ADDIS ABABA UNIVERSITY

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SCHOOL OF CHEMICAL AND BIOENGINEERING

Synthesis and Characterization of Sulfonated Carbon Catalyst for Hydrolysis of Microcrystalline Cellulose

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This is to certify that the thesis prepared by *Kefyalew H/Mariam*, entitled: *Synthesis and Characterization of Sulfonated Carbon Catalyst for Hydrolysis of Cellulose* and submitted in partial fulfilment of the requirement for the degree of Master of Science (School Chemical and Bio Engineering) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION

I declare that this thesis entitled "*Synthesis and Characterization of Sulfonated Carbon Catalyst for Hydrolysis of Cellulose* ''has not been submitted in any form for another degree, diploma or an award at any university or other institution of the tertiary education. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. Information taken from published and unpublished work of others has been acknowledged in the text and a list of references is given. The work was under the guidance of Dr.Beteley Tekola (Ass. Professor) instructor of AAiT in Addis Ababa University, School of Chemical and Bio Engineering.

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ABSTRACT

Microcrystalline cellulose can be converted into valuable products such as glucose via hydrolysis reaction at mild condition using sulfonated carbon catalyst. A sulfonated carbon material was prepared by carbonization of bamboo sawdust followed by sulfonation. Prepared catalyst was studied for its ability to catalyze microcrystalline cellulose yield via hydrolysis reaction. Three carbon-based catalysts at three different temperatures (400, 450 and 500℃) were prepared. Investigations on the sulfonated carbon catalyst were conducted to determine the effect of reaction time (4, 6 and 8 hr), reaction temperature (120, 135 and 150℃) and catalyst to microcrystalline cellulose (1:1, 1.25:1 and 1.5:1) on the hydrolysis reaction. The sulfonated catalysts were characterized using the following analyses: elemental analysis, total acid density, FT-IR, TGA, SEM and XRD. Based on the above characterization results, sulfonated carbon prepared at 500℃ and sulfonated via ultra-sonication was found to have higher acid density that is suitable to catalyze the hydrolysis reaction. The first step in catalyst development approach was to increase the hydrolysis reaction by employing a stronger sulfonation procedure during catalyst preparation. The total acid density obtained for sulfonated carbon catalyst at 500℃ was 4.16 mmol/g which significantly increases glucose yield. According to the FTIR analysis the sulfonated biochar contained sulfonic, carboxylic, and phenolic groups, which are responsible for the exhibited high catalytic performance during hydrolysis of cellulose. The yield of glucose obtained was 60.5% at 149.0°C in 8hr reaction time.

Keywords: *Bamboo sawdust, Sulfonated biochar catalyst, microcrystalline cellulose, hydrolysis reaction.*

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ACRONYMS

AC	Activated Carbon
ANOVA	Analysis of Variance
AGUs	β-D- anhydroglucopyranose units
ASTM	American Society for Testing and Material
BBD	Box-Behnken Design
BC	Biochar
BC-SO_3H-s	Sulfonated biochar with sonication
BC-SO_3H-st	Sulfonated biochar with stirring (without sonication)
BET	Brunauer-Emmett-Teller
CHNS	Carbon, Hydrogen, Nitrogen and Sulphur
CI	Confidence Interval
DF	Degree of freedom
DNSA	3, 5-Dinitrosalisyclic Acid
DP	Degree of Polymerization
EA	Elemental Analysis
FT-IR	Fourier Transform Infrared Spectroscopy
MCC	Microcrystalline Cellulose
HPAs	Heteropoly Acids
RSM	Response Surface Methodology
SEM	Scanning electron microscopy
TAPPI	Technical Association of the Pulp and Paper industry
TCD	Thermal Conductivity Detector
TGA	Thermo gravimetric Analysis
UV-VIS	Ultraviolet-Visible
XRD	X-ray Diffraction Spectroscopy

CHAPTER 1 INTRODUCTION

1.1 Background

Cellulose is the most abundant component of biomass, accounting for 35−50% of lignocellulosic biomass. The relative proportion of the cellulose, hemicellulose and lignin depends on the biomass origin. Cellulose is a linear β-glucose homo-polymer and constitutes with hemicellulose the carbohydrate part of the biomass(Rinaldi and Schuth 2009). It may be obtained from wood or derived in very high purity from cotton fibers, which are about 92% pure cellulose. Glucose of a monomer is expected as a renewable feedstock molecule, which can be efficiently converted into various chemicals, fuels, foods, and medicines(Huber et al. 2006).For example, glucose, a monomer of cellulose, is a key intermediate of various useful chemicals such as polymers, medicines, surfactants, gasoline, and diesel fuels (corma et al. 2007; Kobayashi et al. 2010). Thus, selective hydrolysis of cellulose into glucose is a key process for the beneficial use of biomass.

The hydrolysis of cellulose to obtain reducing sugars such as glucose is essential for using biomass in chemical processes, since the reducing sugars can be transformed into a wide range of important chemicals such as ethanol, 5-hydroxymethylfurfural and hexitols (Rinaldi and Schuth 2009; Vyver et al. 2011). However, conversion of cellulose offers great challenges due to its recalcitrant nature. Thus far, many efforts have been devoted to the depolymerization of cellulose with mineral acids, bases, enzymes(Ramakrishnan et al. 2010), and supercritical water(Sasaki et al. 2000). Liquid acid catalyzed hydrolysis of cellulose is efficient; however, corrosion, waste disposal and solvent recycle make this method unattractive. The hydrolysis of cellulose with enzyme is efficient, but it is slow and sensitive to inhibitors such as furfural and 5- hydroxymethylfurfural. De-polymerization of cellulose in sub- or supercritical water is highly efficient and only requires reaction times on the order of several seconds, but the process gives a complex product distribution and generally has low glucose selectivity due to the high reaction temperature(Peterson et al. 2008).

Solid acid catalytic systems for cellulose hydrolysis have advantages of simplicity, and efficiency since they are easily separated from reaction products and they show good catalytic activity for many substrates. Various solid acids have been examined for cellulose

hydrolysis, including ion-exchange resin(Qi et al. 2011), heteropolyacids (Belderok 2007), sulfonated activated carbon (Onda et al. 2008; Y. Wu et al. 2012), and layered transition-metal oxides(Lai et al. 2011; Tagusagawa et al. 2010). In these research works, the hydrolysis of cellulose is generally carried out in water. Because cellulose is insoluble in water, only the acid sites at the exterior of the catalyst particle surface can apparently be reached by the solid substrate, which leads to low efficiency or the requirement to use long reaction time (24 h) and high reaction temperatures (>200 ℃) to achieve sugar yields above 50% for hydrolysis of cellulose (Geboers et al. 2011).

Biochar, a by-product from biomass pyrolysis process, is another potential carbon source to be used as a support for solid acid catalyst (Lai et al. 2011; Tagusagawa et al. 2010). Pyrolysis of agricultural waste (biomass) is one of the promising thermo-chemical methods to produce bio-oil, biochar and combustible gases. The fast pyrolysis process typically yields up to 75 wt% bio-oil as its main product, and the non-condensable gases (10-20 wt%) and the biochar (15-25 wt%) as by-products (Mohan et al. 2006). Numerous recent studies (Demirbas, A. 2002; Gaskin et al. 2008; Mohan et al. 2006; Yaman 2004) have focused on the utilization of these products, but biochar has received very little attention. As the main scope of this study, the upgrading of biochar as a value added catalyst is investigated in order to increase both the environmental and economic viability of the biomass pyrolysis process. Utilization of biochar support as a catalyst would not only help the commercialization of glucose production, but also increase the environmental viability of the pyrolysis process.

In general, biochar's defining property is its high carbon content; similar to graphite, it is composed mainly of the aromatic carbon rings linked together; but unlike graphite, these rings are irregularly stacked and randomly arranged. Structurally, porous carbons such as biochars are considered nongraphitic (i.e., possessing two-dimensional long-range ordering of carbon atoms in planar hexagonal networks, but without any measurable crystallographic ordering in the c-direction, apart from more or less parallel stacking) and can be further classified as either graphitizable or non-graphitizable. Biochars are essentially non-graphitizable carbons, meaning that they are not capable of being transformed into graphitic carbon solely by treating at temperatures up to 2700 ℃ under atmospheric pressures or lower(Dehkhoda et al. 2010; Yu et al. 2010, 2011).

Sulfonated carbon materials derived from activated carbon, sugar or cellulose are solid acid catalysts that have the potential to eliminate many of the problem associated with solvent recycle and separation and can be made from renewable resources. (Toda et al. 2005) prepared a sulfonated carbon material through the pyrolysis of sugars at 400 ℃ and demonstrated that the material had high catalytic activity for the production of glucose from cellulose. Carbon-based materials containing sulfonic acid (SO_3H) groups are especially attractive catalysts due to their low cost, metal-free composition, and expected high stability and strong acidity.

The conventional processes for the hydrolysis of cellulose involve the use of sulfonated carbon catalyst (Fan et al. 2009). The corrosion and waste disposal problems, however, significantly lower the attraction of liquid acid-catalyzed hydrolysis. In order to minimize the environmental impact, new green approaches have been developed, such as the use of cellulose enzymes, hydrolysis in sub- or super-critical water, ionic liquid promoted dissolution and hydrolysis of cellulose, hydrogenolysis into polyols as well as hydrolysis into glucose by solid catalysts. Among others, the solid catalysts present the advantages of catalyst recovery, less or even no corrosion, and milder reaction conditions. Various solid acids have been investigated in the hydrolysis of cellulose, including ion-exchange resins, heteropolyacids, and layered transition-metal oxide(Onda et al. 2009). A significant challenge associated with the application of solid acids is their much lower efficiency than the sulfonated carbon catalyst. Actually, the highest yield of glucose derived from hydrolysis of cellulose over various solid acids was reported to be only about 40%, which is far less than that on sulfonated carbon catalyst (Hara 2009; Nakajima and Hara 2012). In this study, a process for the hydrolysis of microcrystalline cellulose into glucose is proposed, in which water as reaction medium, using sulfonated carbon materials bearing SO_3H, COOH and OH groups as catalyst instead of enzymes or conventional homogeneous acid catalysts.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes in which a response of interest is influenced by several variables, and the objective is to optimize this response (Bas, D. and H. 2007). Analyzing the effects of the independent variables, this experimental methodology generates a mathematical model which describes the chemical processes within the experimental range (Myers et al. 2009).

Therefore, the aims of this work were to hydrolysis microcrystalline cellulose into glucose using sulphonated carbon catalyst and optimize the process conditions using RSM. Moreover, the conversion of hydrolysis microcrystalline cellulose were also calculated based on the weight difference of the solid part before and after reaction. The glucose obtained from optimized condition were also characterized.

1.2 Statement of the Problem

Homogeneous acid catalysts are generally difficult to recycle and require high operating temperatures and pressures and also give rise to serious corrosion problems. Moreover, water washing processes that are required with homogenous catalysts generate substantial amounts of wastewater and cause loss of glucose. Heterogeneous solid base catalysts are very sensitive to water and, like their homogeneous counterparts; usually do not work well for hydrolysis of cellulose. The established methods for cellulose hydrolysis are known to be catalyzed by cellulose enzymes. But limitations of the enzymes systems are obvious in that the processes are always low efficiency and have a high enzyme cost (Salvador et al. 2010). Heterogeneous solid acid catalysts have gained ever-increasing attention because of their significant advantages in facilitating the separation of the catalyst and eliminating problems with corrosion, toxicity, costly, and environmental harm.

Compared with homogeneous catalysts, heterogeneous solid catalysts have several advantages. In particular, solid catalysts can be easily separated from the liquid mixture after the reaction, thereby allowing the possible reuse of catalysts, along with the minimization of corrosion. These merits have stimulated the research and development of recyclable solid acids as replacements for the unrecyclable liquid acid catalysts in the catalytic hydrolysis of lignocellulosic biomass.

The research project presented in the following report seeks to address this problem by studying the hydrolysis of cellulose to glucose via sulfonated carbon catalyst. The sulfonated carbon catalysts can make hydrolysis of cellulose more energy efficient, and therefore less expensive, by eliminating the need for expensive purification processes that separate the catalyst from reaction products typical in the use of homogeneous catalysts. This makes the hydrolysis of cellulose to glucose using sulfonated carbon catalyst more economically and environmentally feasible as compared with that of homogeneous

catalysts based glucose. In addition, several aspects also need to be of concern such as economy, simplicity, efficiency and environmental friendliness.

The hydrolysis of cellulose to glucose is a critical step for the production of cellulosic bioethanol and many other value added molecules. Solid acids have many advantages over conventional liquid acids, in separation, recycling and being environmental benign. Magnetic solid acids are promising materials for the reaction due to their easier separation and recyclability. Heteropoly acids (HPAs) have been proven to be active catalysts for cellulose conversion, but their water solubility limits their large-scale application. Incorporation of HPAs into supports or the use of insoluble salts of HPAs may solve the problem and require further investigation(Huang and Fu 2013). Recent advances in the introduction of ionic liquids, microwave irradiation, supercritical water and nanoparticles to the cellulose hydrolysis system may allow the reaction to proceed more easily under milder conditions. Besides, cellulose pretreatment can reduce the degree of polymerization of cellulose and increase the surface area which would significantly improve the reaction efficiency and reduce the operation cost. All these technologies help to overcome the recalcitrance of cellulose and depolymerize it to a certain extent. Although cellulose hydrolysis has been developed with solid acids toward the greener synthesis of sugars, the reaction efficiency and selectivity still need to be improved by optimizing the operating conditions.

1.3 Objectives

1.3.1 General Objective

The general objective of this study is to synthesize and characterize sulfonated carbon catalyst for hydrolysis of cellulose into soluble sugars (glucose).

1.3.2 Specific Objectives

The following are the specific objective of this study

- To prepare (or synthesis) sulphonated carbon catalyst using two synthesis protocol (stirring and sonication) starting from bamboo sawdust.
- To characterize the physico-chemical, surface functional group and crystalline properties of prepared catalyst.
- To investigate the performance of sulphonated carbon catalyst for conversion of cellulose (microcrystalline cellulose) to glucose via of hydrolysis.
- To study the main and interaction effect of hydrolysis condition (reaction time, reaction temperature, cellulose to catalyst ratio).
- To find the optimal operating condition of hydrolysis of microcrystalline cellulose (high yield of glucose) using a response surface methodology (RSM).

1.4 Scope of the Study

The scope of this study to synthesize and to characterize the biomass, biochar and sulfonated carbon catalyst. Select the best sulfonated carbon catalyst to perform hydrolysis of microcrystalline cellulose to glucose. Then after hydrolysis the microcrystalline cellulose and characterize the product. Finally, the product of the hydrolysis reaction (i.e. glucose) is analyzed.

1.5 Significance of the Research

The significance of hydrolysis of cellulose to glucose using sulphonated carbon catalyst is to develop effective, safe, environmental friendly and economic technologies to convert cellulose into target monomers (glucose). The conventional processes for the hydrolysis of cellulose involve the use of sulfonated carbon catalyst. The corrosion and waste disposal problems, however, significantly lower the attraction of liquid acid-catalyzed hydrolysis. In order to minimize the environmental impact, the solid catalysts present the advantages of catalyst recovery, less or even no corrosion, and milder reaction conditions. Therefore,

it is desirable to develop a highly active and recyclable solid acid catalyst for the hydrolysis of cellulose. By sulfonation activated carbon in appropriate conditions, the surface acidity and hydrophilicity/hydrophobicity may be tuned to best facilitate the hydrolysis of cellulose.

Compared with liquid acid catalysts, solid acid catalysts have distinct advantages in recycling, separation, and environmental friendliness. Solid acid catalysts are easily separated from the products mixture for reuse after reaction. However, some of the key challenges are in the design of the biomass feedstock processes that are environmentally friendly, economic, versatile and efficient in view of the variable composition and diffuse distribution of biomass. The use of solid acid catalysts can address some of these challenges due to their use of mild operating conditions coupled with selective and efficient reactions.

Generally, the results of this study will provide essential baseline information for the potential of sulfonated carbon catalysts in the hydrolysis of microcrystalline cellulose into glucose with locally available raw material, such as biomass of carbonized. This shows that, the direction of an alternative hydrolysis of microcrystalline cellulose to glucose which is technically feasible, economically competitive, environmentally acceptable, efficient and easily available raw material.

CHAPTER 2 LITERATURE REVIEW

2.1 Cellulose

Cellulose is the primary substance of which the walls of vegetable cells are constructed and is largely composed of glucose residues. Cellulose is the most abundant component of biomass, accounting for 35−50% of lignocellulosic biomass (Rinaldi and Schuth 2009).

Cellulose can be used in different industry widely in the food, chemical and pharmaceutical industries. Moreover, de-polymerization of cellulose under hydrolysis into fermentable sugars such as soluble oligosaccharides and glucose. This fermentable sugar (glucose) can be converted fermented into bioethanol or dehydrated into platform molecules(Huang and Fu 2013). Besides, cellulosic biofuels have been considered as the most promising candidates for the replacement of current petroleum based fuels i.e. biodiesel. Thus, the hydrolysis of cellulose to glucose is a critical step for the production of cellulosic bioethanol and many other value added molecules.

Cellulose consists mainly of glucose units linked together by β-1,4- glucosidic bonds. Unlike starch, cellulose is a crystalline material with an extended, flat, 2-fold helical conformation (H. Guo et al. 2012). Hydrogen bonds help maintain and reinforce the flat, linear conformation of the chain. The top and bottom of the cellulose chains are essentially completely hydrophobic. The sides of the cellulose chains are hydrophilic and capable of hydrogen bonding, because all the aliphatic hydrogen atoms are in axial positions, and the polar hydroxyl groups are in equatorial positions. The degree of polymerization of cellulose is approximately 10,000 to 15,000 glucopyranose monomer units in wood and cotton, respectively (Huang and Fu 2013; Lai et al. 2011). Upon partial acid hydrolysis, cellulose is broken into cellobiose (glucose dimer), cellotriose (glucose trimer), and cellotetrose (glucose tetramer), whereas upon complete acid hydrolysis it is broken down into glucose (Pang et al. 2010).

2.1.1 Structure of Cellulose

Early exploration work on the structure of cellulose was carried out by Anseleme Payen. He obtained a resistant fibrous solid material with the formula $C_6H_{10}O_5$ after the treatment of various plant tissues with acids and ammonia. It was the earliest “cellulose”. The

structure of cellulose was elucidated by Hermann Staudinger. He found that cellulose was composed of D-glucose units, linked to each other to form long chains (Figure 2.1).

The structure of cellulose was made up of many glucose mediates linked in the form of β-D- anhydroglucopyranose units (AGUs). The AGUs are linked to each other through glycosidic bridges on the C1 and C4 carbon atoms. The number of repeating AGUs is defined as the degree of polymerization (DP) of cellulose. Generally, the DP of cellulose varies according to the original type of materials, for example, the common range of DP for wood pulp is about 300–1,700 and is 800–10,000 for cotton or other plant fibers (Huang and Fu 2013). Due to the huge number of hydroxyl groups and linkage of β-1,4-glycosidic bonds, it is easy to form intra- and inter molecule hydrogen bonds which make the cellulose resistant during chemical and biological treatment and make it highly insoluble in common solvents (Huang and Fu 2013).

The more generally known and discussed forms of cellulose are microcrystalline Avicel cellulose and α-cellulose which come from pure bacterial cellulose and the treatment of wood with alkali extraction (the figure 2.1 was taken from (Yao-Bing and Yao 2013).

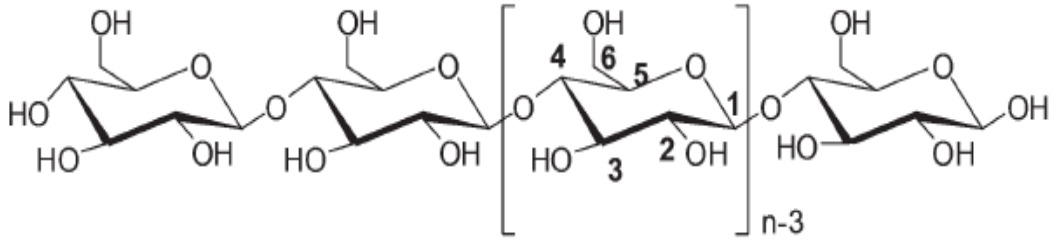


Figure 2.1: Structure of cellulose (degree of polymerization n = DP)

2.1.2 Microcrystalline cellulose

Microcrystalline cellulose (MCC), prepared from natural cellulose through acid hydrolysis, has been widely used in the food, chemical and pharmaceutical industries because of its high degree of crystallinity, small particle size and other characteristics. MCC is a kind of fine white, odorless, water-insoluble powder or particle material (Wang et al. 2010). In general, natural cellulose is first hydrolyzed by acid to obtain hydrocellulose, and then treated along with post-treatment such as grinding and screening processes to prepare MCC for different applications (Abd et al. 2014). MCC has many unique characteristics, such as a high degree of crystallinity (CI) of more than 60 %, small particle size of around 20–80 μm and leveling off degree of polymerization (LODP) below

350 (Nada et al. 2009; Picker and freyer 2007). It has been widely used in the food, chemical and pharmaceutics industries because of these characteristics (Siro and Plackett 2010).

