h followed by calcination under nitrogen flow at 400 °C for 3 h. Subsequently, 12 g of the solid was immersed into 20 mL of deionized water and 20 ml of hydrogen peroxide was added dropwise. The mixture was stirred at 200 rpm at room temperature for 12 h. The solid was separated and dried again at 80 °C for 16

h to obtain the final product which was named magnetic activated carbon-supported p-toluene sulfonic acid catalyst (Magnetic A).

In addition, a second type of magnetic activated carbon-supported p-toluene sulfonic acid catalyst (Magnetic B) was prepared by modifying the above procedure by adding granular sodium hydroxide and during the last step and hydrogen peroxide was added twice (10 ml for each) followed by drying and calcination as described previously.

### 5.2.3 Pretreatment

Pretreatment was performed in batch reactors placed on a hot plate capable of heating and mixing the reactor contents. Biomass and catalyst were mixed in 50 mL for 2 h at 90 °C, stirred at 350 rpm. After pretreatment, catalyst was separated from biomass. For regular catalyst pretreatment, the separation was performed manually followed by the solid wet biomass separation using vacuum filtration. For magnetized catalyst pretreatment, the solid wet biomass was first filtered and the settled catalyst particles were separated by a conventional magnet. The catalyst was stored for subsequent use and pretreated biomass was hydrolyzed.

### 5.2.4 Liquefaction and Total Sugar Oligomer

Soluble polysaccharide in the liquid hydrolysate after treatment consisted of both simple sugars and sugars oligomer. Simple sugars such as glucose and xylose were measured via YSI 2950. To determine total oligomer, all sugars oligomers in the hydrolysate were

converted into monomeric sugar by adapting 4% acid hydrolysis NREL procedures (Sluiter et al., 2006) as below:

$$\text{Liquefaction} = \text{Total oligomer} + \text{CSS} \quad (1)$$

$$\text{Simple sugars} = \text{Glucose} \quad (2)$$

$$\text{Carbohydrate simple sugar (CSS)} = \text{Glucose} * 0.9 \quad (3)$$

$$\text{Total oligomer} = (\text{Glucose} * 0.9) + (\text{Xylose} * 0.88) \quad (4)$$

### 5.2.5 Enzymatic hydrolysis

Enzymatic hydrolysis was performed at 50 °C for 72 h (150 rpm). Biomass samples (1 g dry basis) were mixed with 20 fpu of Cellic Ctec 2 (activity ~ 119 fpu/ml). To avoid microbial growth, 40 µg/ml of tetracycline was added as an antibiotic. 50 mM of citric acid monohydrate buffer (pH = 5.0) was added to adjust the total volume of 20 mL. After 72 h, slurry samples were cooled down to 4 °C and kept refrigerated until further analysis.

### 5.2.6 Sugar analysis

Monomeric soluble sugars in the liquids collected from all experiments were analyzed using a 2950 YSI biochemistry analyzer capable of determining concentrations of soluble sugars such as glucose and xylose. Typically, 1 ml of each sample was prepared in an eppendorf tube and exposing the sample to the enzyme immobilized sensor to obtain the concentrations of glucose and xylose in g/L.

### 5.2.7 Biomass characterization

Iron leaching tests in the liquid hydrolysate and pretreated samples after pretreatment used magnetic catalysts have performed used Perkin Elmer 3100 Atomic Absorption Spectroscopy. Final concentrations of iron leaching found in the solid was reported in mg/g while in the liquid hydrolysates was in mg/L.

### 5.2.8 Statistics Analysis

All experiments were performed in triplicate. Proc GLIMMIX method with Tukey adjustment was used to analyze the data. Data was analyzed to study the effect of 3 different catalysts (regular, magnetic A and magnetic B) on 4 different biomasses (Switchgrass, Gamagrass, Miscanthus x giganteus, and Triticale Hay). In addition, effect of reusability of magnetic catalysts was also tested by analyzing the data for magnetizing procedure (2 levels: MagneticA and MagneticB), feedstock (4 levels: Switchgrass, Gamagrass, Miscanthus x giganteus, Triticale Hay) and Reuse (2 levels: 1 and 2).

## 5.3 Results and discussions

### 5.3.1 Effect of regular and magnetic catalysts on the pretreatment stage

After completion of pretreatment, liquid samples were analyzed to measure the sugar content in the liquid samples. Data for liquefaction, total oligomer and simple sugar of glucose are presented in Fig 5.1. In the present context (see eqs 1-4), liquefaction referred to a mixture of total oligomers and carbohydrate simple sugar (glucose), while total oligomers consisted of

short polymers including xylose oligomer (from xylan) and glucose oligomer (from glucan). It appeared that regular and magnetic A catalysts facilitated solubilization of carbohydrate (Fig 5.1 A and B) corresponding to total sugar yields of  $48.87 \pm 1.42$  mg g<sup>-1</sup> and  $53.37 \pm 0.58$  mg g<sup>-1</sup> respectively. Wang et al. (2012) reported the use of Perfluoroalkylsulfonic (PFS) and alkylsulfonic (AS) acid-functionalized magnetic nanoparticles for pretreatment of wheat straw and attempted to solubilize hemicellulose. Their (Wang et al) results show that after 24-h reaction at lower temperature (80 °C),  $3.5 \pm 0.1\%$  and  $1.0 \pm 0.2\%$ , of monosaccharides from xylan were obtained from the two catalysts. However, at higher temperature (160 °C for 2 h) xylose yields were observed to be 0.3% and 1.2% from PFS and AS catalysts respectively (Wang et al., 2012). In addition, Tan and Lee (2015) reported 0.77 g glucose (glucose yield of 0.77%) in the pretreatment liquid, when 100 g of macroalgae cellulosic residue was treated using Dowex (TM) Dr-G8 solid acid catalyst.

In comparison, the catalysts synthesized in our study performed reasonably well to hydrolyze cellulose (glucan). Particularly, Magnetic B catalyst when used to pretreat Triticale hay provided the highest glucose yield of  $33.62 \pm 0.08$  mg g<sup>-1</sup> (glucose yield of  $10.82 \pm 0.02\%$ ) and maximum xylan oligomer of  $1.79 \pm 0.2$  mg g<sup>-1</sup> in the liquid treatment.