2.2 Raw and Pretreated Cellulosic Material

Lignocellulosic biomass is composed of three main components: cellulose, hemicellulose and lignin. The relative proportion of cellulose varies according to the biomass origin, for example from 80% to 95% for cotton seed hairs and from 20% to 45% for grasses (Kumar et al., 2009). Cellulose is a linear β-glucose homopolymer with subunits called cellobiose, β-1,4 linked glucose. Cellulose is often characterized by its degree of polymerization (DP), defined as the average number of glucose units in a cellulose macromolecule. In raw cellulose, DP is comprised in the 2000–9000 range (Kraessig et al. 2004). In biomass, cellulose is present in cell walls, mostly in organized fibrous structures. Indeed, long glucose polymer chains are stuck together with inter and intra hydrogen bonds and van der Waals interactions, creating a resistant microfibrils network. Although the cellulose structure in biomass is mainly crystalline but some amorphous areas exist (the figure 2.2 was adopted from (Kumar et al. 2009).

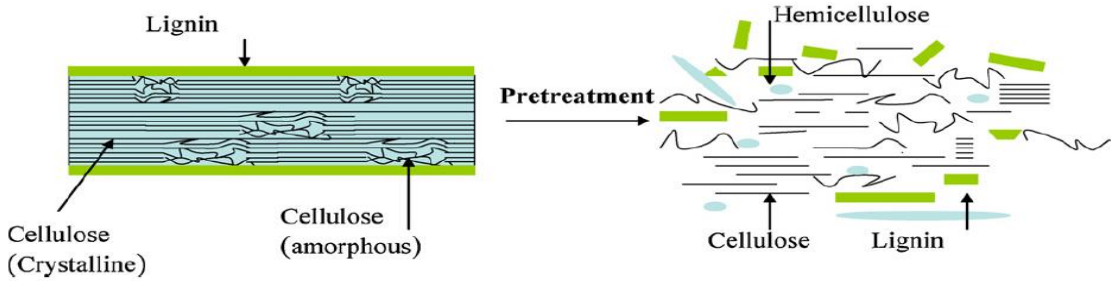


Figure 2.2: Schematic of pretreatment effects on the lignocellulosic structure

Cellulosic microfibrils are protected from external microbial attacks by lignin coverage. The hemicellulose polymer surrounds cellulosic microfibrils and plays a role of "cement" within the plant. In comparison to cellobiose or starch, raw cellulose is more resistant to hydrolysis. Cellobiose is a glucose dimer and starch is a partially crystalline glucose polymer with more reactive β-1, 4 linked glucose. Because of its micro-fibril crystalline structure and its interconnection with lignin and hemicellulose, cellulose in biomass is not

as reactive as starch or cellobiose. However, amorphous part of cellulose is found to be more reactive to hydrolysis than crystalline one(Kumar et al. 2009).

Biomass is often treated in order to improve its reactivity. All actions done in this purpose are called pretreatment processes. Their nature depends on the targeted product and process on interest but pretreatment actions always refer to solubilisation and/or separation processes to make the remaining solid biomass more accessible to further chemical or biological treatments. To achieve high yields of glucose, lignocellulose must first be pretreated. The goal of pretreatment is to decrease the crystallinity of cellulose, increase biomass surface area, remove hemicellulose, and break the lignin seal(Kumar et al. 2009; Shi et al. 2014).

2.3 Proximate analysis of precursor

Proximate analysis is the most often used analysis for characterizing a material in connection with their utilization. The proximate analysis of a substance means of determining the distribution of products obtained when the char sample is heated under specified conditions. When analysis of biomass involves the determination of ash, moisture, volatile matter and fixed carbon, it is called proximate analysis(Mahanim et al. 2016).

2.4 Process Mechanism of Hydrolysis

Hydrolysis can allow the selective and energy efficient conversion of biomass into monomer units, which can then be selectively converted into fuels or chemicals. The hydrolysis reaction for cellulose conversion into sugar polymers is shown in eqn.2.1. Hydrolysis of cellulose is significantly more difficult than for starches because cellulose is in a crystalline form with hydrogen bonding. The hydrolysis reaction can be catalyzed by acids or enzymes, and a recent review has been written by Wyman et al.

$$(C_6H_{10}O_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6 \qquad (2.1)$$

A variety of cellulosic materials are hydrolysis by both enzymatically and acidically catalyzed processes. Enzymatic hydrolysis is a process in which enzymes facilitate the cleavage of bonds in molecules with the addition of the elements of water. Enzymatic

hydrolysis that converts lignocellulosic biomass to fermentable sugars may be the most complex step in this process due to substrate-related and enzyme-related effects and their interactions. The mechanism of enzymatic hydrolysis and the relationship between the substrate structure and function of various glycosyl hydrolase components is not well understood. The established methods for cellulose hydrolysis are known to be catalyzed by cellulose enzymes. But limitations of the enzymes systems are obvious in that the processes are always low efficiency and have a high enzyme cost(Salvador et al. 2010). There are two classes of acid hydrolysis processes are available for saccharification of cellulosic materials. One consists of the dilute acid-high temperature processes, and the other of the concentrated acid-low temperature processes. Sulfuric acid and hydrochloric acid have been the acids of the choice in both classes of processes (Okamura et al. 2006). Most importantly the process of the invention is used in the production of glucose from cellulose.

The hydrolysis reaction is that it involves the splitting of the water molecule into hydrogen cations (H^+) and hydroxide anions (OH^-). As shown in fig-2.3, In the presence of cellulose, the H^+ attacks the oxygen atom in the 1,4'-β-glycosidic linkage. Thus the 1, 4'-β-glycosidic linkage is broken to form a cyclic carbonium cation with a chair shape. This step is considered as the rate determining step. Finally, glucose is formed by rapid ion transfer of OH^- from the dissociation of water molecules to the glucose unit-based carbonium cation. According to such a reaction mechanism, that a solid acid catalyst and reaction conditions favor the splitting of water will favor the hydrolysis of cellulose. The splitting of a water molecule into hydrogen cations (H+) and hydroxide anions (OH^-) can be regarded as deprotonation. These findings suggested that stronger Brønsted acidity is more favorable to the catalytic hydrolysis of cellulose. In addition, to some extent, an increase in the amount of water had a positive effect on the breakage of 1,4'-β-glycosidic bonds and intramolecular hydrogen bonds in insoluble cellulose, thereby leading to more hydrolytic products(the figure 2.3. was adapted from (Zhou et al. 2011)).

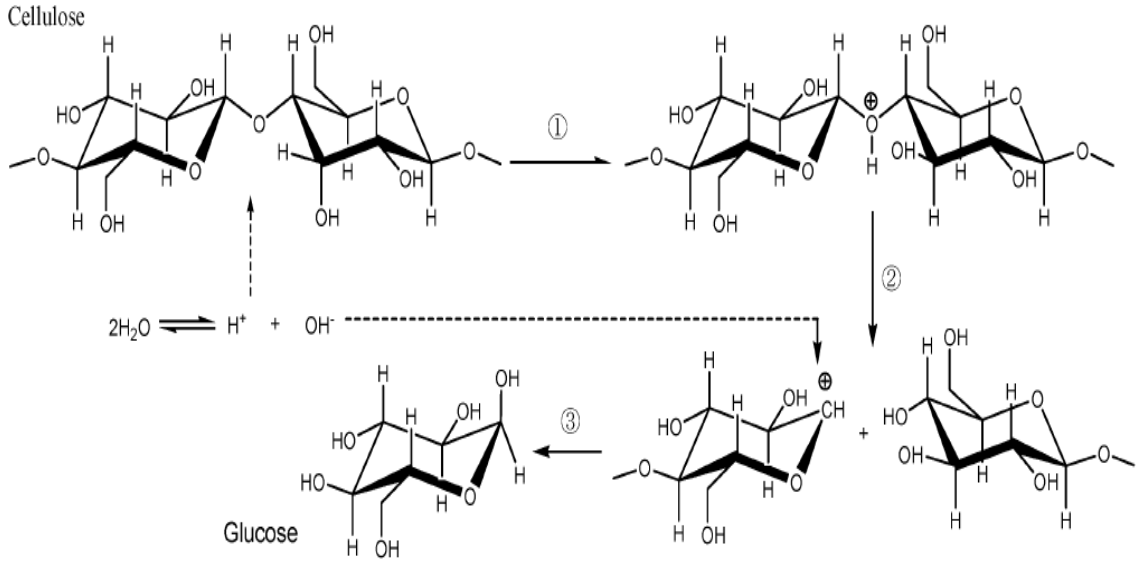


Figure 2.3: Schematic mechanism of breakage of 1, 4'-**β**-glycosidic bonds and formation of glucose in the hydrolysis of cellulose.

2.5 Preparation of Biochar

Biochar is defined as a carbon-rich, porous solid produced by the thermal decomposition of biomass in a reactor with little or no available air and at moderate temperatures. It also distinguishes biochar from activated carbon and carbon black, two typical amorphous carbon materials. From a structural viewpoint, there is no fundamental distinction between biochar and activated carbon, because both of them are amorphous carbon with abundant porosity. Even activated carbon derived from biomass can be considered as a certain type of activated biochar. However, unlike activated carbon, biochar usually has abundant surface functional groups (C−O, C=O, COOH, and OH, etc.), which being highly modifiable act as a platform for the synthesis of various functionalized carbon materials. The technical, economic, and climate-related aspects of biochar production technologies and their application in soil remediation were summarized by (Meyer et al. 2011). Current progress and innovation in biochar production and utilization for improving environmental quality have been reviewed by Hyland and Sarmah. However, these reviews focused on only some specific aspects of biochar, mainly on biochar production and applications for soil remediation and pollutant removal. To date, no comprehensive and critical review on the biochar formation mechanism, its characteristics, or the functionalization of biochar materials for catalysis and energy storage applications is available. In general, it is found

that biochar obtained directly from biomass pyrolysis has poor surface functionality with only limited C −O, C=O, and OH groups present.

Biomass char sulfonic acid (BC-SO_3H) is a novel, recoverable, and renewable solid acid (Xu et al. 2008). As the main scope of this thesis, the upgrading of biochar as a value added catalyst is investigated in order to increase both the environmental and economic viability of the biomass pyrolysis process(Youyu Wu et al. 2012). The effectiveness of sulfonated biochar as catalyst for hydrolysis of microcrystal cellulose into glucose was studied in this research. Bamboo bought from local market and was grinded by milling machine and the dust was collected and dried for 12hr at temperature of 50℃ to avoid create some microbial decay. Then, the dried bamboo was carbonized at 400, 450 and 500℃ for two hours under nitrogen gas flow.

2.6 Development of sulfonated biochar catalyst for hydrolysis reaction

The biochar and the concentrated sulfuric acid (96% H_2SO_4) will be placed in a glass tube, and then will be heated under the protection of nitrogen flow at specified temperature and time. After cooling and filtration, the solid will be washed thoroughly with plenty of hot distilled water (80℃) until no SO_4^{2-} will be detected in the filtrate. The biochar-based catalyst is prepared through sulfonation of as received biochar material under inert gas. Three acidic groups of sulfonic, carboxylic, and phenolic have introduced into the carbon matrix due to sulfonation (the figure 2.4 was reproduced from (Okamura et al. 2006).

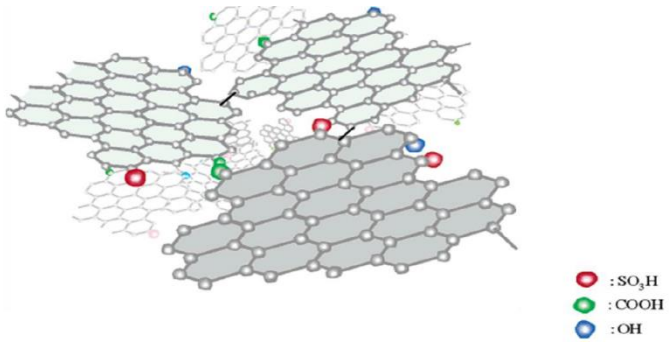


Figure 2.4: Structure of biochar-based catalyst

2.6.1 Factors that Affect Hydrolysis Reaction

2.6.1.1 Temperature

Different solid acid catalysts in hydrolysis reaction operate in different temperature; the range of temperature varies from 60-220 ˚C depending on solvent media and the residence time(Bhaumikparesh and Dhepe 2016; Hu et al. 2015). The breakdown of inter and intra molecular link of a cellulose is directly related with temperature so when the reaction temperature is increased the breakage these linkages highly facilitates(Yamaguchi et al. 2009). However, as stated by a few researchers, increasing temperature has also effect on the amount of glucose produces during hydrolysis this is due to at high temperature degradation glucose to other products like 5-hydroxymethylfurfural (HMF) and levulinic acid easily facilitated(Huber et al. 2006). Hence, it is necessary to optimize the hydrolysis temperature to maximize the amount of glucose and other chemicals produces. Optimizing of hydrolysis temperature depends on several other factors like: type of solid acid catalyst used, residence time of the reaction, and degree of crystallinity of the cellulose raw materials. (Yamaguchi et al. 2009), optimizes cellulose hydrolysis reaction with a temperature range of 323-393 K, and found out that the relation between glucose formations by varying reaction temperature.

Some solid acid catalysts are not hydrothermally stable, consequently, when the reaction temperature passes certain level, there will be active site leaching of the catalyst and this decrease the conversion rate of cellulose to glucose and other chemicals(Bhaumikparesh and Dhepe 2016). However, there are certain solid acid catalysts like sulfonated activated carbon which are hydrothermally stable and can be frequently recycled without losing its active sites. To sum it up, the role of temperature in hydrolysis reaction is indispensable. Hence, when solid acid catalyst used as a catalyst the reaction temperature should be first optimize related to the maximum output glucose and chemicals with losing the active site.

2.6.1.2 Types of Catalyst

Among a series of typical solid acid catalysts like H-form zeolite, sulfated zirconia, sulfonated activated carbon and Amberlyst polymer- based materials (Amberlyst, a commercially available sulfonated resin): sulfonated activated carbon showed remarkably high yield (40.5%) of glucose(H. Guo et al. 2012). Over such sulfonated activated carbon catalysts, a selectivity of higher than 90% to glucose was obtained. The catalysts could act in a way similar to sulfonic acids containing the group -SO_3H. This has fairly strong

acidity. The remarkably catalytic properties can be ascribed to the strong acidity of -SO_3H functional groups and the hydrophobic planes on the surfaces of such a catalyst, as well as its high hydrothermal stability as shown in Figure 2.5 from(Zhou et al. 2011).

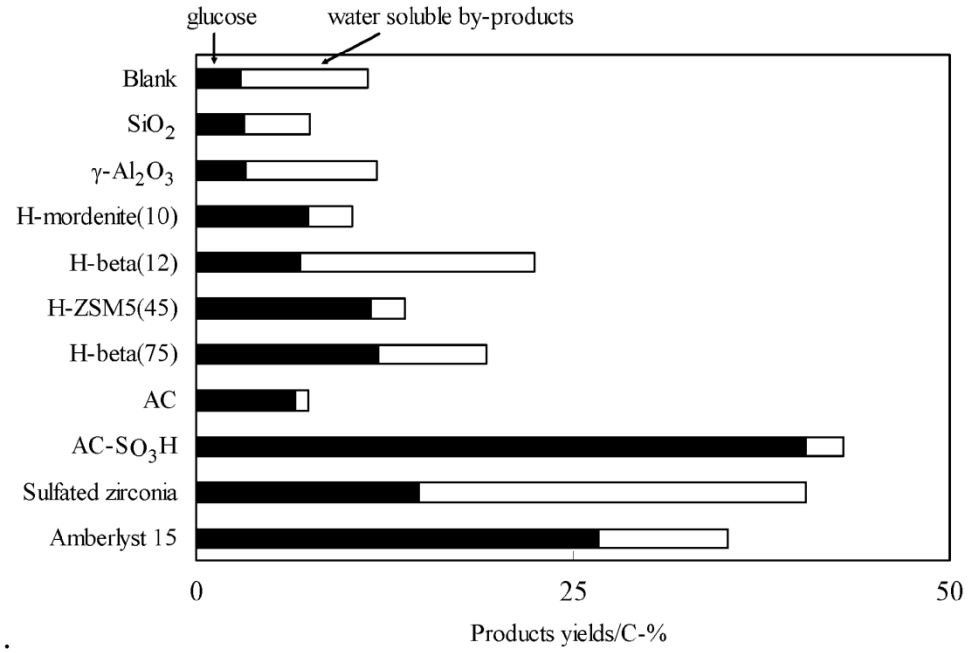


Figure 2.5: Catalytic hydrolysis of cellulose over various solid acid catalysts at 423 K.

Such systematic evaluation of a series of catalysts provided valuable information and clues for the design and preparation of a class of effective solid acid catalyst for catalytic hydrolysis of cellulose by adjusting its surface acidity and hydrophilicity/ hydrophobicity(Huang and Fu 2013). It is worth noting that lignocellulosic biomass itself can be made into carbon materials by carbonization. Bio-char or activated carbon from lignocellulosic biomass such as bamboo, wood, and coconut shells are well-documented catalyst supports(Zhou et al. 2011).

In addition, the surface of carbon solids can be functionalized with several functional groups. For example, a modified amorphous carbon material can simultaneously bear –SO_3H,–COOH, and –OH groups by means of partial carbonization of cellulose, followed by sulfonation of the resulting amorphous carbon .This strategy of catalyst preparation could efficiently enhance the performance of catalysts(Zhou et al. 2011).

2.6.1.3 Degree of Crystallinity

When cellulose is used as a substrate, due to its insolubility in most of the solvents, the reaction system becomes heterogeneous with respect to the substrate itself. This eventually

affects the reaction rate of cellulose hydrolysis in the presence of solid acid catalysts(Bhaumikparesh and Dhepe 2016). However, before hydrolysis reaction takes place the strong crystalline structure of the cellulose should be eliminated and the β-1, 4 glycosidic linkages of the cellulose must be accessible to the active sites of the catalyst. Degree of crystallinity of the cellulose can be minimized either by dissolving the cellulose in ionic liquid or reducing the size of the size of cellulose using ball milling.

Cellulose hydrolysis reaction with solid acid catalyst can be more facilitated by pre dissolving of the cellulose in ionic liquids. Ionic liquids can dissolve crystalline cellulose which is a major problem to achieve higher yields of glucose with other catalyst. Cellulose which is initially dissolves in ionic liquids and hydrolyzed with solid acid catalyst has given higher amount glucose than that of cellulose not dissolved in ionic liquids(Rinaldi and Schuth 2009).

Pretreatment of cellulose before hydrolysis with solid acid catalyst improves the output product of the glucose and other related chemicals. However, applying pretreatment techniques incur additional cost to the process. When using ball milling intensive energy would be spent to completely destroy the crystalline structure of the cellulose. On the other hand, due to it cost applying ionic liquids requires proper recycling technique to recover the ionic liquids after it dissolves the cellulose.

At last, there are also other factors which affect cellulose hydrolysis in solid acid catalyst. Water has both pro and con in the hydrolysis process, very small amount of water can slow the hydrolysis of cellulose because water is a reactant, but too much water can also slow the hydrolysis of cellulose, supposedly because of the hydration of the acid sites on solid acid catalysts, which remains to be proved. The ratio of cellulose to catalyst has to be given proper attentions since varying ratio combination would give different glucose output(Hu et al. 2015).

2.7 Review Different Mechanism of Glucose Production from Cellulose

The first step for cellulose utilization is to depolymerize it into soluble oligosaccharides and glucose. However, the natural polymer forms a robust crystal structure with high chemical stability, thus making the depolymerization processes more difficult. The established methods for cellulose hydrolysis are known to be catalyzed by cellulose

enzymes. But limitations of the enzymes systems are obvious in that the processes are always low efficiency and have a high enzyme cost (Salvador et al. 2010). At the same time, much work has been concentrated on the hydrolysis of cellulose to glucose with mineral acids. Sulfuric acid is known as a typical acid for this reaction. However, the large-scale use of acid suffers from several problems such as reactor corrosion, catalyst recovery and requires treatment of the acid residue, producing lots of waste (Harris et al. 2006). From the industrial point of view, the above limitations must be taken into account when designing production processes. In addition, several aspects also need to be of concern such as economy, simplicity, efficiency and environmental friendliness.

The utilization of heterogeneous acids has the potential to overcome some of the above limitations due to the ease of separation of catalysts. Significant development of this transformation has been made by using various types of catalysts with large pore size and strong acid strength. Associated with these solid acids, many pretreatment technologies have also been developed to reduce the crystallinity of cellulose and increase its surface area in order to improve the reaction efficiency and selectivity, for example, ball-milling, solubilization/ precipitation in ionic liquids, liquid acids/alkaline solutions, the non-thermal atmospheric plasma method and so on (X. Zhang et al. 2013). It is worth noting that most hydrolysis processes with solid acids require the pretreatment of cellulose. Apart from that, microwave irradiation induced assistance of the hydrolysis of cellulose is an effective heating method compared to conventional oil heating (Z. Zhang and Zhao 2009).