The data was also analyzed to investigate the effectiveness of these catalysts to disrupt lignin in the biomasses. As presented in Table 5.2, the highest reduction in total lignin was found in Triticale hay treated with magnetic A catalyst ( $17.73 \pm 0.63\%$ ) followed by regular catalyst ( $15.11 \pm 0.86\%$ ). Our results are similar to Chen et al (2007) who reported the use of 0.5 – 2% alkali to obtain a 10.16 – 24.06% reduction in total lignin for Triticale hay.

Table 5.2 Total Lignin Reduction

Biomass feedstock	Total Lignin Reduction (%) after pretreatment used activated carbon supported p-Toluene sulfonic acid catalysts		
	Regular	Magnetic A	Magnetic B
Switchgrass	10.02 ± 0.95	10.75 ± 1.84	9.50 ± 1.02
Gamagrass	9.83 ± 0.69	13.02 ± 0.19	9.27 ± 0.36
Miscanthus x giganteus	9.19 ± 0.63	11.76 ± 0.82	9.21 ± 0.13
Triticale hay	15.11 ± 0.86	17.73 ± 0.63	12.25 ± 0.34

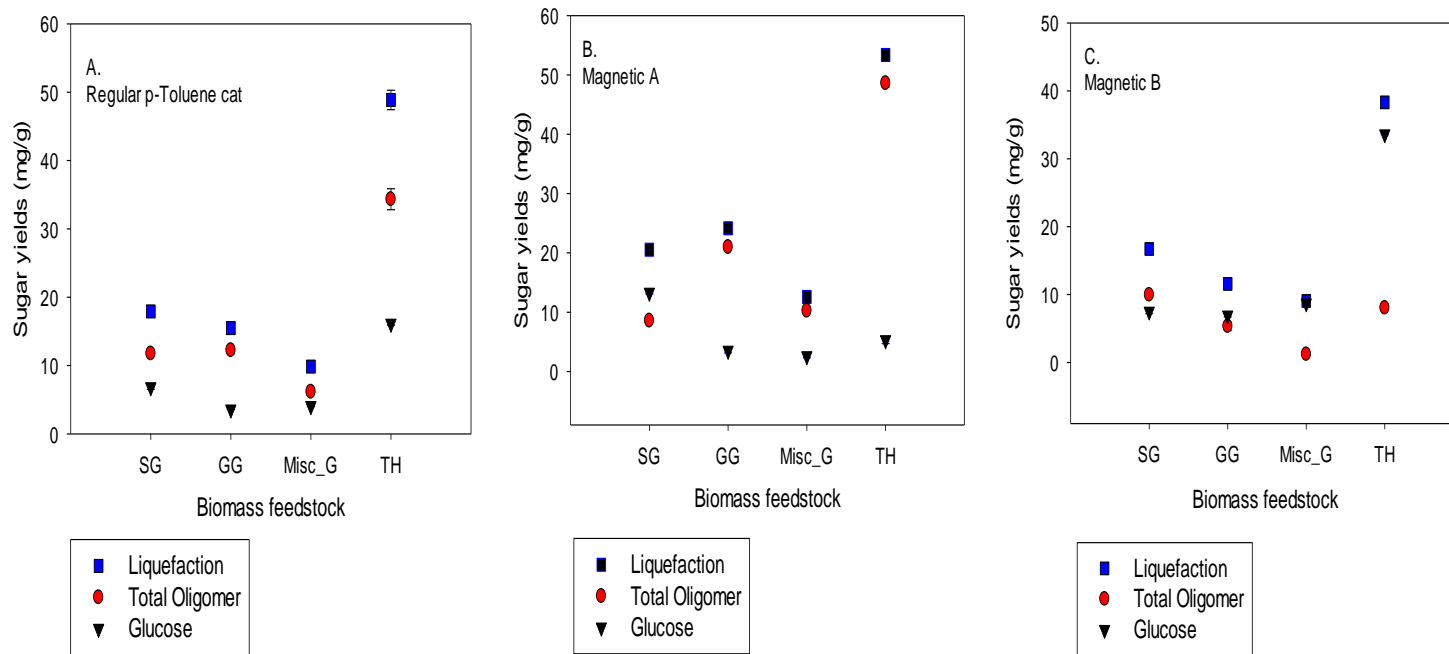


Figure 5.1 Sugar presented in the liquid treatment (A) used Regular catalyst, (B) Magnetic A first use and (C) Magnetic B catalyst first use

### 5.3.2 Effect of regular and magnetic catalysts on the enzymatic hydrolysis stage

As presented from Fig 5.2, the glucose yields obtained after hydrolysis of switchgrass pretreated with regular and magnetic B catalysts were similar ( $p = 0.93$ ). However, for magnetic A, the yields were significantly higher than the yields obtained from regular and magnetic B catalysts ( $p < 0.05$ ). For Gamagrass there was no significant difference between the glucose yields for all three catalysts tested ( $p > 0.1$ ). Meanwhile, glucose yields for triticale hay treated with regular and magnetic A catalyst were not significant ( $p > 0.05$ ). In addition, the statistical analyses of glucose yields for all biomasses tested in this research are presented in Table 5.3.

Table 5.3 SAS Output for comparison of three sulfonic acid catalysts

Simple Effect Comparisons of Magnet*Feedstock Least Squares Means By Feedstock													
Adjustment for Multiple Comparisons: Holm-Tukey													
Simple Effect Level	Magnet	_Magnet	Estimate	Standard Error	DF	t Value	Pr >  t	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
Feedstock Gamagrass	Magnetic A	Magnetic B	0.3181	2.7191	24	0.12	0.9078	0.9925	0.05	-5.2938	5.9300	-6.4722	7.1084
Feedstock Gamagrass	Magnetic A	Regular	-3.8372	2.7191	24	-1.41	0.1710	0.3511	0.05	-9.4491	1.7747	-10.6276	2.9531
Feedstock Gamagrass	Magnetic B	Regular	-4.1554	2.7191	24	-1.53	0.1395	0.2959	0.05	-9.7673	1.4565	-10.9457	2.6350
Feedstock Miscanthus	Magnetic A	Magnetic B	9.2496	2.7191	24	3.40	0.0023	0.0064	0.05	3.6377	14.8615	2.4593	16.0399
Feedstock Miscanthus	Magnetic A	Regular	4.2765	2.7191	24	1.57	0.1289	0.2765	0.05	-1.3354	9.8884	-2.5138	11.0668
Feedstock Miscanthus	Magnetic B	Regular	-4.9731	2.7191	24	-1.83	0.0799	0.1817	0.05	-10.5850	0.6388	-11.7634	1.8173
Feedstock Switchgrass	Magnetic A	Magnetic B	12.1968	2.7191	24	4.49	0.0002	0.0004	0.05	6.5849	17.8087	5.4065	18.9871
Feedstock Switchgrass	Magnetic A	Regular	11.9409	2.7191	24	4.39	0.0002	0.0006	0.05	6.3290	17.5528	5.1506	18.7313
Feedstock Switchgrass	Magnetic B	Regular	-0.2559	2.7191	24	-0.09	0.9258	0.9951	0.05	-5.8678	5.3560	-7.0462	6.5344
Feedstock Triticale	Magnetic A	Magnetic B	13.7374	2.7191	24	5.05	<.0001	0.0001	0.05	8.1255	19.3493	6.9470	20.5277
Feedstock Triticale	Magnetic A	Regular	3.9533	2.7191	24	1.45	0.1589	0.3303	0.05	-1.6586	9.5652	-2.8370	10.7436
Feedstock Triticale	Magnetic B	Regular	-9.7840	2.7191	24	-3.60	0.0014	0.0040	0.05	-15.3959	-4.1721	-16.5744	-2.9937

Overall the maximum glucose yields (for all biomasses) ranged between  $25.3 \pm 0.14 \%$  and  $65.07 \pm 1.63\%$  with Switchgrass providing with maximum glucose yields of  $65.07 \pm 1.63\%$ . It may be noted that when the liquid and pretreated biomass samples were analyzed via atomic absorption spectroscopy, it was found that  $0.4\text{--}6 \text{ mmol L}^{-1}$  and  $4.5\text{--}7 \text{ mg g}^{-1}$  of iron was present in liquid and pretreated biomass suggesting that iron was leaching into the system due to agitation. The yields observed from Miscanthus were between  $25.3 \pm 0.14\%$  -  $34.55 \pm 3.28\%$ . In comparison, Panneerselvam et al (2013a) reported a maximum glucose yield (after enzymatic hydrolysis) of  $13\text{--}26 \%$  ( $60\text{--}80 \text{ mg g}^{-1}$ ) when Miscanthus x giganteus was pretreated with  $40\text{--}58 \text{ mg/L}$  ozone using uniflow and reserve flow configurations. In addition, Miscanthus x giganteus treated with alkali followed by enzymatic hydrolysis was able to reach glucan conversion of  $32.8 \pm 3.49\%$  (Panneerselvam et al., 2013b).

Similarly Gamagrass produced glucose yields between  $160.4\text{--}174.33 \text{ mg g}^{-1}$  ( $47.84 \pm 0.26 \%$  -  $51.99 \pm 4.21 \%$ , see Fig 5.2) after enzymatic hydrolysis with maximum yield that was obtained from Gamagrass treated with regular catalyst. The glucose yields obtained in our research are slightly lower than those reported by other researchers in literature. For example, Xu et al. (2012) reported the glucose yields of  $215.5\text{--}270.5 \text{ mg g}^{-1}$  (Maximum glucan conversion of  $67.7 \%$ ) after enzymatic hydrolysis from many varieties of Gamagrass treated with  $1\%$  NaOH for 60 min at  $121 \text{ }^{\circ}\text{C}$ . Despite reports by Tejirian and Xu (2010) and Chen and Fu (2013) that iron may inhibit enzymatic hydrolysis our data suggested that Cellic Ctec2 can still performed reasonably well. We theorize that the yields could be enhanced by employing a surfactant to minimize the effects of iron on enzymatic hydrolysis as proposed by Chen and Fu (2013). In addition, the amounts of xylose also increased between  $11.48 \pm$

3.66 mg g<sup>-1</sup> - 46.88 ± 0.38 mg g<sup>-1</sup> after enzymatic hydrolysis even without the addition of xylanase (see Table 5.4).

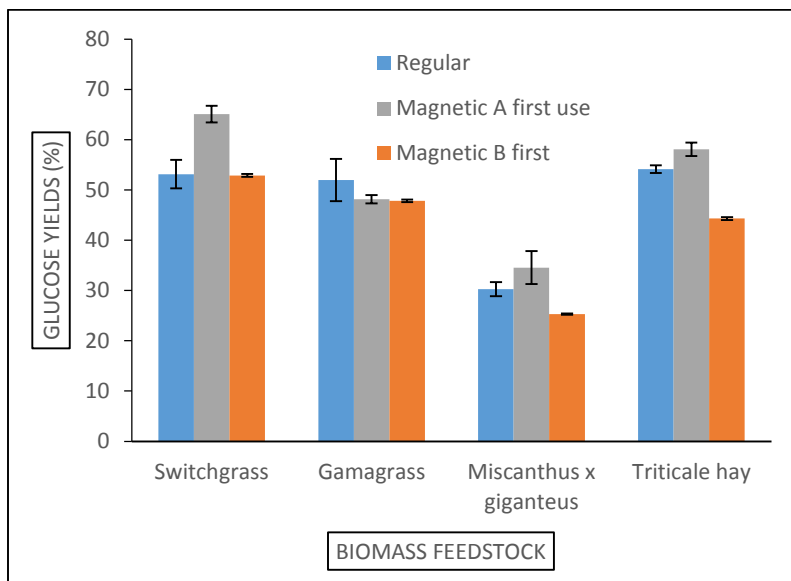


Figure 5.2 Glucose yields produced after enzymatic hydrolysis for four different biomasses used three sulfonic acid catalysts.