2.7.1 Hydrolysis of cellulose by liquid acids

The hydrolysis of cellulose is a process to break the β-1, 4-glycosidic bonds of the polymer which is an essential step for the conversion of cellulose and opens the possibility of subsequent catalytic transformations. Concentrated HCl (6–7 mol L^{-1}) was also effective in the hydrolysis of cellulose in the presence of $CaCl_2$ and LiCl as the additives. The results showed that the salts had a swelling effect by increasing the hydrolysis rate, leading to an 85% yield of glucose at 90 °C. Additionally, concentrated acids had an apparent swelling effect on cellulose. Cellulose swells when the sulfuric acid concentration is above 50%. Camacho et al. studied the effect of H_2SO_4 concentration (from 31%–70%, (w/v)) on the solubilization rate of cellulose, revealing that acid promoted the total solubilization of cellulose when the concentration was above 62%. Although hydrolysis with concentrated acids operates at low temperature and atmospheric pressure, these processes had strict

requirements on water content and displayed severe corrosion. Hydrolysis of cellulose with dilute acid usually requires high temperature(Li and Zhao 2007).

Madison reported a treating process in which wood was treated with 0.5 wt% H_2SO_4 in a continuous reaction. The degradation of products was minimized for the short residence time of cellulose in the reactor. Another successful process using an isothermal plug flow reactor was reported by Grethlein and Thompson. They used 1 wt% H_2SO_4 in the continuous process at 240 °C with a short residence time of 0.22 min; 50% glucose was obtained at last. A few years later (Harris et al. 2006).used a two stage system containing dilute H_2SO_4 in the saccharification of wood. After the hemicellulose was extracted, cellulose was transferred for hydrolysis and a high purity of glucose was obtained. The hydrolysis of cellulose was achieved with gaseous HCl as reported by the Wilke group(Harris et al. 2006). The HCl stream was adsorbed on the dried wood particles and initiated the hydrolysis of hemicelluloses. After extraction of the hemicellulose sugars, the residue was dried and mixed with HCl gas at 45 °C, achieving 90% conversion of the available carbohydrate content of wood to sugars. High pressure HCl systems were also reported for the hydrolysis reaction. Apart from gaseous HCl, anhydrous HF was employed in the hydrolysis of cellulose. The main advantage of using anhydrous HF is its low boiling point (19.5 °C) which facilitated the acid recovery. The yield of sugars was about 45% at 0 °C. The recovery experiment showed that only 0.05–0.1% of HF remained in the system, opening the possibility of reusing mineral acids in the hydrolysis of cellulose.

Current strategies for the hydrolysis of cellulose with acids are mainly accomplished with the acids mentioned above. Although homogeneous-based processes are efficient, the mineral acid systems still suffer from major problems in product/catalyst separation, reactor corrosion, catalyst recycling and the treatment of waste effluent. For example, in the H_2SO_4 based system, the acid in the reaction mixture has to be neutralized with a mixture of $CaO/CaSO_4$ which forms lots of gypsum as waste. For the HCl system it is difficult to reuse the liquid acid while the HF system is very toxic when utilized in large-scale processes. In the research for greener processes for hydrolyzing cellulose, solid acids were developed to address some of the problems and have some unique catalytic properties that mineral acids do not possess(Huang and Fu 2013).

2.7.2 Hydrolysis of cellulose by solid acids

The hydrolysis of cellulose into sugars with solid acids has received increasing attention. Solid acid catalysts have various advantages over liquid acid catalysts: ease of product separation, recyclability, less damage to the reactor. Besides, the use of solid acid catalysts can reduce the pollutants which will have a minimal impact on the environment. Up till now, numerous reviews have been published on the transformation of cellulose with solid acids (F. Guo et al. 2012).

2.7.2.1 Sulfonated carbonaceous based acids

Among various types of solid acid for the hydrolysis of cellulose, carbonaceous solid acids have superior catalytic activities. The good recyclability and cheap naturally occurring raw materials of these carbonaceous acids make them better candidates for the production of biofuel precursors. The first carbonaceous acids from sulfonated D-glucose/sucrose materials were reported by (Suganuma et al. 2012). D-Glucose and sucrose were incompletely carbonized at low temperature to form small polycyclic aromatic carbon rings and subsequently sulfonated with sulfuric acid to introduce sulfonic groups (–SO_3H). They used the carbonaceous acids for the trans-esterification of vegetable oils to biofuels(Yu et al. 2011).

The role of heterogeneous acids for the hydrolysis of cellulose has been intensely described in the past. However, significant development in this area has been witnessed recently using materials with mesoporous morphology along with large pore size and volume which offers controlled reaction and improved selectivity. It was demonstrated that ordered mesoporous materials upon functionalization with sulfonic acid groups act as excellent catalysts for hydrolysis reaction(Huang and Fu 2013).

After their work, much attention was paid to these new types of amorphous solid acid which could be used as catalysts for the cellulose hydrolysis reaction. Carbon-based solid acids were prepared by sulfonation of incompletely carbonized natural polymers, such as sugars, cellulose and starch or by incomplete carbonization of sulfopolycyclic aromatic compounds in concentrated H_2SO_4. Hara et al. reported the preparation method of carbon material bearing –SO_3H groups from microcrystalline cellulose at 723 K over 5 h under N_2 flow. The black powder is then boil in 15 wt% H_2SO_4 at 353 K under N_2 for 10 h; after filtration and washing with hot water, carbon based acid is obtain. Characterization of this solid acid shows that the carbon material is compose of uniform functionalized grapheme

sheets, bearing –SO_3H, –COOH and phenolic –OH groups which are different to conventional solid acids with single functional groups (Figure 2.6 was reproduced from (Hara 2009).

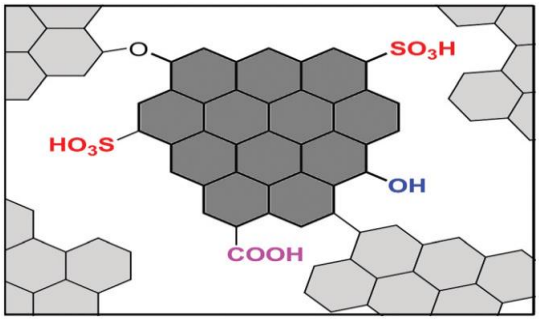


Figure 2.6: Structure of the carbon-based solid acid

The effective surface area of the carbon material during hydrolysis was about 560 m^2 g^{-1}, larger than the BET surface area after hydrolysis (only 2 m^2 g^{-1}). The H_2O vapor adsorption–desorption isotherm showed that a larger amount of water is incorporate into the bulk of the catalyst. The incorporation ability of hydrophilic molecules made it easy for a cellulose chain in solution to be in contact with the –SO_3H groups in the carbon material, which gave rise to high Yao-Bing catalytic performance.

As Huang and Yao Fu reported the strong interactions (hydrogen bonds to oxygen atoms in glycosidic bonds) between the phenolic OH groups and the glycosidic bonds in β-1, 4-glucan led to higher catalytic activities. Such hydrogen bonds are expected to bind cellulose to the surface of the catalysts, making it easier for the hydrolysis to proceed. The activation energy for cellulose hydrolysis with this carbon based acid was 110 kJ mol^{-1} which is lower than that with sulfuric acid (170 kJ mol^{-1}). The amorphous carbon acid is recovering from the reaction mixture and reused at least 25 times without decrease in activity. Elemental analysis and ion chromatography revealed that only 1% –SO_3H groups leached into the solution during the first reaction and no further leaching was detected in subsequent reactions. The excellent performance of the carbon based catalyst opens the possibility of converting cellulose into value-added molecules with cheap and efficient solid acids, which has the potential for use in industrial production in the future.

A mild process for the hydrolysis of cellulose to reducing sugars under microwave irradiation was developed by Fu and Yin et al. They used carbon sulfuric acids (BC–SO_3H) derived from bamboo, cotton and starch as catalysts. The introduction of microwave irradiation greatly accelerated the rate of the hydrolysis reaction. Comparison of hydrolysis results between the two heating methods over BC–SO_3H revealed that the accelerating

effect of microwave irradiation on the reaction was evident. The hydrolysis reaction operated at a lower temperature of 70–100 °C and the yield of reducing sugars was higher than that using conventional heating. The yields of hydrolysis products were different with the different types of carbon acids. The correlation between the hydrolysis product yields and adsorption values confirmed the results from Hara's work that the adsorption of the phenolic OH groups of BC–SO_3H to the oxygen atoms in the β-1,4-glycosidic bonds was responsible for the high catalytic activities of these carbon-based solid acids when hydrolyzing cellulose to glucose.

Cellulose has good solubility in the ionic liquid which enables easier contact with more acid sites. When the reaction was conducted at 110 °C, the total reducing sugars yield was up to 72.7%. Further increase in the reaction temperature led to an increase reaction rates but decreased the TRS yield, due to the rapid decomposition of the formed sugars at higher temperature. Whereas much effort has been invested in the optimization of the hydrolysis reaction conditions of cellulose over carbon based solid acids, the sulfonation conditions have rarely been considered.

Recently, (X. Zhang et al. 2013)reported that by changing the sulfonation temperature of the active carbon (AC), the catalyst exhibited different catalytic activities in the hydrolysis reaction. The active carbon was treated with/without nitric acid and sulfonated at different temperature ranging from 150–300 °C. With the increase of the sulfonation temperature, the acid density of the sulfonated carbon increased. The specific areas of the samples were increased when the temperature was increased from 150 to 250 °C, but decreased on further increasing the temperature. The hydrolysis of ball milled cellulose is conduct at 150 °Cover these solid acids and the AC–N–SO_3H sulfonated at 250 °C gave the best performance; the cellulose conversion and glucose yield were 74.3% and 62.6%, respectively. Apart from the sulfonation conditions, the carbon sources are also evaluated.

Sulfonated carbon catalysts have various advantages over liquid acid catalysts: solid catalysts can be easily separated from the liquid mixture after the reaction, thereby allowing the possible reuse of catalysts, along with the minimization of corrosion, ease of product separation, recyclability, less damage to the reactor(Huang and Fu 2013). Besides, the use of solid acid catalysts can reduce the pollutants which will have a minimal impact on the environment. And also have superior catalytic activities and some unique catalytic

properties that mineral acids do not possess. In this work, porous carbon catalysts preparation using the biomass pyrolysis method via the confined sulfonation process is presented. The resulting catalyst characterized by total acidity, FTIR, XRD, SEM, TGA, EA and sulfur content is tested for the activity on hydrolyses of microcrystalline cellulose. Thus, propose sulfonated carbon catalysts for hydrolysis of cellulose based on the above merits.

Table 2.1: Hydrolysis of cellulose with H_2O over solid acid catalysts

Catalyst	Feedstock	Reaction condition	Typical Products	Yield	Ref.
AC-SO_3H	Cellulose	423k, 24hrs	Glucose	40.5%[c]	(Onda et al. 2008)
AC-SO_3H+COOH +OH	Cellulose	373k, 3hrs	Glucose	4.0%[a]	(Suganuma et al. 2008)
AC-SO_3H	Cellulose	423k, 24hrs	Glucose	61.0%[c]	(Pang et al. 2010)
BC-SO_3H	Cellulose	373k, 1hrs MW(350W)	Glucose	19.8%[c]	(Youyu Wu et al. 2012)

[a]Catalyst: 0.300 g; cellulose:0.025 g; distilled water: 0.7 g. [b]catalyst: 0.125 g; cellulose: 0.1 g; distilled water: 10g. [c]catalyst: 0.300 g; cellulose: 0.25 g; distilled water: 2.25 g.

CHAPTER 3 MATERIALS AND METHODS

3.1 Chemicals and Instruments

The apparatus used in this research are; different size of beakers, graduated cylinder, digital micro-pipet and volumetric pipet, analytical balance (precision is ± 0.01 mg), different styles of burette stopcocks, chiller, spatulas, stirring rods, mechanical and magnetic stirrer, different size volumetric glass flasks, different size of conical and Erlenmeyer flasks, burettes, siever, desiccator used to store samples in a moisture-free environment, muffle furnace, oven, Jenway model 3510 digital pH meter, crucibles, thermometer, Aluminum foil, oil bath, water bath, hotplate magnetic stirrer, autoclave, sonicator (Bandelin sonorex RK 106s), spectrophotometer (Spectro UVD-3000/3200), cuvettes for spectrophotometer, glass and falcon plastic test tubes, plastic bags, soxhlet, condenser, vacuum pump, Tubular furnace (carbolite, maximum temperature 1100°C) was used to prepare the biochar, milling machine, marker, filter paper, funnel, Goggles for eyes protection, protective gloves for hand.

Chemicals used in this research were sulfuric acid (sigma), sodium hydroxide (sigma), ethanol (sigma), sodium chloride(sigma), glacial acetic acid, acetone, sodium chlorite (Aldrich), sodium hydrogen bicarbonate (sigma), hydrochloric acid (sigma), nitric acid (sigma), toluene, D-glucose (anhydrous reagent grade, Aldrich), 3,5-dinitrosalicyclic acid (DNSA), potassium sodium tartrate tetrahydrate or Rochelle salt ($C_4H_4KNaO_6.4H_2O$), nitrogen (N_2) gas, methyl blue powder, Phenolphthalein powder and other chemical reagent necessary for the analysis. All the above chemicals were analytical reagent grade and bought from different chemical stores in Addis Ababa.

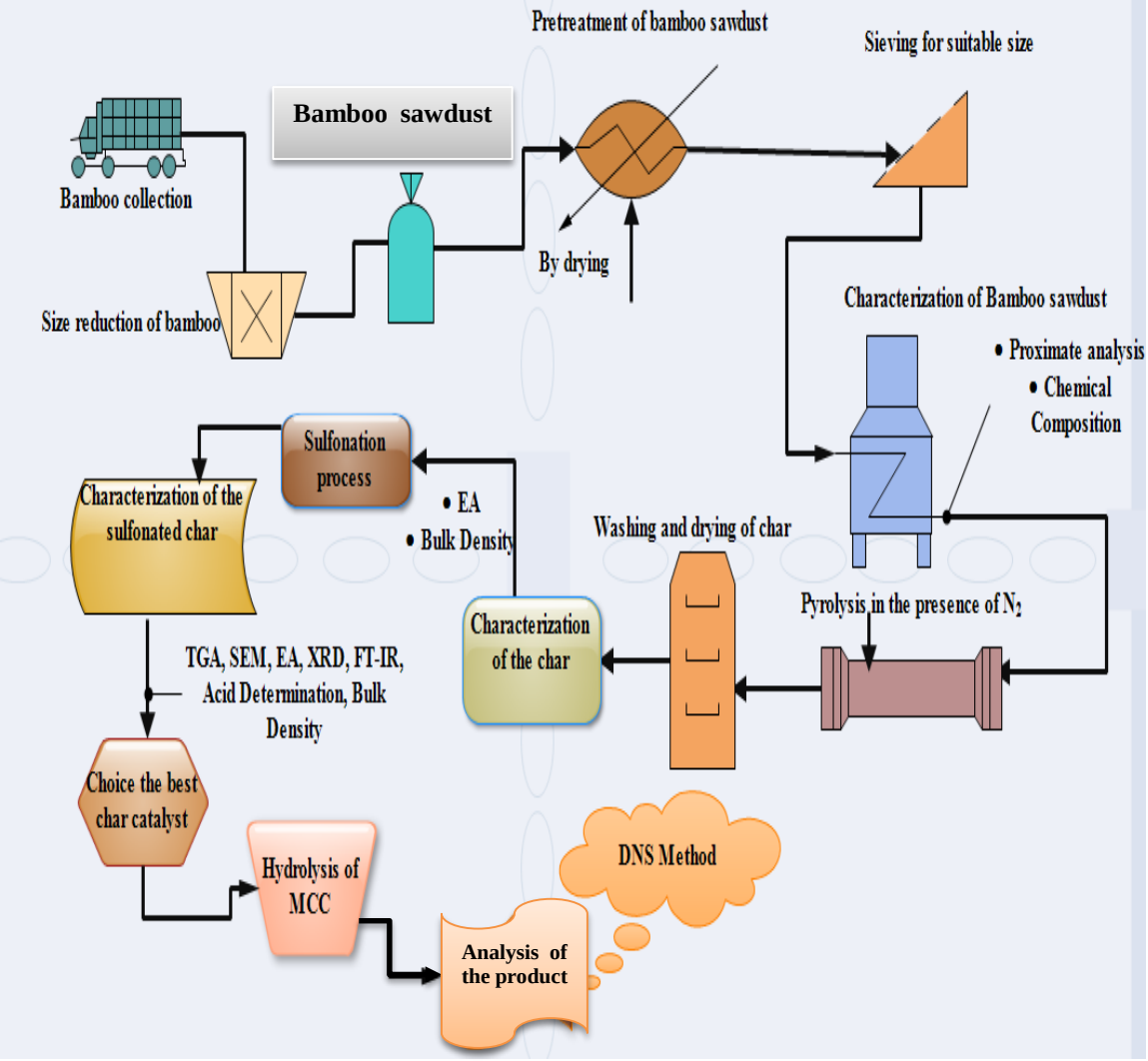


Figure 3.1: Frame work design of the research

3.2 Characterization of Precursor

3.2.1 Proximate Analysis of Bamboo

As defined by ASTM; D1762-84 (Reapproved 2001), proximate analysis separates the products into four groups: moisture, volatile matter, consisting of gases and vapors driven off during pyrolysis, fixed carbon, the nonvolatile fraction of biomass, and ash, the inorganic residue remaining after combustion.

i. Volatile Matter

The sample was measured and placed in a closed crucible. It was then heated up to 900℃ for exactly 7 min in a furnace. The crucible was then cooled in a desiccator and

weighed. The weight of the sample before heating and after heating was used to determine the amount of volatile matter present in the sample.

$$\boldsymbol{Volatile\ matter}, \% = \frac{\boldsymbol{W_1 - W_2}}{\boldsymbol{W_1}} * \mathbf{100} \qquad (3.1)$$

Where: W_1 = weight of dry sample and closed crucible before heating (g), W_2 = weight of dry sample and closed crucible after heating (g).

ii. Ash Content:

Sample was measured and taken in a crucible. It was then heated to 650 °C for 3hr. During this test the crucible was left open. The heating was done in a muffle furnace. After the required heating, the crucible was cooled in a desiccator and then weighed. In this test, the amount of residual substance is equal to the ash present in the sample.

$$\boldsymbol{Ash\ content}, \% = \frac{\boldsymbol{W_2}}{\boldsymbol{W_1}} * \mathbf{100} \qquad (3.2)$$

Where: W_1 = weight of dry sample before heating (g), W_2 = weight of ash (g).

iii. Moisture Content:

Sample was measured and taken in a petri dish. It was dispersed nicely on the petri dish. It was then heated at 105°C for 12hr. The petri-dish was left open during the heating process. After heating, the petri-dish was cooled in desiccator and then weighed. This specifies the amount of moisture content present in the sample.

$$\boldsymbol{Moisture}, \% = \frac{\boldsymbol{W_1 - W_2}}{\boldsymbol{W_1}} * \mathbf{100} \qquad (3.3)$$

Where: W_1 = weight of sample and petri dish before drying (gram), W_2 = weight of sample and petri dish after drying (gram).

iv. Fixed Carbon Content

The fixed carbon content is determined by subtracting the sum of percentage compositions of moisture content, volatile matter content, and ash content form 100. The value obtained is the amount of fixed carbon present in the sample expressed in percentage.

Fixed carbon (%) = 100 – (moisture, % + ash, % + volatile matter, %) (Wet basis) **(3.4)**

Fixed carbon (%) = 100 – (ash, % + volatile matter, %) (Dry basis**)** **(3.5)**

3.3 Preparation of Biochar

Bamboo bought from local market and was grinded by milling machine and the dust was collected and dried for 12hr at temperature of 50℃ to avoid the creation some microbial decay. The dried bamboo sawdust was collected between the 0.5mm and 1.5mm sieved mesh sizes. A 100 gram of pretreated bamboo sawdust was placed in a horizontal stainless steel tubular reactor equipped with tubular furnace, temperature controller, and N_2 gas inlet(Dehkhoda et al. 2010). The carbonization was performed at three different temperatures: 400, 450 and 500°C at the same pyrolysis time of 2hrs with heating rates of 8, 9 and 10°C/min, respectively under a nitrogen flow rate of 250 mL/min. The char samples were then demineralized for 1 hr in 0.01 M HCl, then washed with plenty of hot distilled water until neutral and dried in the oven at 105±1 ℃ for overnight.