Table 5.4 Xylose produced after enzymatic hydrolysis

Biomass feedstock	Xylose produced after enzymatic hydrolysis from four biomasses treated using p-Toluene sulfonic acid catalysts (mg g <sup>-1</sup> dry biomass)				
	Regular	Magnetic A first use	Magnetic A second use	Magnetic B first use	Magnetic B second use
Swithgrass	30.00 ± 1.86	37.00 ± 1.80	23.27 ± 1.27	20.93 ± 0.48	28.33 ± 0.18
Gamagrass	22.34 ± 2.17	24.80 ± 1.50	19.94 ± 5.62	19.85 ± 0.07	12.30 ± 1.46
Miscanthus x giganteus	12.35 ± 0.85	13.85 ± 0.24	15.93 ± 0.90	11.48 ± 3.66	13.25 ± 0.57
Triticale hay	35.60 ± 1.42	46.88 ± 0.38	45.53 ± 0.29	29.80 ± 0.23	33.27 ± 0.70

### 5.3.3 Effect of reusability of magnetic catalysts on sugars yields produced at enzymatic hydrolysis

The data showed that when Magnetic A catalyst was used to pretreat biomasses, the glucose yields after hydrolysis of Gamagrass, *Miscanthus x giganteus* and Triticale hay have maintained yields within 5% difference. Analysis of data using GLIMMIX procedure suggested that glucose yields (after enzymatic hydrolysis) from Gamagrass, *Miscanthus x giganteus*, and Triticale hay treated with magnetic A catalyst were not significantly different between first and second uses ( $p > 0.05$ , table 5.5). In addition, the glucose yield for Switchgrass treated with magnetic A was found to decrease by 11.8% after first use.

The trend exhibited by Magnetic B was different. The data showed that when Magnetic B catalyst was used to pretreat biomasses, the glucose yields after hydrolysis of miscanthus, and triticale hay increased significantly when the catalyst was reused for the second time. However, the hydrolysis yields for switchgrass was similar for both reuses (53.86%,  $p = 0.42$ , table 5.5).

Recently, Tan and Lee (2015) reported the use of solid acid catalyst (Dowex (TM) Dr-G8) to treat macroalgae cellulosic residue at 120 °C for 30 min followed by enzymatic hydrolysis for 30 h using 45 FPU/g of cellulase and 52 CBU/g of  $\beta$ -glucosidase. The authors observed a glucose yield of 94% even after fifth reuse of the catalyst. Although our glucose are lower when compared to Tan and Lee (2015), it may be noted that the feedstock employed by the authors, i.e., macroalgae cellulosic residue did not contain lignin. In addition, the enzyme loading in our study was is lower than that of Tan and Lee (2015). Further, we also observed xylose in our research (table 5.4).

Overall, our results suggest that Magnetic A exhibited consistent activity for Gamagrass, Miscanthus x giganteus and Triticale hay while Magnetic B was observed to be consistent for Switchgrass. In addition, possibility that accumulated iron in wet biomass, which may also have affected the yield.

Table 5.5 SAS Output reusability test

Simple Effect Comparisons of Magnet*Feedsto*Reuse Least Squares Means By Magnet*Feedstock													
Adjustment for Multiple Comparisons: Holm-Tukey													
Simple Effect Level	Reuse	_Reuse	Estimate	Standard Error	DF	t Value	Pr >  t	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
Magnet*Feedstock Magnetic A Gamagrass	Reuse_1	Reuse_2	-3.9565	2.4132	32	-1.64	0.1109	0.1109	0.05	-8.8721	0.9590	-8.8720	0.9589
Magnet*Feedstock Magnetic A Miscanthus	Reuse_1	Reuse_2	4.5195	2.4132	32	1.87	0.0703	0.0703	0.05	-0.3960	9.4350	-0.3960	9.4350
Magnet*Feedstock Magnetic A Switchgrass	Reuse_1	Reuse_2	11.8130	2.4132	32	4.90	<.0001	<.0001	0.05	6.8975	16.7285	6.8975	16.7285
Magnet*Feedstock Magnetic A Triticale	Reuse_1	Reuse_2	3.9533	2.4132	32	1.64	0.1112	0.1112	0.05	-0.9622	8.8688	-0.9622	8.8688
Magnet*Feedstock Magnetic B Gamagrass	Reuse_1	Reuse_2	9.8615	2.4132	32	4.09	0.0003	0.0003	0.05	4.9460	14.7770	4.9461	14.7770
Magnet*Feedstock Magnetic B Miscanthus	Reuse_1	Reuse_2	-13.9635	2.4132	32	-5.79	<.0001	<.0001	0.05	-18.8790	-9.0479	-18.8789	-9.0480
Magnet*Feedstock Magnetic B Switchgrass	Reuse_1	Reuse_2	-1.9617	2.4132	32	-0.81	0.4223	0.4223	0.05	-6.8773	2.9538	-6.8772	2.9537
Magnet*Feedstock Magnetic B Triticale	Reuse_1	Reuse_2	-11.1572	2.4132	32	-4.62	<.0001	<.0001	0.05	-16.0728	-6.2417	-16.0727	-6.2418

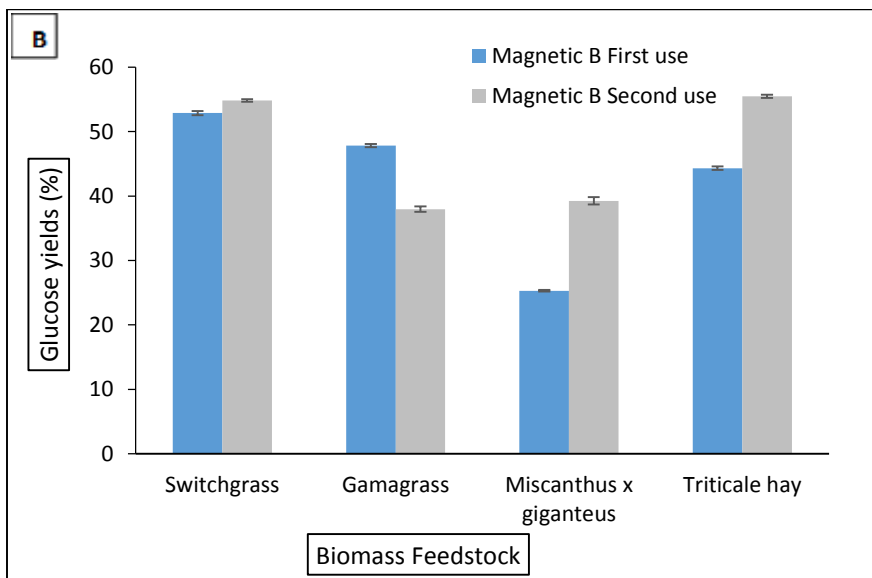
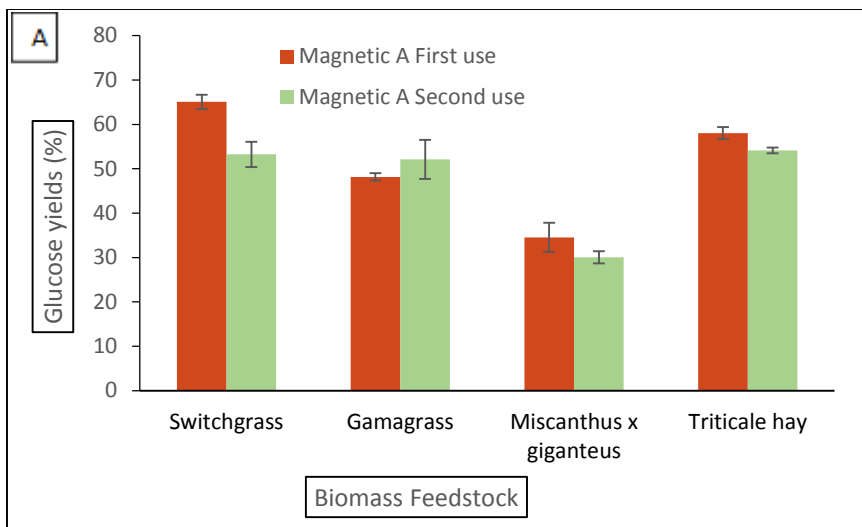


Figure 5.3 Glucose yields for reusability A) Magnetic A and B) Magnetic B catalysts

## 5.4 Conclusions

Magnetic catalysts were found to provide similar or higher yield of sugars compared with regular catalyst. Although xylose was detected in the liquid after enzymatic hydrolysis, adding xylanases might help in improving the formation of 5 carbon sugars. Reusability of magnetic catalysts were tested although, future studies are needed to enhance the activities.

## 5.5 References

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## Chapter 6

### Conclusions and Proposed Future Ideas

#### 6.1 Conclusions

The goal of the present research was to explore the use of activated carbon supported sulfonic acid catalysts for pretreatment and hydrolysis of biomass. The research was performed in three phases. In the first phase sulfonic acid catalysts were synthesized via impregnation of various sulfonic acids on coconut shell activated carbon. The catalysts were characterized via Boehm titration, BET surface area, TGA, and FTIR to determine the surface chemical properties of the catalysts. The catalyst was systematically tested for pretreatment of switchgrass. Effects of temperature and pretreatment time on final yield of glucose were studied. It was observed that catalytic pretreatment at 90 °C for 120 min using pTSA resulted in maximum glucose yields of 50.9 – 61.5%.

In the second phase of the research, the sulfonic acid catalyst was tested as a hydrolysis agent for model (cellulose, starch and cellobiose) and real (switchgrass) feedstocks. In addition, effects of physical (ultrasonication) and chemical (NaOH and H<sub>2</sub>SO<sub>4</sub>) pretreatments on catalytic hydrolysis were also studied. For model biomasses, i.e., starch and cellobiose, it was observed that catalytic hydrolysis resulted in glucose yields of  $190.07 \pm 2.02 \text{ mg g}^{-1}$  and  $237.1 \pm 0.86 \text{ mg g}^{-1}$ , respectively, although the catalyst exhibited almost no activity towards cellulose. For raw switchgrass, however, a glucose yield of  $72.67 \pm 1.03 \text{ mg g}^{-1}$  (conversion of  $23.25 \pm 0.33 \%$ ) was obtained after catalytic hydrolysis. Ultrasonication

pretreatment prior to catalytic hydrolysis resulted in a glucose yield of  $16.91 \pm 0.05$  %, but chemical treatments completely inhibited subsequent catalytic hydrolysis.

In the third and final phase, effective separation of the catalyst was investigated. To enhance separation from biomass, magnetic particles were impregnated on the activated carbon surface along with sulfonic acids. The catalysts were tested as pretreatment agents for Switchgrass, Gamagrass, Miscanthus and Triticale hay for 2 h at 90 °C after which enzymatic hydrolysis using Ctec2 was performed. It was observed that glucose yields of magnetic catalysts were somewhat similar to regular catalyst, with a maximum yield of  $65.07 \pm 1.63$  % (Switchgrass). In addition, results from reusability studies using magnetic catalysts indicated that there was a slight reduction in catalytic activity during the second run.

## 6.2 Proposed future ideas

The yields of glucose obtained in the present research were lower than other conventional pretreatments. One reason might be the lack of adequate contact between biomass and catalyst and lack of adequate solubility. Hence solutions such as ionic liquids may be employed to facilitate the catalytic pretreatment and hydrolysis of biomass. In addition, when magnetized catalysts were employed as pretreatment agents, there appeared to be an inhibition during the hydrolysis, perhaps due to presence of iron particles. Hence suitable surfactants may be investigated to facilitate optimum hydrolysis along with efficient catalyst recovery.