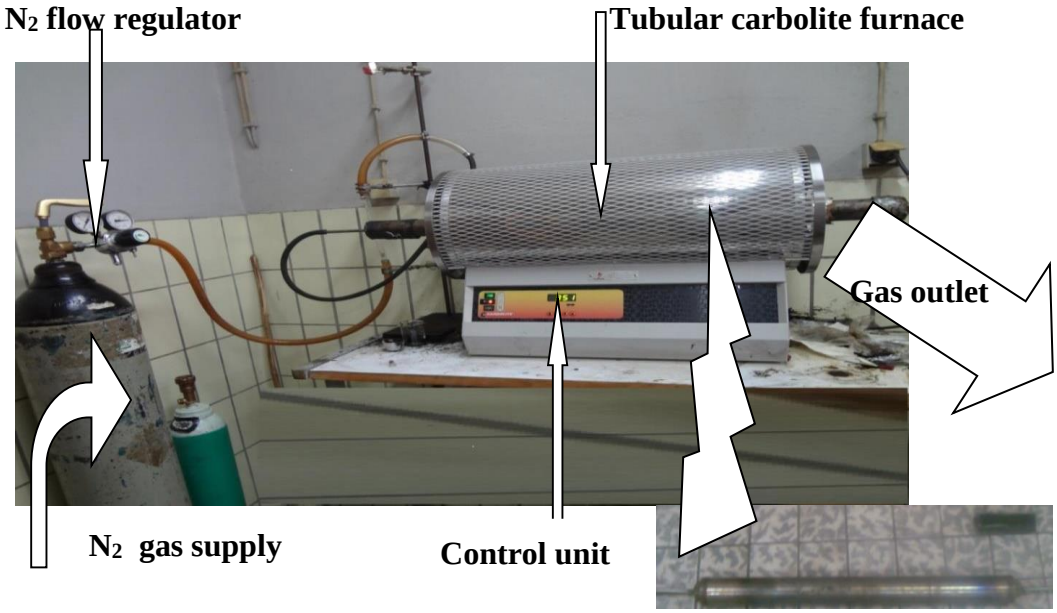


Figure 3.2: Carbonization process equipment setup

3.3.1 Ultimate (CHNS) analysis of biochar

The Ultimate Analysis of a sample determines the elemental composition of the sample. It is based on the principle of Dumas method which involves the complete and instantaneous oxidation of the sample by flash combustion. The results are in percentage composition of Carbon, Hydrogen, Nitrogen and Sulphur. From these results the oxygen composition is determined by subtracting the sum of Carbon, Hydrogen, Nitrogen, and Sulphur

compositions from 100. The Ultimate Analysis was carried out in EA 1112 Flash CHNS/O- analyzer under the condition of carrier gas (He-gas) flow rate of 120 ml/min, reference flow rate of 100 ml/min, and oxygen flow rate of 250 ml/min with furnace temperature of 900° C and oven temperature of 75 °C. The sample is fed into the analyzer along with excess supply of oxygen. The reaction of oxygen with other elements (namely carbon, hydrogen, nitrogen, and sulphur) present in the sample produces carbon dioxide, water, nitrogen dioxide, and sulphur dioxide respectively. The combustion products are separated by a chromatographic column and are detected by the thermal conductivity detector (TCD), which gives an output signal proportional to the concentration of the individual components of the mixture. This determines the equivalent compositions of elements in the sample.

3.4 Catalyst Preparation

Next, the biochar generated above was further reacted to attach strong acidic functional groups on the carbon surface based on (Kitano et al. 2009; Nakajima and Hara 2012; Suganuma et al. 2008, 2012) with some modification. For the strong acid catalyst (-SO_3H), the biochars were sulfonated by two protocols, without sonication and with sonication for each of biochar produced above at 400,450 and 500℃ (see section 3.3).

10 g of the milled BC-450 was taken and poured in 102 ml of 96% of H_2SO_4(1:10 ratio of wt./v) at temperature of 140°C for 15hr in the presence of N_2 gas flow; the sample has been shaken for 10min in the process of sulfonation by first protocol . After sulfonation the reactor cool down to room temperature and 1L of distilled water was added and waited for some times and started to wash the sulfonation product by hot distilled water not less than 80℃ until the pH neutral nearly 6.8 pH. Then, the cake is dried in an oven at 105℃ for 4hr.

15g of the milled BC-450 was taken and poured in 153.125 ml of 96% of H_2SO_4 (1:10 ratio of wt./v) and sulfonated at temperature of 140°C for 15hr in the presence of N_2 gas flow. During the process of sulfonation, the sulfonated sample has been taken and sonicated for a period of 40 minutes by second protocol. Finally, after the sulfonated carbon material was cooled and added to 1L of distilled water and washed by hot distilled water (>80℃) until the filtrate pH became neutral. Then after, the cake is dried in an oven at 105℃ for 4hr. Similarly for other biochar i.e. BC-400 and BC-500.

3.5 Characterization of Biochar Catalyst

3.5.1 Determination of the acidity

Titration method of every functional group over BC-SO_3H samples was determined by the method reported in the (Benak et al. 2002; Onda et al. 2009; Toda et al. 2005) with some modification.

The total acid density (-SO_3H, -COOH and -OH groups) of catalyst samples was determined using the standard acid-base back titration method. The catalyst samples were pre-dried in the oven at 105°C for two hours anterior to analysis, then 0.05 g of catalyst was added into 20 mL of 0.01N NaOH and sonicated for 1hr at room temperature before back-titration with 0.01N HCl using phenolphthalein as an indicative. Three different acidic groups of sulfonic, carboxylic and phenolic constitute the total acid density. The total acidity (N_{total}) of the sulfonated carbon was calculated as follows:

$$N_{total}(\boldsymbol{mmol/g}) = \frac{(\boldsymbol{volume\ NaOH * Normality\ of\ NaOH - volume\ of\ HCl * Normality\ of\ HCl})}{\boldsymbol{Mass\ of\ the\ catalyst}} \quad (3.6)$$

The sulfonic group density can be determined using the standard base direct titration method. The catalyst was pre-dried in the oven at 105°C for two hours prior to analysis, then 20ml of 0.01N of sodium chloride was added to a 0.05g of catalyst and sonicated for 1hr at room temperature before direct titrated by 0.01N of sodium hydroxide aqueous solution using phenolphthalein as an indicative. The density of SO_3H group (*N* mmol/g) was calculated as follows:

$$N_{SO3H}\ (\mathbf{mmol/g}) = \frac{(\boldsymbol{volume\ of NaOH\ used\ for\ the\ titration * Normality\ of\ NaOH})}{\boldsymbol{Mass\ of\ catalyst}} \quad (3.7)$$

The density of -SO_3H plus -COOH groups can be determined using the standard acid titration method. The catalyst was pre-dried in the oven at 105°C for two hours prior to analysis. Then, 20ml of 0.01ml sodium bicarbonate ($NaHCO_3$) was added to 0.05 g of a catalyst. The solution was sonicated for 1hr at room temperature before acid titrated by 0.01N of HCL aqueous solution using pH meter (until 4 pH). The density of -SO_3H plus -COOH groups (*N* mmol/g) was calculated as follows:

$$N\text{-}_{SO3H,\text{-}COOH}(\boldsymbol{mmol/g}) = \frac{(\boldsymbol{volume\ NaHCO3 * Normality\ of\ NaHCO3 - volume\ of\ HCl * Normality\ of\ HCl})}{\boldsymbol{Mass\ of\ the\ catalyst}} \quad (3.8)$$

According to the above titration results, the content of -OH groups = the content of the total functional groups – the content of -SO_3H plus –COOH groups; the content of -COOH groups = the content of -SO_3H plus –COOH groups – the content of -SO_3H groups.

3.5.2 Ultimate (CHNS) analysis of sulfonated carbon catalyst

The elemental analysis of biochar and sulfonated biochar were measured by using EA 1112 Flash CHNS/O- analyzer under the condition of carrier gas (He-gas) flow rate of 120 ml/min, reference flow rate of 100 ml/min, and oxygen flow rate of 250 ml/min with furnace temperature of 900° C and oven temperature of 75 °C. For every component four calibration points were calibrated. Samples were run in duplicate and the average values are to be taken to minimize the data error. Elemental Analysis was conducted in Addis Ababa University, college of natural science in the department of chemistry.

3.5.3 Fourier Transform Infrared (FT-IR) Spectroscopy

The chemical compositions of bamboo sawdust, biochar and sulfonated biochar were determined using FT-IR spectroscopy (FTIR-65, Perkin-Elmer) with the more conventional KBr technique. FT-IR spectroscopy is an important method to determine the presence and absence of particular bands of functional groups. FTIR is most useful for identifying chemicals that are either organic or inorganic. The instrument used was able to record spectra from wave numbers of 4000 to 400 cm^{-1}. Spectrum is produced as a result of the absorption of infrared radiation. The functioning group is determined based on the interpretation of the infrared spectrum obtained by comparing it with the standard spectrum group frequencies. The raw material and laboratory prepared sulfonated biochar catalyst samples for the analysis was first milled in a ceramic pestle and mortal to powdery conditions. The powder was then mixed with KBr particles to make it suitable to infrared analysis. The mixture was then pressed to a small thickness, slightly below 1mm, required for FTIR analysis. FTIR Analysis conducted in Addis Ababa University, college of natural science in the department of chemistry. The FT-IR data will be graphed by origin pro 8.0 and essential ft-ir setup.

3.5.4 X-ray Diffraction (XRD) Spectroscopy

This analysis is one of the most commonly used techniques for either crystalline or amorphous phase identification. In this technique, the catalyst sample is irradiated with X-ray of a known wavelength (λ). The X-ray diffraction (XRD) patterns of sulfonated catalyst were obtained using MiniFlex 300/600, Japan operated at 40 kV and 15 mA, using Ni-filtered Cu-Ka radiation (1.54059-1.54441). The samples were placed on a sample holder made up of silicon wafer and the measurements were taken continuously from 10^o to 80^o angles over the range of 2θ. The resultant intensity data was processed by using in-built diffraction software Match! 3 to graph and analyze the peak position. A custom-made sample holder with a shallow well was used for the samples, and rotated to improve particle statistics. These analyses have been performed in Addis Ababa University, college of natural science in the department of chemistry.

3.5.5 Characterization of the Morphology Catalyst

Scanning Electron Microscopy (SEM) has been extensively used to characterize the microstructure of biochar and sulfonated biochar catalyst. The surface morphology, such as the surface shape, pattern, and feature of the selected biochar and biochar sulfonated catalyst was observed using JEOL JSM-IT300 SEM attached with Energy Dispersive X-ray (EDX). SEM is commonly used for observing microscopic pores that exist on the carbon surface. SEM was performed in Leather Industry Development Institute (LIDI) laboratory.

3.5.6 Thermo Gravimetric Analysis (TGA)

TGA is an instrument which is designed to measures the amount & rate of change in the mass of a sample as a function of temperature or time in a controlled atmosphere. The thermal stability of raw material was tested by measuring the mass loss during a heating ramp rate. Thermal stability of the sulfonated biochar catalyst was determined by thermogravimetric analyzer (TA instrument, SDT Q600 V20.9 Build 20) using temperature programming from 25 to 800^{o}C at the heating rate of 20^{o}C/min to which the sample was heated. For the thermo gravimetric analysis to be carried out, small amount of the sample (22-30mg) was placed in a vial, which was present in the TGA analyzer. This vial is connected to sensors which detects the weight of the sample at all times. Testing was carried out under inert atmosphere (N_2) with a flow rate of 2 ml/min to remove all

corrosive gases and avoid thermoxidative degradation and the retention time of the sample at the maximum temperature. These values were used as basis for the analysis. TGA was performed in Leather Industry Development Institute (LIDI) laboratory. The thermal degradation onset temperature and the thermal degradation weight loss of composites were recorded and analyzed using origin pro 8.0 software.

3.6 Hydrolysis of microcrystalline cellulose

The microcrystalline cellulose (Weissenborn, Germany, VIVAPUR®102; pH, 6.3, bulk density, 0.30g/ml, particle size, 20-50 μm; crystallinity, 77%; degree of polymerization, max.350) was provided by the Ethiopian Pharmaceutical Industry Manufacturing. The X-ray diffraction, XRD analysis was carried out using a Cu-K∂ source (1.541874Å) to validate the structure of microcrystalline cellulose.

The hydrolysis of microcrystalline cellulose was carried out in a stainless-steel autoclave with autogenously pressure. Microcrystalline cellulose, catalyst, and distilled water were charged into the glass reactor lined with Teflon based on Konwar with some modification. Before the autoclave reactor was adjusted the mixture of catalyst to microcrystalline cellulose ratio (1:1, 1.25:1, and 1.5:1) and 10 ml of distilled water was sonicated for 20min to amorphously the mcc and mixing well. The reaction was proceeded in the autoclave at specified temperature (120, 135, and 150℃) and time (4, 6, and 8 hrs). Then cooled by discharging the steam formed in the autoclave to 323 K for few minutes. After the reaction, the reaction mixture was filtered (containing the catalyst and the unreacted cellulose) and the filtrate solution was analyzed by DNS analysis (Li and Zhao 2007). An absolute calibration method was employed for calculation of product yields. The conversion of cellulose was determined based on the weight difference of the solid part before and after reaction (Eq. 3.14).

3.6.1 Analysis of Reducing Sugar

The reducing sugar (glucose) was measured using 3, 5-dinitrosalicylic acid (DNS) method. When alkaline solution of 3, 5-dinitrosalicylic acid reacts with reducing sugars (eg. Glucose) it is converted into 3-amino-5-nitrosalicylic acid with orange-red color. Intensity of the colour is an index of reducing sugar. This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the

aldehyde functional group present in glucose(Anamaria Negrulescu et al. 2012; Marsden et al. 2000). Simultaneously, 3, 5-dinitrosalicylic acid (DNS) is reduced to 3-amino, 5-nitrosalicylic acid under alkaline conditions:

Oxidation

Aldehyde group ----------> Carboxyl group

Reduction

3, 5-dinitrosalicylic acid ----------> 3-amino, 5-nitrosalicylic acid

The above reaction scheme shows that one mole of sugar will react with one mole of 3, 5-dinitrosalicylic acid. Different reducing sugars generally yield different color intensities; thus, it is necessary to calibrate for each sugar. The DNS method is carried out by preparing a set of solutions with known glucose concentrations and mixing them with the DNS reagent. A standard curve is plotted and the concentrations of unknown sugar samples can be derived from the standard curve.

Procedure Description

Reducing sugars have the property to reduce many of the reagents. A reducing sugar is one that in a basic solution forms an aldehyde or ketone. The aldehyde group of glucose converts 3, 5-dinitrosalicylic acid (DNS) to 3-amino-5-nitrosalicylic acid, which is the reduced form of DNS. Water is used up as a reactant and oxygen gas is released during the reaction. The formation of 3-amino-5-nitrosalicylic acid results in a change in the amount of light absorbed, at wavelength 540 nm.

A series of solutions containing varying concentrations of glucose are prepared in test tubes and a known quantity of DNS is added to each. These test tubes are then heated on a water bath for few minutes and their optical densities are measured using a colorimeter. A graph is then plotted with amount of glucose on x-axis and the observed optical density at y-axis. The plot thus obtained is called a standard glucose curve(Anamaria Negrulescu et al. 2012; Marsden et al. 2000).

a) Standard stock solution of glucose

0.1 % D-glucose was prepared by putting 50ml distilled water in a 100ml of volumetric flask with a stir bar (Note: do not add the powered glucose before the water or you will

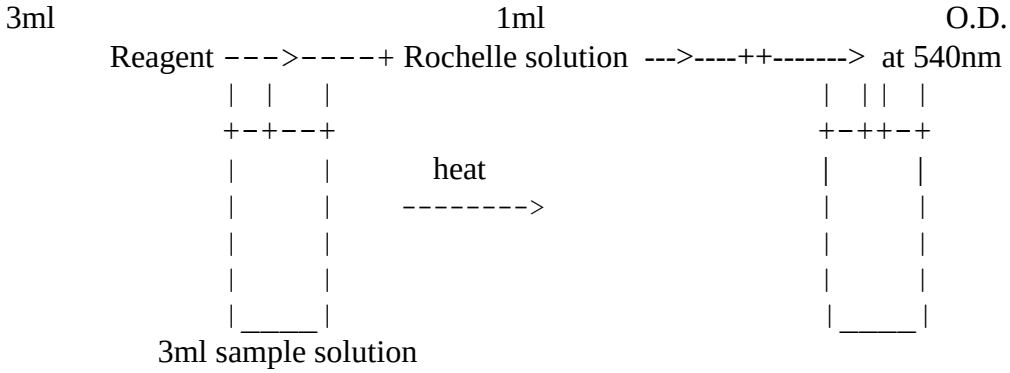
make rock candy!!!!!!). Placed 50ml of distilled water on stir/hot plate and turned on heat and start stirring. 50ml of distilled water was allowed to get warm, but not necessary to have boiled. Finally, it was added 0.1 g of glucose (dextrose) and filled the volumetric flask up to 100ml with distilled water.

b) Preparation of dinitrosalicylic acid (DNS) reagent

1g of 3, 5-dinitrosalicylic acid, 200 mg of crystalline phenol, and 50 mg of sodium sulphite were simultaneously dissolved in 100 mL of 1% NaOH solution by stirring. Forty per cent of Rochelle salt solution (sodium-potassium tartrate solution) was prepared. The DNS solution was stored in dark glass bottle at temperature below 20 °C.

c) Preparation of standard curve

Standard curve was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the standard stock solution of glucose and the volume was made up to 3 mL by adding distilled water. 3 mL of DNS reagent was added and the mixture was heated for 10 minutes in a boiling water bath. After the development of the color, 1 ml of a 40% potassium sodium tartrate tetra hydrate (Rochelle salt) solution was added (in warm contents) before the mixture is cooled and mixed thoroughly to stabilize the color. After cooling the tubes, the absorbance was recorded at a wavelength of 540 nm using Spectro UV-VIS double beam PC 8 Scanning auto cell (Spectro UVD-3200).



Generally, reduced sugar (glucose) can be investigated by the DNS method employing D-glucose as the standard. DNS reacts with free carbonyl group of the RS under alkaline condition, forming 3-amino-5-nitrosalicylic acid, an aromatic compound with maximum absorption at 540 nm, allowing a quantitative spectrophotometer measurement of the amount of RS present. Sugar reduces the organic DNS which absorbs maximally at yellow wave length. Results in change (shift) in absorption spectrum from yellow to red/brown at 540nm. The concentration of glucose was measured at 540nm for only reacted DNS by detecting the reducing end of the monosaccharide, but unreacted DNS not seen at this wavelength. The amount of absorbance directly related to amount of reducing sugar (glucose).

Generally the conversion de-polymerization cellulose can be estimated by:

$$\text{Conversion, \%}=\frac{\mathbf{M_{residue}-M_{catalyst}}}{\mathbf{M_{cellulose}}}\boldsymbol{x100} \qquad (3.9)$$

Where $M_{residue}$ (unit: g) is mass of dried residue and $M_{catalyst}$ (g) is mass of catalyst charged.

3.7 Experimental design and statistical analysis

The RSM was applied to evaluate the effects of hydrolysis reaction parameters and optimize conditions for various responses. Box-Behnken experimental design (BBD) with three numeric factors on three levels was used. This design consisted of fifteen randomized runs with three replicates at the central point to minimize the error. Independent variables used in experimental design was shown in Table 3.2 below and the run sheet (see table 3.1). Each variable was coded variables in dimensionless numbers from -1 to 1(Bas, D. and H. 2007). The response variable was fitted to the following second-order polynomial model (Eq.3.15) which is generally able to describe relationship between the responses and the independent variables (Anamaria Negrulescu et al. 2012; Aslan and Cebeci 2007; Marsden et al. 2000):

$$Y=\beta_o+\beta_1A+\beta_2B+\beta_3C+\beta_4AB+\beta_5AC+\beta_6BC+\beta_7A^2+\beta_8B^2+\beta_9C^2 \qquad (3.10)$$

where Y represents the response variable, A, B and C are the independent variables affecting the response, and β_o, β_1, β_2, β_3, β_4, β_5, β_6, β_7, β_8, and β_9 are the regression coefficients for intercept, linear, quadratic and interaction terms.

Optimal hydrolysis reaction conditions were determined considering glucose yield as response. Treatment of multiple responses and selection of optimal conditions were based on desirability function. The experimental design and multiple linear regression analysis were performed using design expert 10.0.6.0 (stat-Ease, Inc 2017).

Table 3.1: Run sheet

	Run #1	Run #3	Run #4
Temperature	120 ℃	150 ℃	120 ℃
Time	4hrs	4hrs	8hrs
Catalyst to cellulose ratio	1.25	1.25	1.25
yield of glucose			
	Run #8	Run #7	Run #6
Temperature	150 ℃	120 ℃	150 ℃
Time	8hrs	6hrs	6hrs
Catalyst to cellulose ratio	1.25	1	1
yield of glucose			
	Run #15	Run #9	Run #11
Temperature	120 ℃	150 ℃	135 ℃
Time	6hrs	6hrs	4hrs
Catalyst to cellulose ratio	1.5	1.5	1
Yield of glucose			
	Run #14	Run #13	Run #5
Temperature	135 ℃	135 ℃	135 ℃
Time	8hrs	4hrs	8hrs
Catalyst to cellulose ratio	1	1.5	1.5
Yield of glucose			
	Run #12	Run #2	Run #10
Temperature	135 ℃	135 ℃	135℃
Time	6hrs	6hrs	6hrs
Catalyst to cellulose ratio	1.25	1.25	1.25
Yield of glucose			

Table 3.2: Experimental domain with natural and coded values of independent variables used in Box-Behnken design (BBD)

Factors	**Symbol**	**Coded Variable level**		
		Level 1	**Level 2**	**Level 3**
		-1	**0**	**+1**
Temperature (℃)	A	120	135	150
Residence time (hr)	B	4	6	8
Catalyst to Cellulose ratio (mass ratio)	C	1	1.25	1.5

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Characterization of Precursor (bamboo sawdust)

4.1.1 Proximate Analysis of Bamboo sawdust

The proximate analysis of bamboo was done, before carbonized in order to know the moisture content, volatile matter, ash content and the fixed carbon content of the bamboo. The proximate analysis was carried out using muffle furnace. The proximate analysis was done using (ASTM; D1762-84 (Reapproved 2001)) and the result obtained is shown in Table 4.1. The detailed calculation part is also included under appendix-A. (Based on average values of three replicates, errors lower than 1% in all components, weight percent on dry basis).