# Appendices

## Appendix A

### Statistical analysis codes

```
title ' Effect of Sulfonic Solid Acid Catalyst Direct Hydrolysing on
Glucose of Alamo Switchgrass';
data hydrolysing;
input rep temp time Glu_noCat Glu_Cat;
datalines;
1 75 6 6.601 9.530
2 75 6 5.765 9.883
3 75 6 6.069 9.474
1 75 12 5.940 9.470
2 75 12 5.957 9.754
3 75 12 6.203 9.103
1 75 18 5.803 11.662
2 75 18 6.010 11.713
3 75 18 5.710 10.966
1 75 24 5.975 10.988
2 75 24 5.809 10.976
3 75 24 6.129 10.944
1 90 6 12.499 30.693
2 90 6 12.214 27.730
3 90 6 11.985 30.294
1 90 12 12.929 34.637
2 90 12 11.798 34.667
3 90 12 11.617 34.531
1 90 18 11.902 73.931
2 90 18 11.750 73.472
3 90 18 12.869 70.626
1 90 24 11.826 74.189
2 90 24 12.192 68.418
3 90 24 12.185 66.018
;

proc sgplot data= hydrolysing;
scatter y=Glu_noCat x = time/ group= temp;
run;

proc sgplot data= hydrolysing;
scatter y= Glu_Cat x = time/ group= temp;
run;

proc sgplot data= hydrolysing;
scatter y=Glu_Cat x = temp/ group= time;
run;

proc sgpanel data= hydrolysing;
panelby temp;
scatter y=Glu_Cat x = time;
run;
proc sgpanel data= hydrolysing;
panelby temp;
scatter y=Glu_noCat x = time ;
```

```

run;

*** use this model;
Proc glimmix data=hydrolysing plots=all;
class rep temp time;
model Glu_Cat =temp|time;
random _residual_/ subject= rep*temp*time group= temp;
LSMEANS temp time/diff adj=tukey cl lines;
LSMEANS temp*time/diff adj=tukey slicediff= (temp time) cl lines;
title Glucose With Catalyst;
run;

*** use this model;
Proc glimmix data=hydrolysing plots=all;
class rep temp time;
model Glu_noCat =temp|time;
random _residual_/ subject= rep*temp*time group= temp;
LSMEANS temp time/diff adj=tukey cl lines;
LSMEANS temp*time/diff adj=tukey cl slicediff= (temp time) lines;
title Glucose Without Catalyst;
run;

title ' Effect of Sulfonic Solid Acid Catalyst Direct Hydrolysing on
Glucose of Model Feedstock';
data hydrolysing2;
input rep feedstock$ time Glu_noCat Glu_Cat;
datalines;
1 Cellulose 6 0.498 0.744
2 Cellulose 6 0.195 0.546
3 Cellulose 6 0.000 0.499
1 Cellulose 24 0.000 1.546
2 Cellulose 24 0.243 1.446
3 Cellulose 24 0.145 1.586
1 Starch 6 0.000 129.611
2 Starch 6 0.000 115.653
3 Starch 6 0.000 114.874
1 Starch 24 0.000 186.035
2 Starch 24 0.097 192.231
3 Starch 24 0.048 191.964
1 Celloboise 6 1.171 53.016
2 Celloboise 6 1.183 62.747
3 Celloboise 6 0.642 46.358
1 Celloboise 24 1.855 238.857
2 Celloboise 24 1.626 236.248
3 Celloboise 24 1.437 236.301
;

proc sgplot data= hydrolysing2;
scatter y= Glu_noCat x = time/ group= feedstock ;
run;

proc sgplot data= hydrolysing2;
scatter y= Glu_Cat x = time/ group= feedstock ;
run;
*** use this model;
Proc glimmix data=hydrolysing2 plots=all;

```

```

class rep feedstock time;
model Glu_Cat =feedstock|time;
  random _residual_/ subject= rep*feedstock*time group= feedstock;
LSMEANS feedstock time/diff adj=tukey cl lines;
LSMEANS feedstock*time/diff adj=tukey slicediff= (feedstock time) cl
lines;
title Glucose With Catalyst;
run;

```

```

*** use this model;
Proc glimmix data=hydrolysing2 plots=all;
class rep feedstock time;
model Glu_noCat =feedstock |time;
  random _residual_/ subject= rep*feedstock*time group= feedstock;
LSMEANS feedstock time/diff adj=tukey cl lines;
LSMEANS feedstock*time/diff adj=tukey cl slicediff= (feedstock time)
lines;
title Glucose Without Catalyst;
run;

```

```

data Ultrasonic;
input
US_time      rep    Glu_noCat_noHyd    Glu_Cat_noHyd;
datalines;
5 1          4.297766    22.50912587
5 2          4.29745247    23.36359475
5 3          4.318771699    22.79308676
15 1         6.655053155    23.42806588
15 2         6.547087512    24.01720737
15 3         6.695983656    24.26665437
25 1         4.249487963    25.06994303
25 2         5.007176954    25.74292814
25 3         5.154135211    24.4140625
;

```

```

data USNOHYD (drop = Glu_noCat_noHyd    Glu_Cat_noHyd);
length treatment $16;
set Ultrasonic;
Treatment = "US_NoCat_noHyd";
H_time = 0;
glucose = Glu_noCat_noHyd;
output;
Treatment = "US_Cat_noHyd";
H_time = 0;
glucose = Glu_Cat_noHyd;
output;
run;

```

```

data USHYD;
length treat treatment $16;
input Rep Treat $ H_Time    Glucose;
US_TIME= input( scan(treat , 2, "_" ), 7.0);

```

```

Treatment = scan( treat , 1, "_" ) ;
datalines;
1   Ultrasound_5   6   41.143
2   Ultrasound_5   6   42.329
3   Ultrasound_5   6   38.079
1   Ultrasound_5   24  53.037
2   Ultrasound_5   24  52.756
3   Ultrasound_5   24  51.855
1   Ultrasound_15  6   45.984
2   Ultrasound_15  6   47.583
3   Ultrasound_15  6   48.138
1   Ultrasound_15  24  52.949
2   Ultrasound_15  24  53.113
3   Ultrasound_15  24  52.565
1   Ultrasound_25  6   51.464
2   Ultrasound_25  6   53.135
3   Ultrasound_25  6   52.591
1   Ultrasound_25  24  52.600
2   Ultrasound_25  24  52.591
3   Ultrasound_25  24  52.417
;
proc print data= ushyd;
run;
proc contents data= ushyd;
run;
data all;
  set USNOHYD USHYD;
run;

proc sgplot data= all;
scatter y= Glucose x =US_time /group= treatment ;
run;

proc sgplot data= all;
scatter y= Glucose x =US_time /group= treatment ;
run;

proc sgpanel data= all;
panelby H_TIME/ coulms=3;
scatter y= Glucose x =US_time /group= treatment ;
run;

proc freq data=all;
  tables treatment*H_TIME*US_TIME/LIST;
run;
*** Treatment (H_Time) represents 4 treatments from combination of
"TREATMENT" and "HTIME" ***;
*** use this model;
Proc glimmix data= all plots=all;
class rep US_time H_Time treatment;
model Glucose = Treatment (H_Time)| US_time ;
random _residual_/ subject= rep* Treatment* H_Time* US_time group=
Treatment* H_time;
LSMEANS US_TIME /diff adj=tukey cl lines;
LSMEANS Treatment(H_TIME) /diff adj=tukey cl lines slicediff= (H_TIME);