Table 4.1: proximate analysis of bamboo

Component	Average
Moisture content %	9.96
Volatile Matter* %	80.97
Ash Content*%	1.6
Fixed Carbon* %	17.433

***based on dry basis**

The Proximate Analysis result of bamboo determines the distribution of its contents. It may be noted that the volatile matter present in bamboo, contributes maximum to its contents. The moisture content present in the sample can also be considered as water vapor when it is heated to high temperatures. Hence, 80.97% is volatile matter and 9.96% is moisture content. Larger weight loss of the substance implies greater volatile matter content. The values from the Table 4.1 indicates that the ash content of the sample is 1.6% and also fixed carbon content of the sample is 17.433%. It can be seen that the fixed carbon and ash content decreased with increased the volatile content.

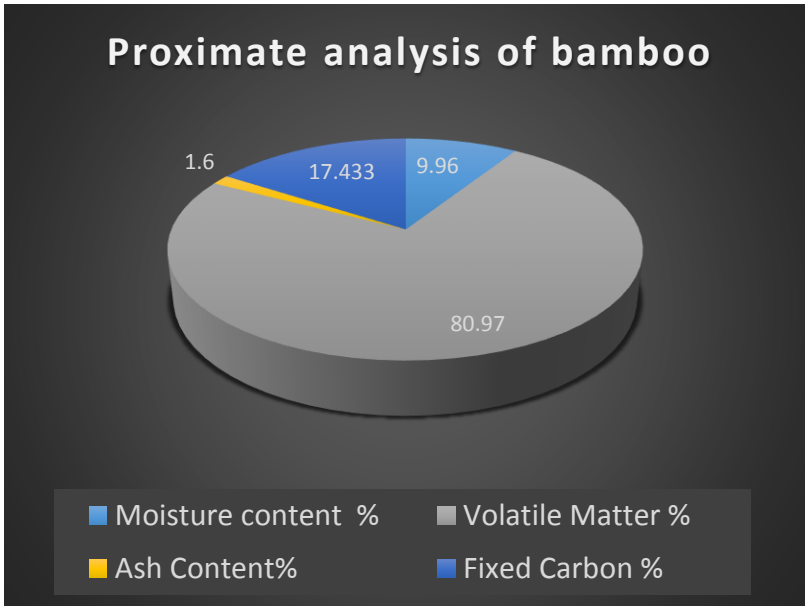


Figure 4.1: Proximate analysis of bamboo

In general, the Table 4.1 shows that the fixed carbon content for bamboo is 17.433%. The bamboo sample was rich in volatiles (80.97%) but low in ash content (1.6%). The low ash content would result in minimal effects of inorganic impurities on pore development during pyrolysis process. Previous studies suggested that suitable biochar should be low in ash but rich in carbon and volatiles. The ash consists of mainly minerals such as silica, alumina, iron, magnesium and calcium. Ash in char is undesirable and taken as impurity. Ash may interfere with carbon adsorption through competitive adsorption and catalysis of adverse reactions. Usually materials with the lowest ash content produce the most active products. Fixed carbon content is changed when samples exposed to high temperatures during carbonization. Thus, the fixed carbon content increased steadily while volatiles declined with temperature. At higher temperatures, organic substances become unstable as the heat provides energy to the molecules to break their bonding and linkages and once these are broken, the substances will be discharged both as gas and liquid products. This gives an overview about the properties and components of bamboo. The above result shows that, the moisture and ash contents of bamboo are found to be very low as compared to other precursors in the literature. This makes bamboo one of the best raw materials for biochar production.

4.2 Ultimate analysis of prepared biochar

For total content of elements in biochar samples, C, H, N and S were measured by using EA 1112 Flash CHNS/O- analyzer under the condition of carrier gas (He-gas) flow rate of 120 ml/min, reference flow rate of 100 ml/min, and oxygen flow rate of 250 ml/min with furnace temperature of 900° C and oven temperature of 75 °C. For every component four calibration points were calibrated. Samples were run in duplicate and the average values are to be taken. The percentage of oxygen content was estimated by difference as follows: O (%) = 100 - (C + H + N + S) (Calvelo Pereira et al. 2011).

Table 4.2: Elemental analysis of the biochar

Sample	**N_2 (%)**	**C (%)**	**H (%)**	**S (%)**	**O_2 (%)**
BC-400℃	0.1510	77.4445	3.4955	0.0000	18.8390
BC-450℃	0.1425	79.8805	3.1875	0.0000	16.7895
BC-500℃	0.3345	84.4785	2.8065	0.0000	12.3805

The elemental content of biochars is presented in Table 4.2 showed that carbon content increased with temperature, but the loss of O, and H was recorded. The increase in carbon content with temperature is due to increasing degree of carbonization. However, these decline in O and H elements may be attributed to breaking of weaker bonds in biochar structure and highly carbonaceous materials yielded with increased temperature (Demirbas, A. 2002). The decrease in S with temperature could be explained by the volatilization of sulfur during pyrolysis process. But, the result shows no sulfur content in the carbonization temperature of 450 and 500℃. It indicates that the volatilization of sulfur above 400℃ is very high. Though it has been reported by many authors that pyrolysis temperature lead to reduce N content of biochars, relative increases in N content were observed in samples of biochar with temperature in this study. Our results suggest that temperature could conserve N in wood chars. This can be explained by incorporation of N into complex structures that are resistant to heating and not easily volatilized (Gaskin et al. 2008). In consistent with our results, (Calvelo Pereira et al. 2011) also found that N enrichment relative to the original feedstock was recorded upon charring of woody

material. Char is a porous carbon structure that remains after the hydrogen and oxygen fractions have left the fuel. It is generally assumed that char completely exists of carbon, but since char is often defined as the solid residue after pyrolysis, it is often polluted with other components. Char is usually polluted with mineral fractions and after incomplete pyrolysis large fractions of hydrogen and oxygen can still exist.

4.3 Bulk density of biochar and its catalyst

Bulk density is used to characterize the particles of char and its catalyst and also useful for the estimation of tank or packing volume. Bulk density, or packing density, includes all pores and voids (inter-particle spaces in its calculation). For determination of bulk density method described by ASTM Test Methods (D 2854 – 96 (Reapproved 2000) was used with a slight modification. Each sample was tested in triplicate. It is determined by filling a graduated cylinder, with tapping. It follows that the value obtained is dependent upon the form of the catalyst (powder, tablets, extrudates) because of the different contribution of inter-particle void space to the pore volume. The use of the term bulk density should be encouraged, accompanied by the detailed description of the conditions of its determination.

The bulk density is defined as the mass of a unit volume of the sample in air, including both the pore system and the voids between the particles. The result of bulk density was expressed as g/mL on dry basis. The bulk density is not an intrinsic property of a material; it can change depending on how the material handled. The bulk density of biochar and sulfonated biochar depends on the shape, size and density of the individual particles.

Table 4.3: Bulk density of biochar and its catalyst

Sample no.	Biochar	Average bulk density of biochar,(g/ml)	Catalyst	Average bulk density of catalyst (g/ml)
1	BC-400	0.2698	BC-400-SO_3H-st	0.3161
2			BC-400-SO_3H-s	0.3472
3	BC-450	0.2837	BC-450-SO_3H-st	0.3250
4			BC-450-SO_3H-s	0.3493
5	BC-500	0.2839	BC-500-SO_3H-st	0.3429
6			BC-500-SO_3H-s	0.3650

As shown in the Table 4.3 the density of sulfonated biochars are higher than its biochars due to sulfonation of biochar. This indicates the sulfonated catalyst (BC-SO_3H) has been -SO_3H functional group on the surface biochar. Thus, the bulk density of sulfonated biochar can be related to its functional groups.

The density of biochars was increased as carbonization temperature (from 0.2698 to 0.2839 g/ml). Because, the particle size of biochars were decreased with the pyrolysis temperature but the pore sizes were increased and also more volatile matter was vaporized with temperature increment. The effect of sulfonation on the density of carbon material was also seen. Different bulk density could be seen, when the biochars were sulfonated with sonication and without sonication for every biochars. The density of sulfonated biochar with sonication was greater than without sonication, this shows more –SO_3H group was formed on the surface biochar with sonication. Thus, the bulk density result tells the availability of the functional group on surface of biochar Catalyst.

4.4 Characterization of sulfonated biochar catalyst

4.4.1 Determination of the acidity

Titration method was described in section 3.5.1. Each sample was tested in triplicate. The total acid site density, consisting of carboxylic, phenolic, and sulfonic groups, was measured via back titration method. The sulfonic group density can be determined using the standard base direct titration method. The Table 4.4 shows higher value of the total acidity than the density of SO_3H group can be interpreted as due to the presence of many acidic groups like phenolic OH or CO_2H groups in addition to SO_3H groups in the materials.

Table 4.4: The acid density of the biochar and sulfonated biochar catalyst

Samples	Total acid density (mmol/g)	SO_3H (mmol/g)	COOH and OH (mmol/g)
BC- 400℃	1.8	0.000	1.7560
BC- 450℃	1.9	0.000	1.8560
BC-500℃	1.71	0.000	1.6660
BC-400-SO_3H-s	4.0000	0.5900	3.4100
BC-400-SO_3H-st	4.1600	0.4950	3.6650
BC-450-SO_3H-s	2.9100	0.4370	2.4730
BC-450-SO_3H-st	2.9800	0.3980	2.5820
BC-500-SO_3H-s	3.7100	0.5280	3.1820
BC-500-SO_3H-st	3.55	0.447	3.1030

The contents of –SO_3H, -OH, -COOH groups on BC-SO_3H are usually among 0.1-4.9, 0-0.9, 0-7.8 mmol/g, respectively (Benak et al. 2002; Nakajima and Hara 2012; Onda et al. 2008, 2009; Suganuma et al. 2012; Toda et al. 2005; Youyu Wu et al. 2012; Yamaguchi et al. 2009). Though -SO_3H groups are considered as the key active acidic site, the existence of -OH and -COOH groups would provide hydrophilic reactants accessing to the-SO_3H groups, which would be in favor of effective catalytic performance. According to literature(Nakajima and Hara 2012; Onda et al. 2009), it is assumed that all sulfur content of each sample is in the form of –SO_3H. The total acid density is consisted of three different acidic groups of sulfonic, carboxylic and phenolic. However, only the sulfonic group has enough acid strength to participate in hydrolysis reaction. This explains the difference between the total acid density and the density of sulfonic groups. Generally, the Table 4.4 shows the results of -SO_3H group and the total acid density which sulfonated with sonication was higher than sulfonation without sonication in every carbonized temperature biochar. Thus, the new developed technique of sulfonation with sonication was best method for sulfonation of biochar.

4.4.2 Elemental Analysis (CHNS analysis) of sulfonated carbon catalyst

For total content of elements in sulfonated biochar samples, C, H, N and S were measured based on described in section 4.2.

Table 4.5: Elemental analysis of the sulfonated catalyst

Sample	**N_2 (%)**	**C (%)**	**H (%)**	**S (%)**	**O_2 (%)**
BC-400-$S0_3$H-s	0.3205	59.4582	2.7021	1.8679	35.9719
BC-400-$S0_3$H-st	0.409	58.4561	2.6086	1.7239	37.2114
BC-450-$S0_3$H-s	0.496	62.5243	2.6179	1.8080	32.5499
BC-450-$S0_3$H-st	0.506	64.2819	2.6465	1.9317	31.1399
BC-500-$S0_3$H-s	0.514	65.6839	1.8802	2.1045	30.3314
BC-500-$S0_3$H-st	0.517	58.3071	1.7384	2.0623	32.6019

The elemental content of the sulfonated catalyst is presented in Table 4.5 showed that sulfur content increased from BC-400-SO_3H to BC-500-$S0_3$H, but the loss of C, N and H was recorded. The increase in sulfur content with temperature is due to increasing degree of carbonization. However, these decline in C and H elements may be attributed to breaking of weaker bonds in biochar structure. The decrease in N with sulfonation could be explained by the volatilization of N during sulfonation process. But, the result shows an increasing of nitrogen content with sulfonation as biochar carbonized temperature increases. It indicates that the volatilization of nitrogen during sulfonation is very low. Though it has been reported by many authors that sulfonation lead to reduce C, H and N content of sulfonated biochars (Song, W., Guo 2012), relative increases in oxygen content were observed in samples of catalyst in this study. Generally, the result of elemental analysis and acid density are related, because –SO_3H group increases from BC-400 to Bc-500 in both case analysis. In addition to this –SO_3H groups of sulfonated biochar with sonication are greater than –SO_3H groups of sulfonated biochar w/o sonication (by stirring) in both of them.

4.4.3 Thermo gravimetric Analysis

Thermogravimetric analysis (TGA) was performed in thermo gravimetric analyzer (TA instrument, SDT Q600 V20.9 Build 20) using temperature programming from 25 to 800°C at the heating rate of 20°C/min to which the sample was heated under a 2mL/min flow of nitrogen. The TGA result of biochar (Figure 4.2) clearly indicates that the initial weight loss begins at a temperature around 50°C. From 100 to 350 °C, there was a steady in change of weight percentage biochar. But the drastic change in weight is felt only when the

temperature reaches around 400°C. Henceforth there is a steep and steady weight loss of biochar of bamboo sawdust. The quality of biochar depends on the process and technique of removal of volatiles from the sample. It also varies with the rate of dehydration of the sample. Hence taking these into consideration, it can be identified that there is maximum loss of weight in the temperature range of 360°C to 800°C. Overall of the analysis, the sample lost about 16% of its weight still left at 800℃.

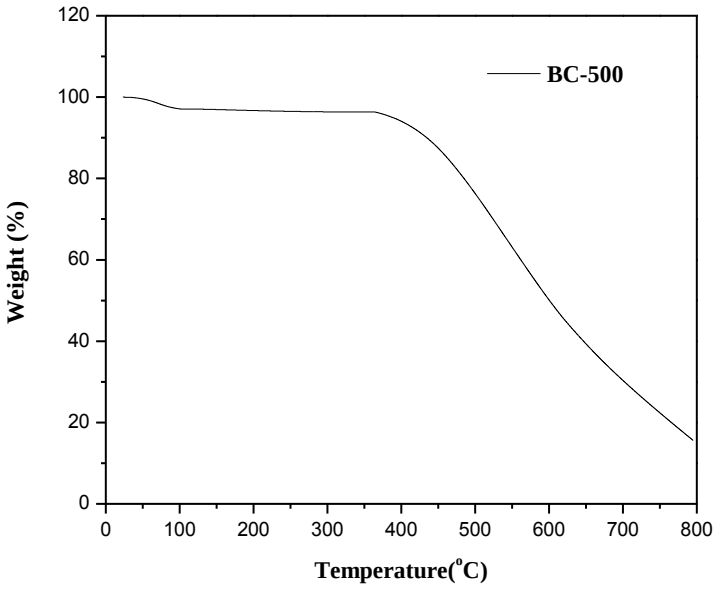


Figure 4.2: TGA thermograph of BC-500

The thermal stability of the catalyst prepared from char carbonized at 500 °C (BC-500-SO_3H) was investigated via thermogravimetric analysis (TGA) under nitrogen flow. As shown in Figure 4.3, the catalyst started slightly to lose 10% of its weight between 50 and 150 °C, most likely from the loss of the free moisture adsorbed on the surface of catalyst and due to water desorption. From 150 to 250 °C, there was a marginal increase in the rate of loss followed by a steady. Then, the weight continued to drop slightly plateaued between 250 and 350 °C can be interpreted as due to the decomposition of the SO_3H groups and rapid loss of weight from 400 to 800 °C. This TGA result resembles that of the sulfonated biochar made by sulfonation of aromatic hydrocarbons such as naphthalene with concentrated sulfuric acid. Overall of the analysis, the sample lost about 18% of its weight still left at 800℃.

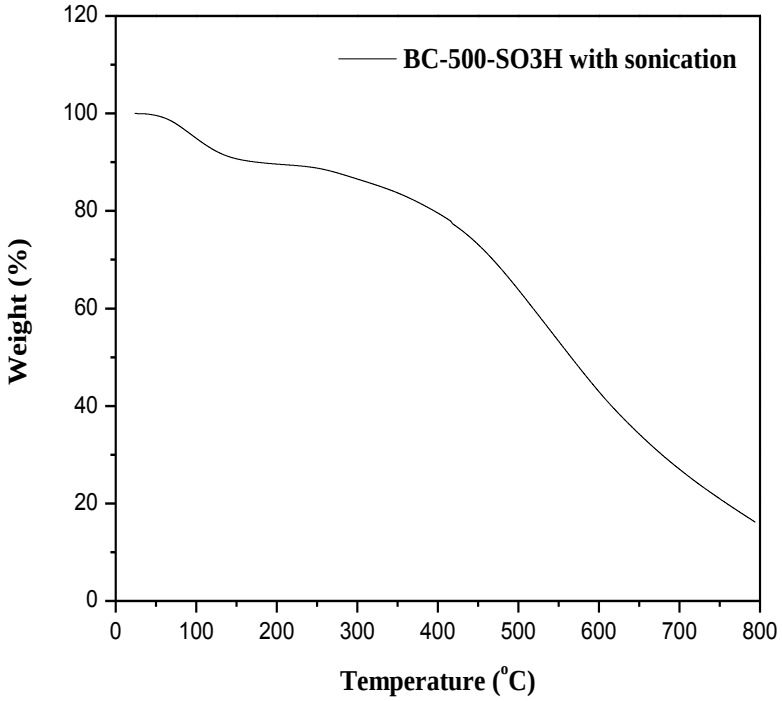


Figure 4.3: TGA thermograph of BC-500-SO_3H-s

Thermal gravimetric analysis was used to understand the impact of the sulfonation on the thermal stability of the solid acid catalysts. Sulfonation of the biochars using H_2SO_4 lowered the temperature at which mass loss occurred in the TGA analysis. Using the mass loss (weight %) versus temperature data, the onset of thermal decomposition was initiated at 250°C for sulfonation of biochar, compared to 350°C for unsulfonated biochar (see Figure 4.2 and Figure 4.3). These data indicated that sulfonation of the biochars partially oxidized the structure, and reducing thermal stability. In both TGA curves, the weight losses below 50°C apparently resulted from evaporation of adsorbed water.

Generally, TGA is used solely to determine the decomposition of material as the heating temperature changes. For materials which exhibit either mass loss or gain due to decomposition, oxidation or loss of volatiles. And also TGA provides valuable information that can be used to predict lifetime of materials, to select materials for certain end-use applications, to predict product performance and to improve product quality. Thermogravimetric was also depicted weight loss due to functional group decomposition. It is widely applicable in the area of catalysts fields of research and development.

4.4.4 Characteristic of the morphology (SEM)

Scanning Electron Microscopy (SEM) has been extensively used to characterize the microstructure of biochar and sulfonated biochar catalyst. The surface morphology, such as the surface shape, pattern, and feature of the selected biochar and biochar sulfonated catalyst was observed using JEOL JSM-IT300 SEM attached with Energy Dispersive X-ray (EDX).

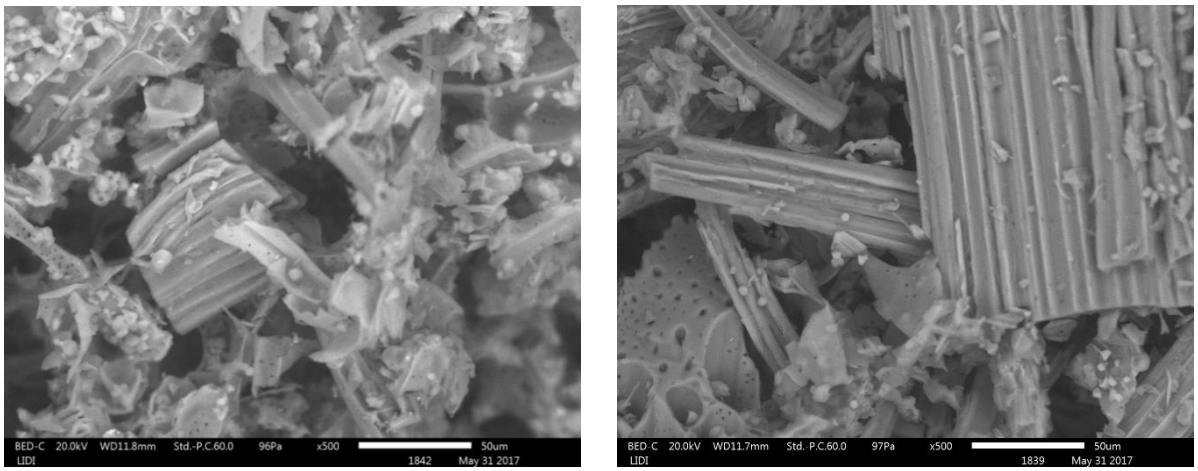


Figure 4.4: SEM images of: A) BC-500 at 50**µm**; and B) BC-500-SO_3H-s at 50**µm** indicating morphologies and pore sizes.

The SEM was used to get proper images of samples at the microscopic level. These images are primarily used to determine the structure and distribution of the pores that are present on the surface of the biochar and sulfonated biochar. It also determines the nature of the size of the pore, depending on the magnification of the image taken. The SEM images of the biochar and sulfonated biochar are depicted in Figure 4.4. From these images (Figure 4.4), it can be seen that the biochar (BC-500) and sulfonated biochar (BC-500-SO_3H-s) consisted mainly of aggregates of carbonaceous microspheres that had diameters of several mm. The smooth surfaces of the microspheres were partially destroyed after post-treatment sulfonation. When biochar was sulfonated with H_2SO_4 and biochar, a morphological change occurred and the resulting a biochar had a sponge like structure with large conchoidal cavities due to sulfonation agglomeration occur. The bamboo based biochar are predominantly meso/macropore structures and are, therefore, usually suitable for the

adsorption of large molecular species. Such properties are used to advantage in hydrolysis of cellulose processes.

4.4.5 X-ray Diffraction (XRD) Spectroscopy

X-ray diffraction (XRD) is one of the most important non-destructive tools to analyze all kinds of matter - ranging from fluids, to powders and crystals and also to identify crystalline phases and orientation. XRD patterns for BC-400-SO_3H-s (Figure 4.5) exhibited one strong diffraction peak at 2θ=19–30° and one broad, and for BC-400-SO_3H-st exhibited one strong diffraction peak at 2θ=18–29° and one broad. These XRD patterns are typical of amorphous carbonaceous materials that consist of aromatic carbon sheets oriented in a random fashion.

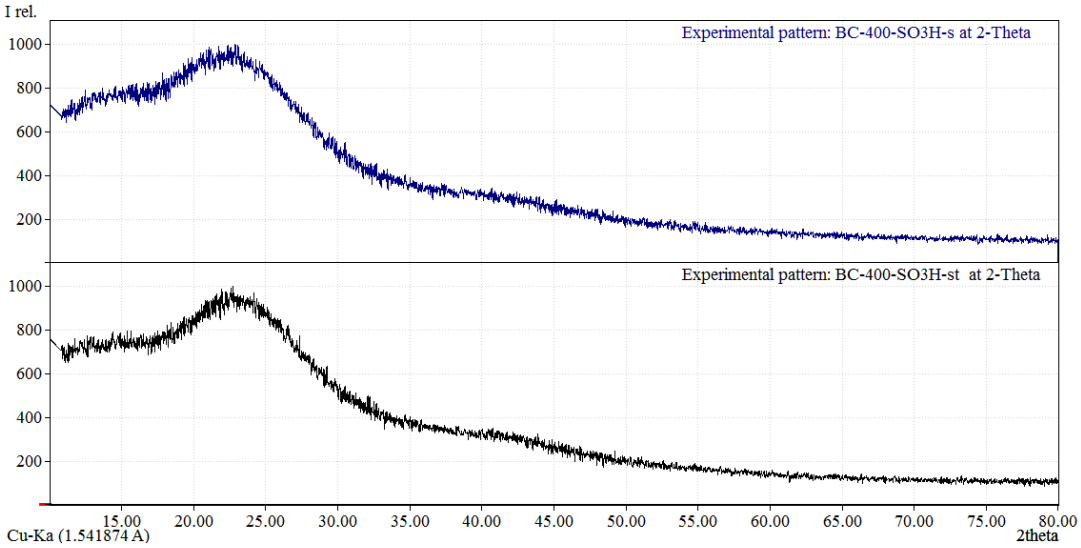


Figure 4.5: XRD patterns of BC-400-SO_3H-s and BC-400-SO_3H-st

Figure 4.6 showed XRD patterns of BC-450-SO_3H-s and BC-450-SO_3H-st. Typical crystal diffraction peaks were not seen, and the width and weak diffraction peak at 2θ=17° - 29° for BC-450-SO_3H-s and 2θ=17° - 29° for BC-450-SO_3H-st, in both patterns implied the structures of both samples were amorphous. The diffraction peak at 2θ=22, and 23 in the pattern of BC-450-SO_3H-s and BC-450-SO_3H-st, respectively assigned to characteristic diffraction peaks of crystalline carbon were still visible, which implied an incomplete carbonization.

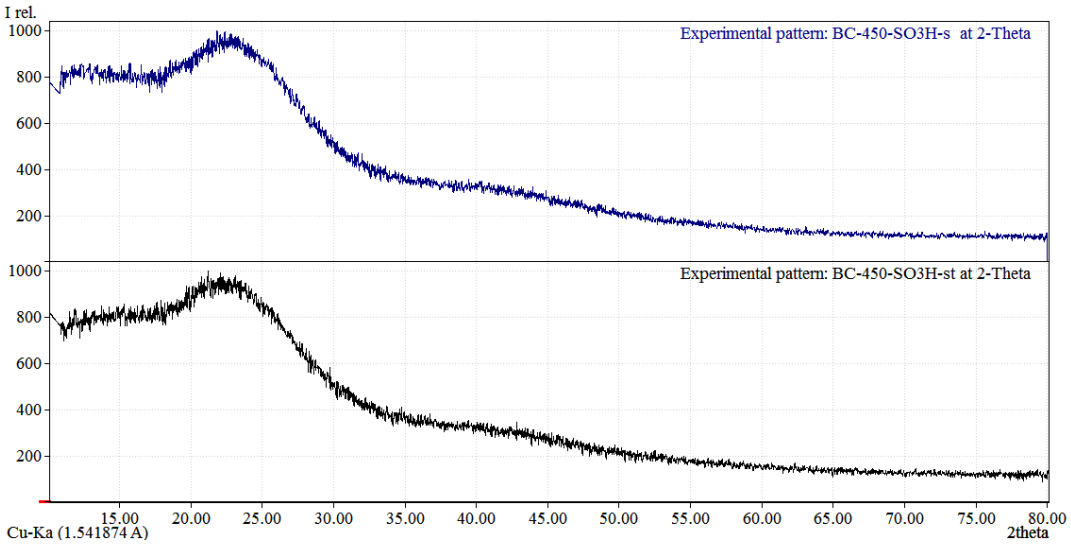


Figure 4.6: XRD patterns of BC-450-SO_3H-s and BC-450-SO_3H-st

Figure 4.7 showed XRD patterns of BC-500-SO_3H and BC-500-SO_3H with and without sonication. Similarly, typical crystal diffraction peaks were not seen, and the width and weak diffraction peak at 2θ=18° - 27° for BC-500-SO_3H-s and 2θ=18° - 27° for BC-500-SO_3H-st, in both patterns implied the structures of both samples were amorphous. The process of carbonization of biomass is sophisticated, especially in the presence of sulfuric acid, and many reactions would occur. The cellulose and hemicellulose chains of branches may dehydrate. Lignin may polymerize or rearrange. Crystal structure decomposed and amorphous hydrocarbon structure was detected. The diffraction peak at 2θ=22, and 23 in the pattern of BC-500-SO_3H-s and BC-500-SO_3H-st, respectively assigned to characteristic diffraction peaks of crystalline carbon were still visible, which implied an incomplete carbonizations similar to BC-400-SO_3H and BC-450-SO_3H catalyst.

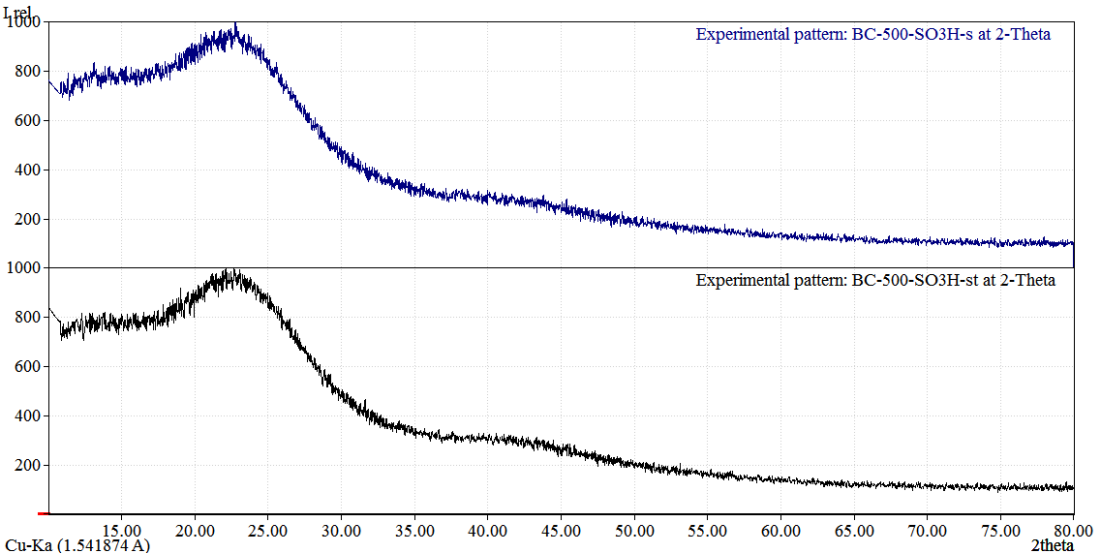


Figure 4.7: XRD patterns of BC-500-SO_3H-s and BC-500-SO_3H-st

4.4.6 Fourier Transform Infrared (FT-IR) Spectroscopy

Samples were measured on Spectrum 65 FT-IR (PerkinElmer) in the range 4000-400cm^{-1} using KBr pellets. Fourier Transform Infrared Spectroscopy (FTIR) is a non-destructive analytical technique used to identify organic and inorganic materials.

FTIR analysis results in absorption spectra which provide information about the chemical bonds and molecular structure of a material and also helps to identify the carbon skeleton structure and groups attached on it.

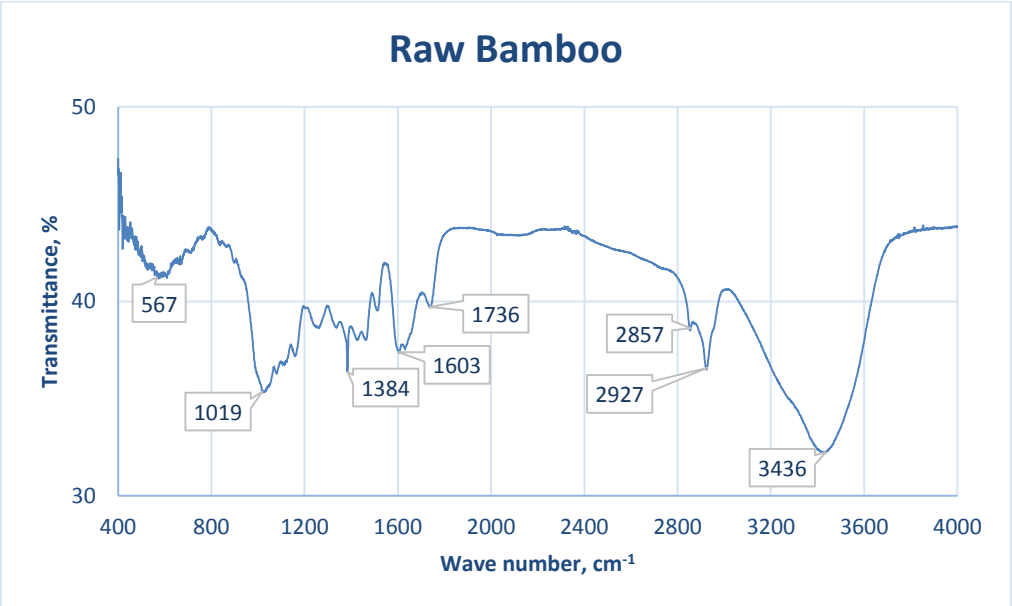


Figure 4.8: FT-IR spectra for raw bamboo sawdust

The FTIR spectra of the bamboo sawdust are shown in Figure 4.8. All of the bands in the bamboo spectrum appeared broader and sharper than the bamboo char, especially those in the absorption bands at 3434cm^{-1}and 2926 cm^{-1}, which are attributed to the stretching vibrational bands of the O–H group and C–H group in–CH_2 and –CH_3, respectively (Liao et al. 2015). In addition, the absorption bands at 1161, 1221, 1030 and 1058 cm^{-1} corresponding to the C–O–C stretching vibrations, considered to be important characteristics of cellulose (Fan et al. 2009; M. Liu et al. 2013), were also more intense in the bamboo spectrum. It has been reported that the hydrolysis effect of solid acid catalysts is mostly on the surface. The hydrogen bonds of bamboo were broken down when the catalysts were in contact with cellulose, which led to more C–H, C–C, C–OH and C–O–C exposed on the surface; as a result the intensity of these peaks increased (Tong et al. 2013).

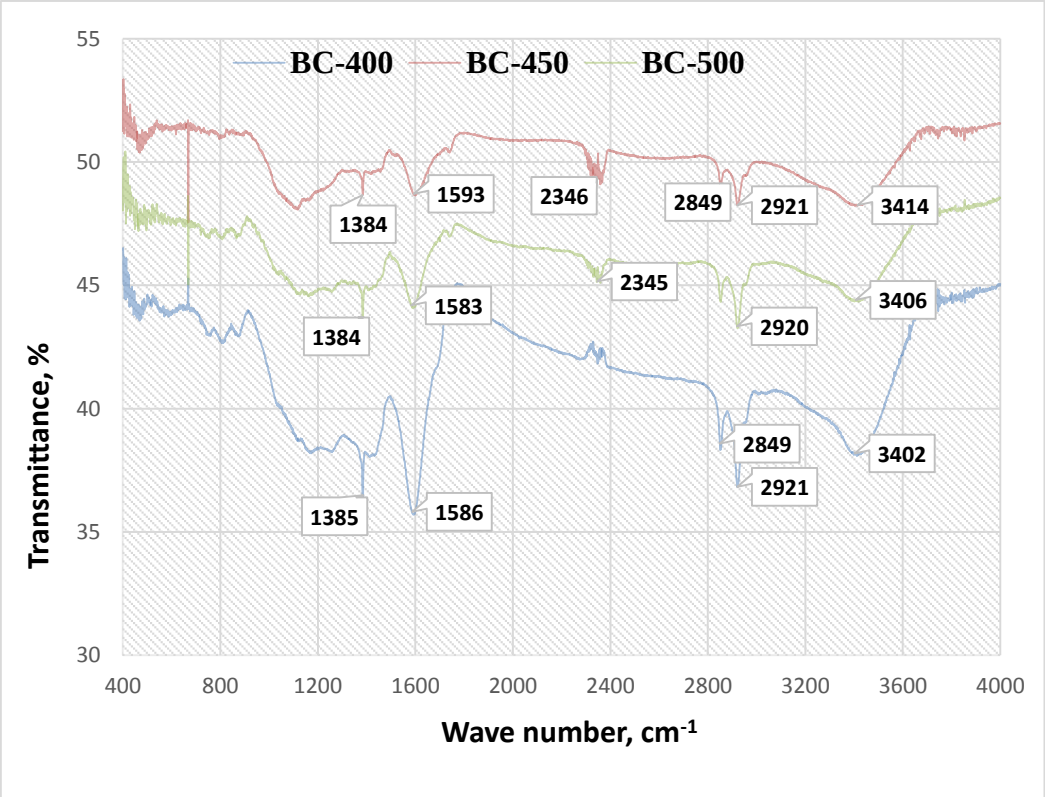


Figure 4.9: FT-IR spectra for biochar carbonized at 400, 450 and 500℃

Figure 4.9 showed the IR spectrum of the char's pyrolysis at 400, 450 and 500ºC before sulfonation. The band 1750 cm^{-1} were C=O stretching vibration peak and 3430 - 2364cm^{-1} belonged to O-H stretching vibration peak. In addition to, phenolic C-OH, C=C, C=O,OH stretching vibration, and carboxylic C-OH bending vibration bands appear at 1191cm^{-1},

1608cm-1, 1750-1788cm^{-1}, 2928cm^{-1}, and 1425cm^{-1}, respectively, demonstrating the presence of COOH and phenolic OH groups on the surface of the catalyst (Qi et al. 2012). These peaks were seen in spectra showed that there were plentiful of oxygen-containing groups such as phenolic hydroxyl, ester, ether, and carboxylic groups in polycyclic aromatic skeleton. And a band at 2924.33 – 2921.51 cm^{-1} is attributable to saturated C-H stretching vibration, showed that the carbonization incomplete (Qi et al. 2012; Tong et al. 2013). This band disappeared after sulfonation indicated cellulose chain continue to convert into polycyclic aromatic hydrocarbon structure during sulfonation.

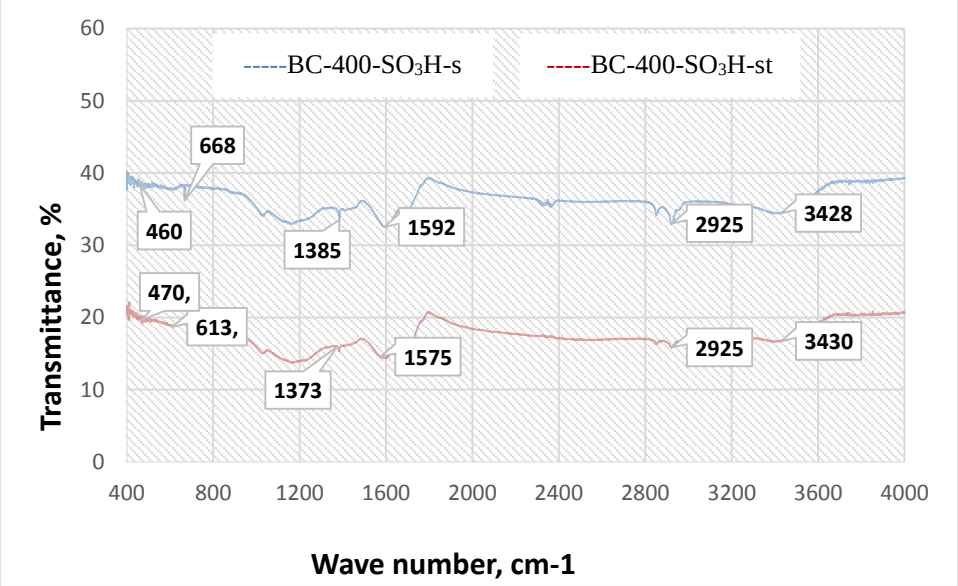


Figure 4.10: FT-IR spectra for BC-400-SO_3H-s and BC-400-SO_3H-st

FTIR spectra of BC-400-SO_3H-s and BC-400-SO_3H-st are shown in Figure 4.10. The band at 3404-3693 cm^{-1} is attributed to -OH stretching vibrations, which indicates that large numbers of hydroxyl groups are present on the as-prepared biochar materials (Qi et al. 2012).The absorption bands at 1711-1795cm^{-1} and 1604 cm^{-1}are attributed to COO and C=C stretching vibrations, respectively(Qi et al. 2012).The vibration bands at 1050 (SO_3–stretching) and 1495 cm^{-1} (O=S=O stretching in SO_3H) indicate that the as-prepared biochar materials have surface-SO_3H groups (Suganuma et al. 2008)and are likely to promote solid-acid catalyzed reactions. Therefore, the prepared sulfonated char materials had an amorphous carbon structure that probably consisted of polycyclic aromatic carbon sheets with randomly attached SO_3H, COOH, and phenolic OH groups.

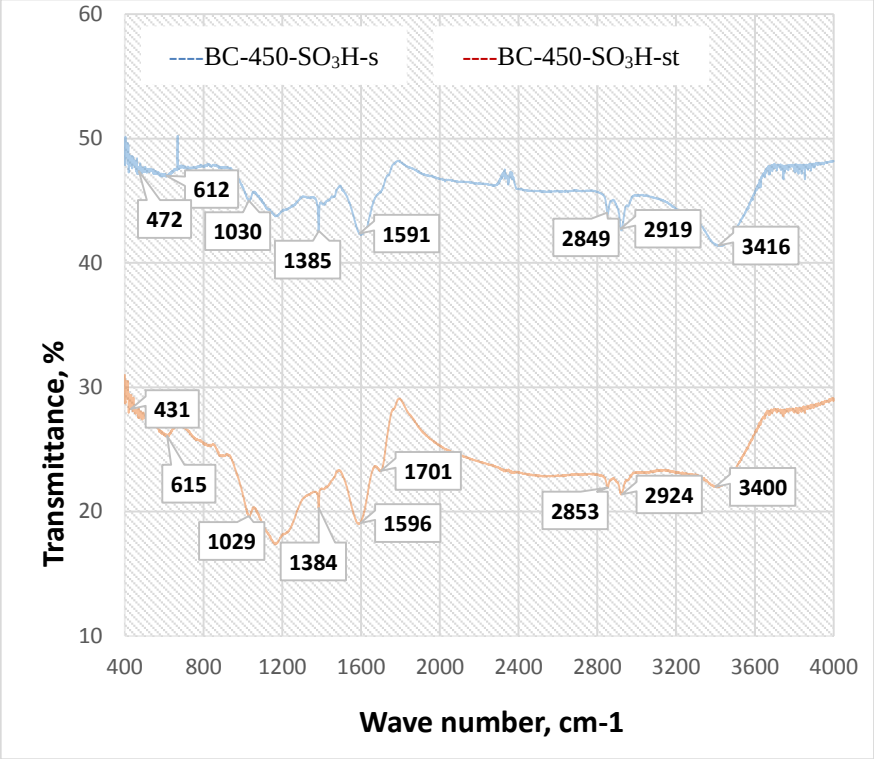


Figure 4.11: FT-IR spectra for BC-450-SO_3H-s and BC-450-SO_3H-st

The FT-IR spectrum of the SO_3H group-bearing biochar material is shown in the Figure 4.11. The absorption bands at 1716 and 1617 cm^{-1}, correspond to COO- and C=C stretching vibrations, respectively (Titirici 2012) and also the peaks at 1696-1800 cm^{-1} and at 1171-1176 cm^{-1} are due to the presence of COOH and C-O functional groups, respectively. The band at 3397 cm^{-1} can be assigned to C–OH stretching vibration and –OH bending, and these imply that large numbers of hydroxyl groups exist on the as-prepared biochar material (X. Liu et al. 2010). Generally, the peaks attributable to aliphatic C-H bond, aromatic C-H bond, O-H bond in a carboxylic acid group, and O-H bond in a phenol group are seen as a broad peak between 3689 $cm^{-1}$and 2348 cm^{-1}. The peaks due to the SO_3H group are seen at 1032-1034 cm^{-1} and at 700-907 cm^{-1}. The vibration bands at 1385cm^{-1} (O=S=O stretching in SO_3H) and 1034 cm^{-1} (SO_3- stretching) are evidence that the resulting material has surface –SO_3H groups (Suganuma et al. 2008) which may have catalytic activity.