```

```

LSMEANS Treatment* US_time (H_TIME) /diff adj=tukey cl lines
slicediff=treatment*H_time;
title US_time With Catalyst No Catalyst noHyd;
run;

```

```

data PRETREATMENT_HYDROLYSIS ;
length treatment $16;
input Rep Treatment $ Time Glucose;
datalines;

```

1	NaOH	6	0.454
2	NaOH	6	0.717
3	NaOH	6	0.294
1	NaOH	24	0.363
2	NaOH	24	0.281
3	NaOH	24	0.130
1	H2SO4	6	1.362
2	H2SO4	6	1.390
3	H2SO4	6	1.868
1	H2SO4	24	2.308
2	H2SO4	24	2.263
3	H2SO4	24	2.862
1	Ultrasound_5min	6	41.143
2	Ultrasound_5min	6	42.329
3	Ultrasound_5min	6	38.079
1	Ultrasound_5min	24	53.037
2	Ultrasound_5min	24	52.756
3	Ultrasound_5min	24	51.855
1	Ultrasound_15min	6	45.984
2	Ultrasound_15min	6	47.583
3	Ultrasound_15min	6	48.138
1	Ultrasound_15min	24	52.949
2	Ultrasound_15min	24	53.113
3	Ultrasound_15min	24	52.565
1	Ultrasound_25min	6	51.464
2	Ultrasound_25min	6	53.135
3	Ultrasound_25min	6	52.591
1	Ultrasound_25min	24	52.600
2	Ultrasound_25min	24	52.591
3	Ultrasound_25min	24	52.417

```
;
```

```

proc sgplot data=PRETREATMENT_HYDROLYSIS ;
scatter y= Glucose x = time/ group= Treatment ;
run;

```

```

proc sgpanel data=PRETREATMENT_HYDROLYSIS ;
panelby treatment;
loess y= Glucose x = time ;
run;

```

```
*** use this model;
```

```

Proc glimmix data=PRETREATMENT_HYDROLYSIS plots=all;
class rep Treatment time;
model Glucose =Treatment|time;

```

```

random _residual_ / subject= rep*Treatment*time group= Treatment;
LSMEANS Treatment time/diff adj=tukey cl lines;
LSMEANS Treatment*time/diff adj=tukey slicediff= (Treatment time) cl
lines;
title Glucose With Catalyst;
run;

```

```

data Ultrasonic;
input
US_time      rep    Glu_noCat_noHyd    Glu_Cat_noHyd;
datalines;
5 1          4.297766    22.50912587
5 2          4.29745247    23.36359475
5 3          4.318771699    22.79308676
15 1         6.655053155    23.42806588
15 2         6.547087512    24.01720737
15 3         6.695983656    24.26665437
25 1         4.249487963    25.06994303
25 2         5.007176954    25.74292814
25 3         5.154135211    24.4140625
;

```

```

proc sgplot data=Ultrasonic;
scatter y= Glu_noCat_noHyd x =US_time ;
run;
*** use this model;
Proc glimmix data=Ultrasonic plots=all;
class rep US_time;
model Glu_noCat_noHyd =US_time;
random _residual_ / subject= rep*US_time group= US_time;
LSMEANS US_time/diff adj=tukey cl lines;
title US_time With Catalyst No Catalyst noHyd;
run;

```

```

proc sgplot data=Ultrasonic;
scatter y= Glu_Cat_noHyd x =US_time ;
run;

```

```

Proc glimmix data=Ultrasonic plots=all;
class rep US_time;
model Glu_Cat_noHyd =US_time;
*random _residual_ / subject= rep*US_time group= US_time;
LSMEANS US_time/diff adj=tukey cl lines;
title US_time With Catalyst No Catalyst noHyd;
run;

```

```

data Hydrolysis;
length type $ 15 FeedstockRep $ 15 Feedstock $ 15 treat $20
Magnet $10;
input Type $ FeedstockRep $ @;

```

```

do treat = 'Regular',      'Magnetic A_first',      'Magnetic A_second',
           'Magnetic B_first',      'Magnetic B_second';
input Total_SCarb @@;
if treat in ('Magnetic A_first' 'Magnetic B_first') then Reuse =
"Reuse_1";
else if treat in ('Magnetic A_second' 'Magnetic B_second') then
Reuse= 'Reuse_2';
else if treat in ('Regular') then Reuse =
"Reuse_0";
if treat in ('Magnetic B_first' 'Magnetic B_second') then Magnet =
"Magnetic B";
else if treat in ('Magnetic A_first' 'Magnetic A_second') then
Magnet = "Magnetic A";
else Magnet = "Regular";
Feedstock = scan (FeedstockRep, 1, "_");
output;
end;
datalines;

```

hydrolisis	Switchgrass_1	48.42476	62.17815	48.55269	52.64673
	55.2055				
hydrolisis	Switchgrass_2	58.21206	67.80745	58.40397	52.51879
	54.62978				
hydrolisis	Switchgrass_3	52.77467	65.24868	52.83864	53.47833
	54.69375				
hydrolisis	Gamagrass_1	44.91366	49.56607	45.0926	48.3135
	37.8754				
hydrolisis	Gamagrass_2	59.46736	46.70305	60.24276	47.77668
	37.27894				
hydrolisis	Gamagrass_3	51.59405	48.19421	50.99758	47.41881
	38.77009				
hydrolisis	Miscanthus_1	32.31685	29.35245	31.92808	25.27032
	39.1204				
hydrolisis	Miscanthus_2	27.65156	40.6269	27.40858	25.5619
	38.39145				
hydrolisis	Miscanthus_3	30.85895	33.67756	30.76175	25.07593
	40.28672				
hydrolisis	Triticale_hay_1	55.42145	56.72492	55.09961	43.77072
	55.87204				
hydrolisis	Triticale_hay_2	52.78234	60.74796	52.91107	44.47877
	54.97087				
hydrolisis	Triticale_hay_3	54.13408	56.72492	54.32719	44.73625
	55.61456				

```
;
```

```

proc freq data= hydrolysis;
tables treat*Reuse/list;
tables treat*Reuse*Feedstock/list;
run;

ods pdf style=seaside file= "YA_output_NOV112015.pdf";
title "Reuse_0 , Reuse_1 , Reuse_2 Hydrolysis ";
title2 " total_SCarb vs Feedstock panelby Reuse group Magnet ";
proc sgpanel data= hydrolysis;
panelby REuse / columns =3 ;