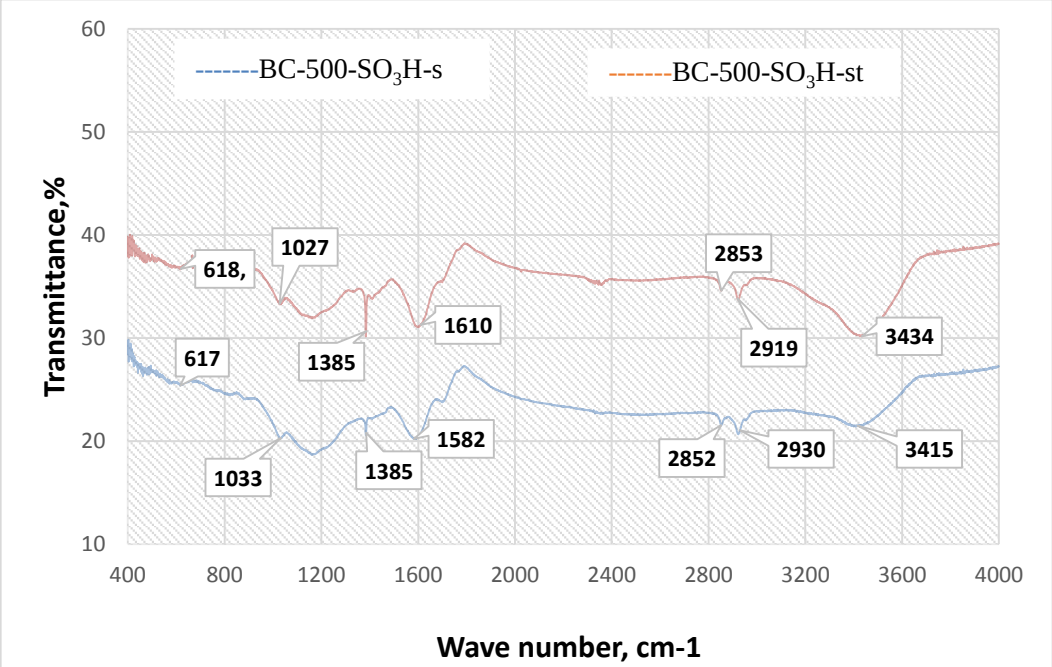


Figure 4.12: FT-IR spectra for BC-500-SO_3Hs- and BC-500-SO3H-st

In spectrum of BC-500-SO_3Hs- and BC-500-SO3H-st, the peaks attributable to aliphatic C-H bond, aromatic C-H bond, O-H bond in a carboxylic acid group, and O-H bond in a phenol group are seen as a broad peak between 3750 cm-1and 2359 $cm^{-1}$and also peaks appeared at 1177-1179 cm^{-1} and 1037 cm^{-1} assigned to S=O double bond stretching vibration, and the characteristic band about 628-668 cm^{-1} was the C-S stretching vibration peak. The vibration bands at 1037 (SO_3- stretching) and 1384 cm^{-1}(O=S=O stretching in SO_3H) in the FTIR spectrum indicate that the resulting material possesses SO_3H groups (Suganuma et al. 2008). As verified section 4.4.1, according to the acid-base titration results, sulfonic acid density of BC-500-SO_3H was also 0.528 $mmol \cdot g^{-1}$ and the total acid density 3.71 $mmol.g^{-1}$. The FT-IR spectrum of BC-500-SO_3H (Figure 4.12) compares with other works, demonstrates bands at 1089cm-1, 1037cm-1, and 1179cm-1, which are consistent with S0$_3$H groups (X. Zhang et al. 2013). It is shown that the carbon-based solid acid have the group of –SO_3H.

4.5 Performance analysis for hydrolysis reaction of microcrystalline cellulose

Based on the acid density, elemental analysis, and FT-IR analysis, the best catalyst (BC-500-SO_3H-s) was choice for suitable hydrolysis reaction. The XRD and FT-IR analysis

were carried out to validate the structure and functional group of microcrystalline cellulose.

The microcrystalline cellulose of vivapur® 102 was white and insoluble in water and not treated by the ball-milling method. The XRD patterns of cellulose before the hydrolyzed reaction is shown in Figure 4.13. The XRD pattern of the raw cellulose showed diffraction peaks of cellulose crystalline around 14.87°, 22.28°, and 34.77°. From the XRD pattern, the crystalline size of cellulose was calculated to be about 62Å (See calculation in Appendix-C). Therefore, to decrease the crystalline peaks of microcrystalline cellulose sonicated the mixture of sulfonated biochar, distilled water and MCC before hydrolyzation reaction performed.

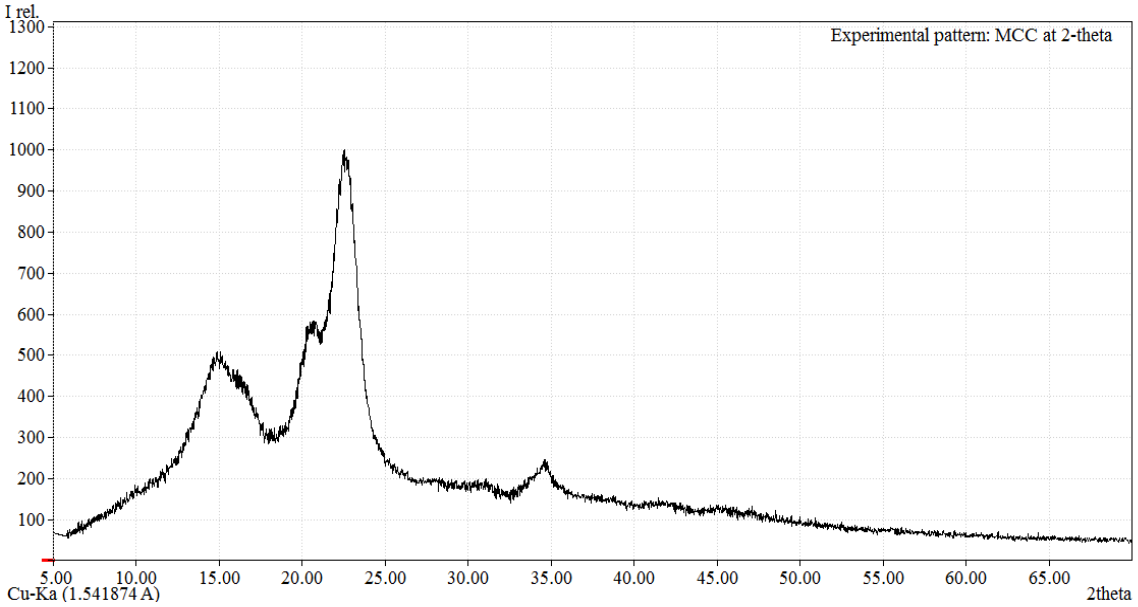


Figure 4.13: XRD patterns of microcrystalline cellulose before hydrolysis

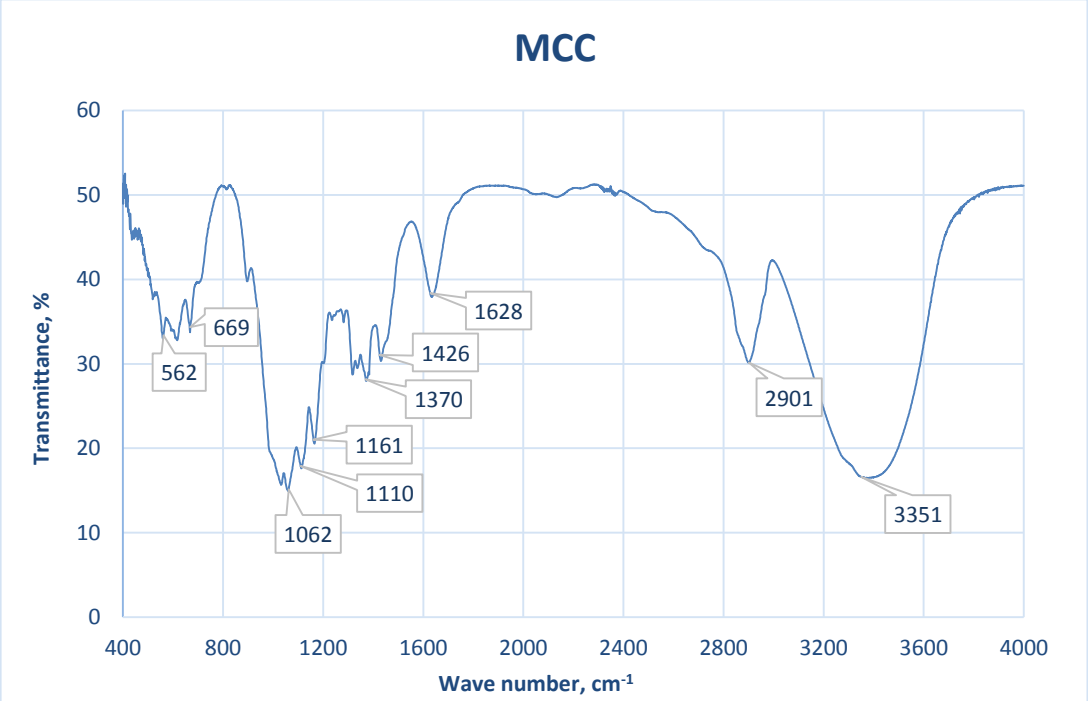


Figure 4.14: FT-IR spectra for microcrystalline cellulose (MCC)

The FTIR spectra of the MCC are shown in Figure 4.14. All of the bands in the MCC spectrum appeared broader and sharper, especially those in the absorption bands at 3386 cm^{-1} and 2905 cm^{-1}, which are attributed to the stretching vibrational bands of the O–H group and C–H group in–CH_2 and –CH_3, respectively (Liao et al. 2015). In addition, the absorption bands at 1166, 1114, 1059 and 1058 cm^{-1} corresponding to the C–O–C stretching vibrations, considered to be important characteristics of cellulose (Liu et al. 2014; Fan et al. 2013), were also more intense in the MCC spectrum. It has been reported that the hydrolysis effect of solid acid catalysts is mostly on the surface. The hydrogen bonds of cellulose were broken down when the catalysts were in contact with cellulose, which led to more C–H, C–C, C–OH and C–O–C exposed on the surface; as a result the intensity of these peaks increased (Tong et al. 2013). Also the intensity of the ‘‘crystallinity band’’ ascribed to the saturated –CH bending vibration at 1433 cm^{-1} increased in MCC, demonstrating a higher degree of crystallinity (Kalita et al. 2013).

The hydrolysis of microcrystalline cellulose was carried out in a stainless-steel autoclave with autogenously pressure. Microcrystalline cellulose, catalyst, and distilled water were charged into the glass reactor lined with Teflon based on(Konwar 2016) with some modification. Before the autoclave reactor was adjusted the mixture of catalyst to

microcrystalline cellulose ratio (1:1, 1.25:1, and 1.5:1) and 10 ml of distilled water was sonicated for 20min to amorphously the microcrystalline cellulose and mixing well. The reaction was proceeded in the autoclave at specified temperature (120, 135, and 150℃) and time (4, 6, and 8 hrs) and after the reaction, the reaction mixture was filtered (containing the catalyst and the unreacted cellulose) and the filtrate solution was analyzed by DNS analysis (see section 3.6.1). An absolute calibration method was employed for calculation of product yields. The conversion of cellulose was determined based on the weight difference of the solid part before and after reaction (Eq.3.14).

Based on calibration curve (in appendix-B of figure B-1), R^2 value of 0.9913 indicates a fit linear correlation. The slope of this graph was used to calculate glucose concentration in unknown samples**.**

$$\boldsymbol{Y = 2.9X + 0.026,\ R2 = 0.9913} \qquad \textbf{Eq. 4.1}$$

Where Y= absorbance at 540nm and X= glucose concentration, mg/ml

Based on Eq.3.14 the conversion of microcrystalline cellulose was shown in appendix-B of table B-2 below. As shown in appendix-B of table B-2 the conversion of MCC was increased as the catalyst to cellulose ratio, reaction temperature, and time increases. From the appendix-B of table B-2, high conversion of MCC, (58%) was attained at catalyst to cellulose ratio (1.25:1), reaction temperature (150℃), and time (8hrs).

The degree of hydrolysis of microcrystalline cellulose by sulfonated biochar catalyst was calculated as relative to the standard curve equation formed. The concentration of unknown samples were calculated based on standard curve equation (Eq.4.1) after absorbance reading. The yield of glucose was determined from the reaction solution of glucose after the reaction. Then after the reaction, the yield of the glucose was determined by Eq.4.2 given below (see appendix-B of table B-3).

$$\text{Yield}_{glu},\ \% = \frac{\boldsymbol{mol\ of\ Desired\ product\ of\ glucose}}{\boldsymbol{mol\ of\ the\ reactant\ (mcc)}} * \mathbf{100} \qquad \textbf{Eq. 4.2}$$

4.5.1 Model fitting and response surface methodology

The regression coefficients of the model for each response are presented in Table 4.8, while results of the analysis of variance (ANOVA) are summarized in Table 4.6. According to particularly high value of coefficient of multiple determinations (R^2) for yield (0.9951) (Table 4.9) model equation provides good representation of experimental values.

Moreover, for the response, mathematical model was statistically acceptable due to significant regression for the model ($p < 0.05$) (Table 4.6). Lack of fit testing confirmed adequacy of fitting experimental data to a second-order polynomial model, where p-value for lack of fit was insignificant ($p > 0.05$) (Table 4.6). Therefore, as suggested by ANOVA it can be concluded that Eq.4.3 would be able to adequately describe behavior of yield of glucose (Myers et al., 2009).

Table 4.6: Analysis of variance (ANOVA) of the fitted second-order polynomial model for yield of glucose.

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	308.42	9	34.27	113.40	< 0.0001	Significant
A-Temperature	*190.12*	*1*	*190.12*	*629.17*	*<0.0001*	
B-Time	*51.57*	*1*	*51.57*	*170.65*	*< 0.0001*	
C-Catalyst to cellulose ratio	*13.72*	*1*	*13.72*	*45.41*	*0.0011*	
AB	*11.08*	*1*	*11.08*	*36.65*	*0.0018*	
AC	*1.90*	*1*	*1.90*	*6.28*	*0.0540*	
BC	*3.76*	*1*	*3.76*	*12.45*	*0.0168*	
A^2	*20.65*	*1*	*20.65*	*68.35*	*0.0004*	
B^2	*10.81*	*1*	*10.81*	*35.77*	*0.0019*	
C^2	*10.10*	*1*	*10.10*	*33.42*	*0.0022*	
Residual	1.51	5	0.30			
Lack of Fit	*0.96*	*3*	*0.32*	*1.17*	*0.4906*	*Not significant*
Pure Error	*0.55*	*2*	*0.27*			
Cor Total	309.93	14				

A, B, C, representing experimental variables: temperature, time, and Catalyst to cellulose ratio, respectively. df- degree of freedom

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, BC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

For more justification of fitting model see Table 4.7. If the coefficient estimate p-values less than 0.01 are very significant and color coded red, p-values less than 0.05 but greater than 0.01 are significant and color coded green, p-values less than 0.10 but greater than 0.05 are marginally significant and color coded blue, and p-values greater than or equal to 0.10 are considered insignificant and color coded black.

The "Lack of Fit F-value" of 1.17 implies the Lack of Fit is not significant relative to the pure error. There is a 49.06% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good because we want the model to fit.

Table 4.7: The estimated coefficients of p-value for yield of glucose

Response	A	B	C	AB	AC	BC	A^2	B^2	C^2
Yield of glucose	4.875	2.539	1.310	1.664	0.689	0.970	-2.365	-1.711	-1.654
p=	<0.0001	<0.0001	0.0011	0.0018	0.0540	0.017	0.0004	0.0019	0.002
Legend	p <0.01, 0.01<= p <0.05, 0.05<= p <0.10, p >=0.10								

Table 4.7 shows that the reaction temperature, reaction time, catalyst to cellulose ratio, interaction between reaction temperature with reaction time, interaction between reaction time with catalyst to cellulose ratio, square of the reaction temperature, square of reaction time, and square of catalyst to cellulose ratio affects the yield of glucose for conversion MCC in hydrolysis reaction significantly.

Table 4.8: Box-Behnken experimental design with predicted and experimentally obtained values of glucose yield.

Response	**1**			**Yield of glucose**		
Run Order	**Temperature, ℃**	**Time, hrs**	**Catalyst to cellulose ratio , (wt/wt)**	**Actual Result**	**Predicted Result**	**Residual**
1	135	8	1.5	57.49	57.05	0.44
2	120	6	1	46.42	46.09	0.33
3	135	4	1.5	49.967	50.04	-0.069
4	135	8	1	52.564	52.49	0.069
5	150	6	1	54.49	54.46	0.033
6	150	4	1.25	52.6	52.20	0.40
7	135	6	1.25	55	55.60	-0.60
8	135	4	1	48.92	49.36	-0.44
9	120	4	1.25	45.876	45.77	0.10
10	120	8	1.25	47.12	47.52	-0.40
11	150	6	1.5	58.12	58.45	-0.33
12	150	8	1.25	60.5	60.60	-0.10
13	135	6	1.25	55.84	55.60	0.24
14	120	6	1.5	47.294	47.33	-0.033
15	135	6	1.25	55.96	55.60	0.36

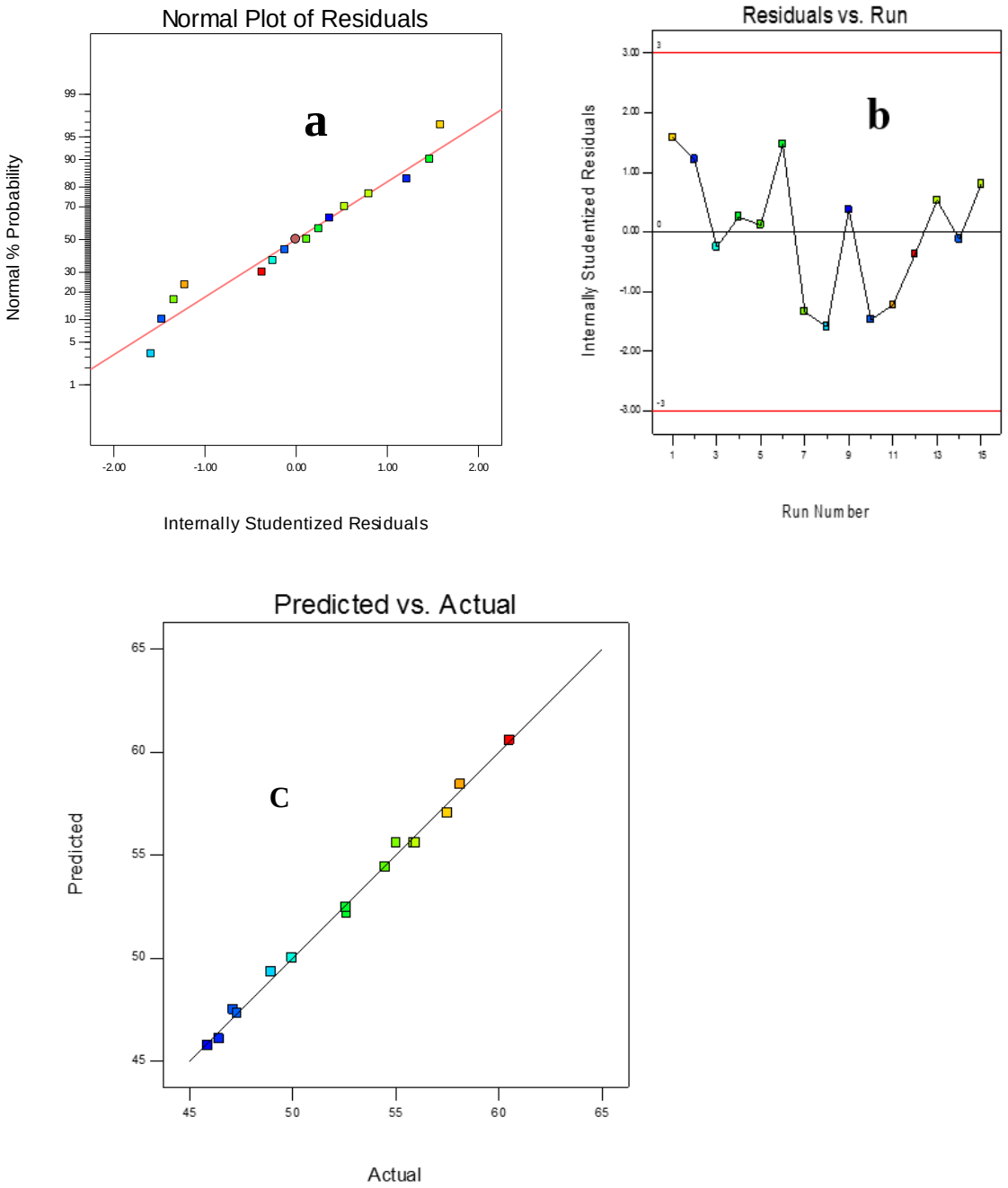


Figure 4.15: Diagnostic plots for the adequacy of proposed model.