```

```

scatter x= feedstock y= total_SCarb/ group = Magnet;
colaxis grid;
rowaxis grid;
run;

title "Reuse_0 , Reuse_1 Hydrolisis";
title2 " total_SCarb vs Feedstock panelby Magnet group Reuse";
proc sgpanel data=hydrolisis; where Reuse in ( 'Reuse_0', 'Reuse_1');
panelby Magnet / columns =2 ;
scatter x= feedstock y= total_SCarb/ group = REuse ;
colaxis grid;
rowaxis grid;
run;

title "Reuse_0 , Reuse_1 Hydrolisis ";

title2 "model total_SCarb = Magnet | Feedstock";
proc GLIMMIX data = hydrolysis plots=studentpanel; where Reuse in (
'Reuse_0', 'Reuse_1');
class Magnet feedstock Reuse;
model total_SCarb = Magnet | Feedstock;
LSMEANS Magnet Feedstock / cl lines diff adj=tukey;* alpha=0.025;
LSMEANS Magnet * Feedstock/ slicediff= (Magnet Feedstock) cl diff
adj=tukey ;
run;

title "Reuse_1 , Reuse_2 Hydrolisis ";
title2 " total_SCarb vs Feedstock panelby Magnet group Reuse";
proc sgpanel data= hydrolysis; where Reuse in ( 'Reuse_1', 'Reuse_2');
panelby Magnet / columns =2 ;
scatter x= feedstock y= total_SCarb/ group = REuse ;
colaxis grid;
rowaxis grid;
run;

title "Reuse_1 , Reuse_2 Hydrolisis ";
title2 "model total_SCarb = Magnet | Feedstock| Reuse";
proc GLIMMIX data = hydrolysis plots=all; where Reuse in ( 'Reuse_1',
'Reuse_2');
class Magnet feedstock Reuse;
model total_SCarb = Magnet | Feedstock| Reuse;
LSMEANS Magnet Feedstock / cl lines diff adj=tukey;
LSMEANS Magnet * Feedstock/ slicediff= (Magnet Feedstock) cl diff
adj=tukey ;
LSMEANS Magnet * Reuse/ slicediff= (Magnet Feedstock) cl diff
adj=tukey;
LSMEANS Feedstock*Reuse/ slicediff= ( Feedstock Reuse) cl diff
adj=tukey;
LSMEANS Magnet * Feedstock*Reuse/ slicediff= (Magnet*Feedstock) cl
diff adj=tukey;
ods output lsmeans = lsmnds6(rename= (estimate = PredMean))
diffs=diffds6;
run;

quit; ods pdf close;

```

Appendix B

Lignocellulosic biomass

Miscanthus x giganteus



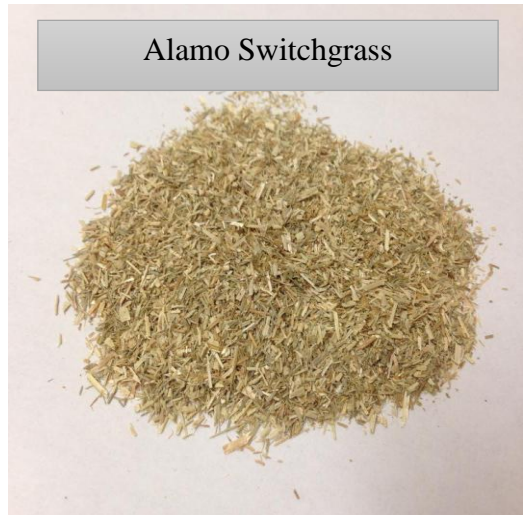
Triticale Hay



Gamagrass



Alamo Switchgrass



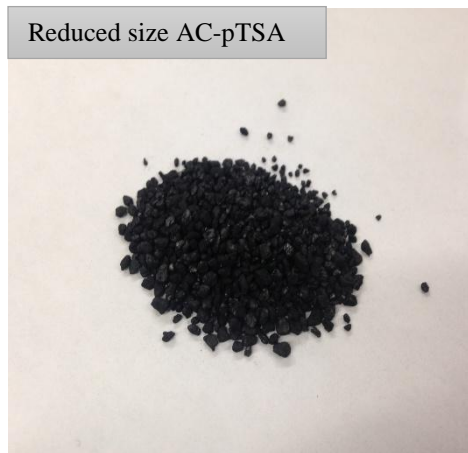
## Appendix C

### Solid acid catalysts

Raw activated carbon



Reduced size AC-pTSA



Magnetic A catalyst



Magnetic B catalyst



## Appendix D

### Solid recoveries after treatments for chapter 5

<b>Catalysts</b>	<b>Solid Recoveries (%)</b>			
	Switchgrass	Gamagrass	Miscanthus x giganteus	Triticale hay
<b>Regular</b>	$89.980 \pm 0.952$	$90.165 \pm 0.685$	$90.808 \pm 0.633$	$84.892 \pm 0.857$
<b>Magnetic A_1</b>	$89.251 \pm 1.839$	$86.982 \pm 0.190$	$88.243 \pm 0.825$	$82.266 \pm 0.629$
<b>Magnetic A_2</b>	$87.726 \pm 1.029$	$87.178 \pm 1.641$	$87.668 \pm 1.335$	$80.627 \pm 0.319$
<b>Magnetic B_1</b>	$90.499 \pm 1.020$	$90.727 \pm 0.563$	$90.785 \pm 0.365$	$87.747 \pm 0.335$
<b>Magnetic B_2</b>	$89.987 \pm 0.444$	$91.132 \pm 0.453$	$90.180 \pm 0.860$	$82.998 \pm 0.318$