4.5.1.1 Development of Regression Model Equation

Experimental values were fitted to a second-order polynomial model (Eq.4.3.) and the model equation that correlates the response (yield glucose) to the hydrolysis reaction process variables in terms of actual value after excluding the insignificant terms was given

below. The predicted model for percentage of yield of glucose in terms of the actual factors is given in Eq.4.3.

Yield of glucose = -160.28037 +2.60068*A -3.51031*B +34.95250*C +0.055467*A*B +0.18373* A*C +1.93950*B *C -0.010512*A^2 -0.42772 *B^2 -26.46200 *C^2 **Eq.4.3**

Where A= Temperature, B= Time, C= Catalyst to cellulose ratio

4.5.1.2 Model Adequacy Check

The model was tested for adequacy by analysis of variance (ANOVA). The regression model was found to be highly significant with the correlation coefficients of determination of R-Squared, adjusted R-Squared and predicted R-Squared (Table 4.9) having a value of 0.9951, 0.9863, and 0.9463 respectively. The Predicted R-Squared of 0.9463 is in reasonable agreement with the Adjusted R-Squared of 0.9863; i.e. the difference is less than 0.2, then the model is fitting the data and can reliably be used to interpolate. Adequacy Precision measures the signal to noise ratio. A ratio greater than 4 is desirable i.e. the model has a strong enough signal to be used for optimization. Your ratio of 33.036 indicates an adequate signal. This model can be used to navigate the design space. The adequacy of the model was further checked with analysis of variance (ANOVA) as shown in Table 4.6. P-value is calculated by model mean square divided by residual mean square.

Table 4.9: Sequential model fitting for glucose yield.

Source	**R^2**	**Adjusted-R^2**	**Predicted-R^2**	**PRESS**	
Linear	0.8241	0.7761	0.7149	88.37	
2FI	0.8781	0.7867	0.7358	81.88	
Quadratic	0.9951	0.9863	0.9463	16.65	Suggested
Cubic	0.9982	0.9876		+	Aliased

The quality of the model developed could be evaluated from their coefficients of correlation. The value of R-squared for the developed correlation is 0.9951. It implies that 99.95% of the total variation in the percentage of yield is attributed to the experimental variables studied.

4.5.2 Effect of process variables on the yield of glucose

One of the most important quality parameters in the hydrolysis reaction are temperature, catalyst activity and reaction medium. In this study, effects of each process variables and their interaction effects on the target response glucose yield, was determined. From acquired experimental data and developed model the three dimensional response surface

and single factor plots were constructed to illustrate the main and interactive effect of independent variables on the response and shown in Figure 4.16 and Figure 4.17.

4.5.2.1 The main Effect of Process Variables for hydrolysis reaction

The hydrolysis of cellulose catalyzed by the prepared carbon material was examined for different reaction temperatures, catalyst to cellulose ratio and reaction times as shown (Figure 4.16). Figure 4.16a shows that the effect of different reaction temperatures on the hydrolysis of cellulose, yield of glucose increased almost linearly with increasing temperature. The yield of reducing sugar (glucose) increased remarkably with increasing reaction temperature from 120 to 150 ℃ at a given reaction time before it reached a maximum value. When the hydrolysis was conducted at optimum condition of 150 °C, glucose yield of 60.5% was obtained at 8hr. From the results, it can be seen that temperature has a significant effect on the cellulose hydrolysis. The influence of different reaction times on the yield of glucose was shown in Figure 4.16b. The yield of hydrolysis of microcrystalline cellulose showed a trend of gradual increased as the time extended to 8.0 hr. Because the sulfonated biochar catalyst of hydrolysis of microcrystalline cellulose to glucose would got enough time to hydrolysis the cellulose, the yield of reducing sugar increased gradually with prolonged reaction time. From the results, it can be seen that time has a significant effect on the cellulose hydrolysis. Figure 4.16c shows the effect of catalyst dosage on the reduced sugar yield from hydrolysis of cellulose. As the catalyst increases, the reducing sugar (glucose) yield remarkably increased for a reaction time. However, further increases in catalyst dosage resulted in a decrease in reducing sugar yield and this is probably due to the accelerated degradation of reducing sugars caused by excess active sites in the system. Moreover, in all cases, reducing sugar (glucose) yield increased rapidly in the initial stages of the reaction, but then decreased with prolonged reaction time that seemed to be related to the degradation of the formed reducing sugars. From the results, it can be seen that catalyst to cellulose ratio has a significant effect on the cellulose hydrolysis.

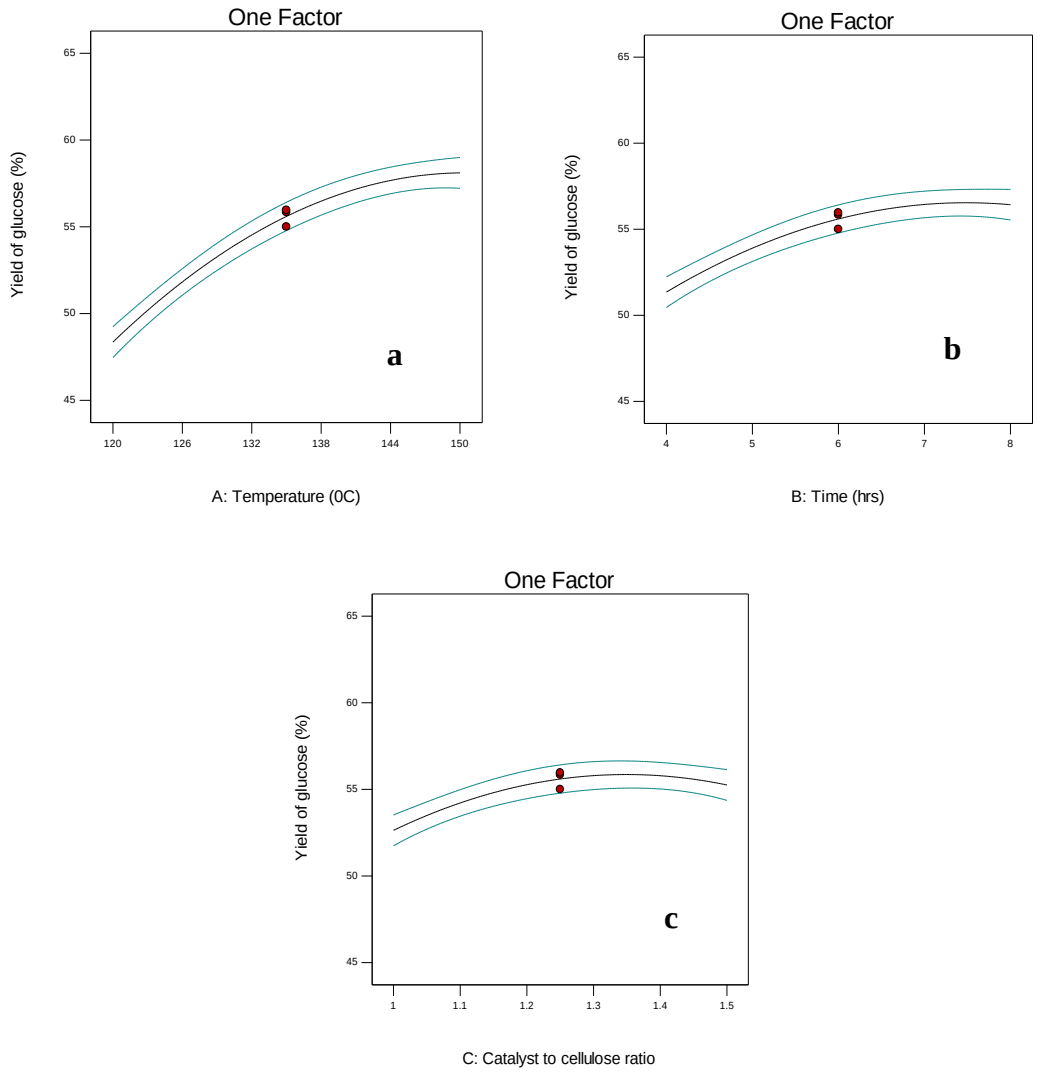


Figure 4.16: Glucose yield versus temperature (A), time (B), and catalyst to cellulose ratio (C)

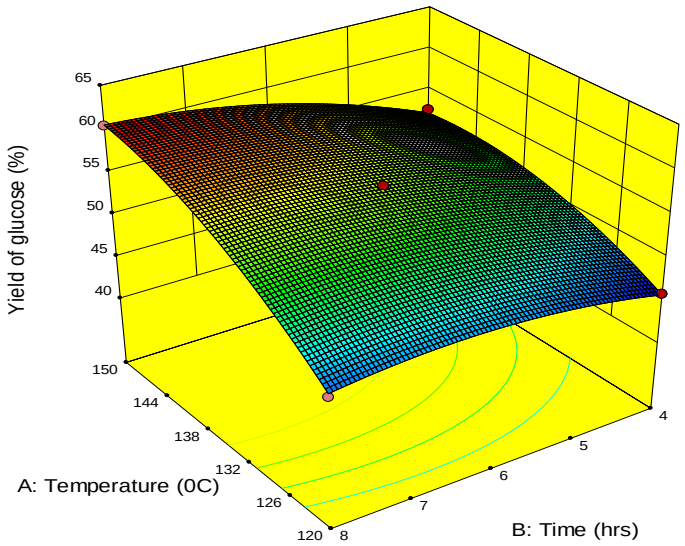
4.5.2.2 The Interaction Effect of Process Variables for hydrolysis reaction

The most common way to summarize the results of a BBD experiment is in the form the three dimensional response surface or contours plot. The process variables were found to have significant interaction effects but less interaction effect of reaction temperature with catalyst to cellulose ratio. Figure 4.17 shows the interaction effect of reaction temperatures with reaction time, the interaction effect of reaction time with catalyst to cellulose ratio on yield of glucose, respectively.

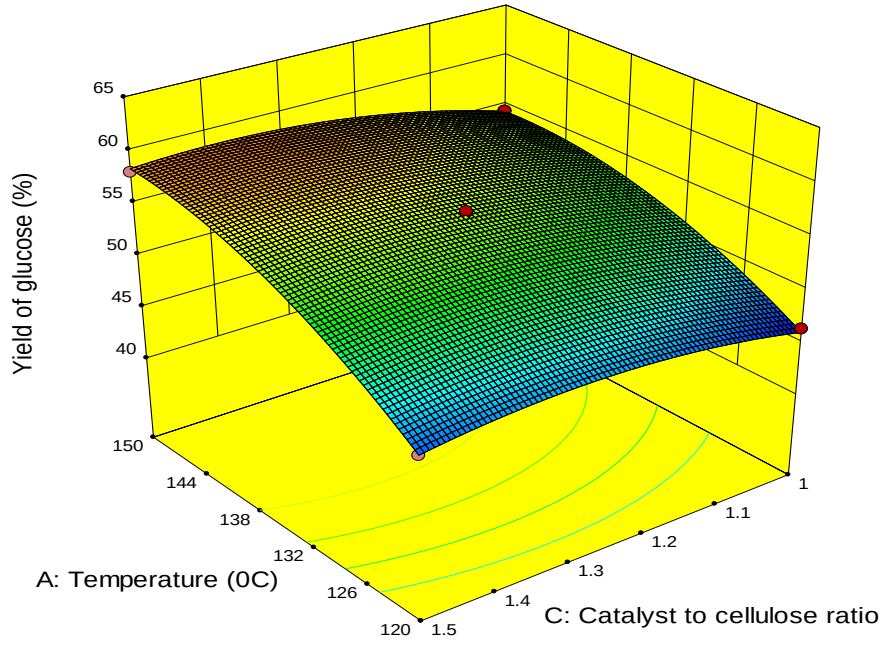
Figure 4.17 (A) shows as the effect of reaction temperature to reaction time on yield of glucose. As shown the figure both temperature and time have increased up to optimum to

increase the yield of glucose. Lower reaction temperatures could not depolymerize cellulose efficiently and thereby reduces the yield of glucose. And on the other hand higher reaction temperatures increases the depolymerize cellulose that can be hydrolyzed and increases the yield of glucose, but if reaction temperatures increases beyond optimum value it will degrade the glucose into other chemicals.

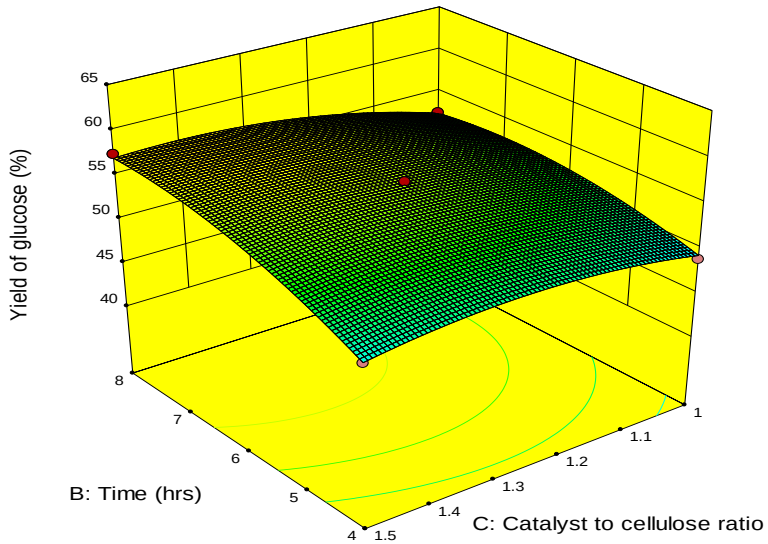
Figure 4.17 (B) shows as the interaction effect of reaction time with catalyst to cellulose ratio on yield of glucose. Similarly, as lower reaction time could not depolymerize cellulose efficiently because the catalyst need enough time to involve on the hydrolysis reaction and thereby reduces the yield of glucose. But, higher reaction time increases the activity of catalyst to depolymerize cellulose that can be hydrolyzed. Thus, increases the yield of glucose.



A



B



C

Figure 4.17: Three dimensional response surface plots representing interaction effects: (A) temperature with time (B) temperature with catalyst to cellulose ratio, and (C) time with catalyst to cellulose ratio**.**

Generally, sulfonated char had acidic surface functional groups, such as phenolic carboxylic and $–SO_3H$ groups. The hydrothermal hydrolysis of cellulose was carried out at 423 K. The blank reaction without catalysts resulted in few production of glucose. The biochar catalyst had a significant catalytic activity for the cellulose hydrolysis into glucose.

4.5.3 Diagnostics of model adequacy

The adequacy of the model was checked by constructing different diagnostic plots show in Figure 4.15. The normal % probability plot of residuals for response was normally distributed, as they lie reasonably close to the straight line and shows no deviation of the variance (Figure 4.15 a). Internally studentized residuals plot was constructed to facilitate the satisfactory fit of the developed model and the plot (Figure 4.15b) shows that, all the data points lie within the limits (±3). The predicted values obtained from the developed model were quite close to the experimental values and lie reasonably close to the straight line and indicated the adequate agreement with real data (Figure 4.15c).

4.5.4 Optimal process condition for the hydrolysis reaction

After checking the design evaluation, ANOVA statistics, and diagnostics graphs, i.e. tools for making sure the models provide a good estimate of the true response surface. The results above have shown that the three hydrolysis process variables (the reaction temperature, reaction time, catalyst to cellulose ratio) and the two interaction among the variables affect the yield of glucose. Therefore, the next step is to optimize the process variables (interaction between reaction temperature and reaction time, interaction between reaction time and catalyst to cellulose ratio)in order to obtain the highest yield of glucose using the model regression developed (Eq.4.3). This function searches for combination of independent parameter levels that simultaneously satisfy the requirement for response in the design (Yamaguchi et al. 2009). So in order to obtain the maximum yield of glucose, the predicted combination of parameters was as follows: temperature of 149.787^oC, reaction time of 8hr and catalyst to cellulose ratio of 1.469:1. Under these conditions, the model predicted of 61.898% with a desirability value of 0.999(Figure 4.18). To validate the optimum conditions predicted by the model using desirability ramp, triplicate experiments were conducted using the optimized hydrolysis process conditions and mean yield of glucose value of 60.68% was obtained. The results are closely related with the data obtained from optimization analysis using desirability functions, indicating BBD incorporate with desirability function could be effectively used to optimize the parameters

that affect yield of glucose. Thus, this research shows that MCC can definitely be used for synthesis of glucose and optimum yield of glucose can be obtained from the hydrolysis of MCC using sulfonated biochar catalyst and also at optimum yield of glucose, high conversion of MCC was achieved.

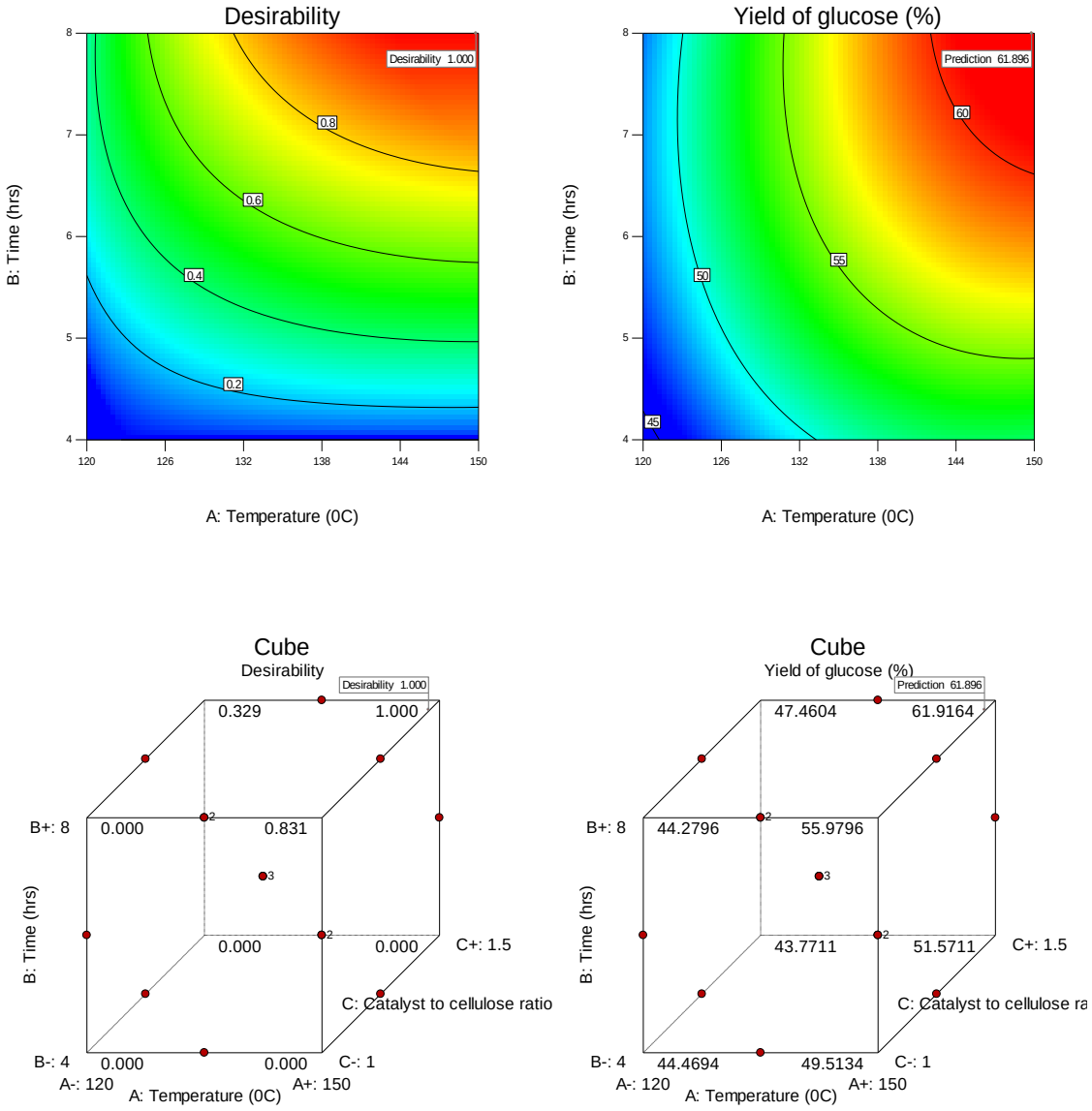


Figure 4.18: The desirability and the optimum yield of glucose at optimum process conditions of cubic and contour plots.

Based on the above optimized value of glucose yield, other typical hydrolysis of cellulose with H_2O over solid acid catalyst work was compared with this work as given in Table 4.10.

Table 4.10: Typical hydrolysis of cellulose with H_2O over solid acid catalyst

Catalyst	Feedstock	Reaction condition	Typical Products	Yield	Ref.
AC-SO3H	Cellulose	423k, 24hrs	Glucose	40.5%[c]	(Onda et al. 2008)
AC-SO3H+COOH+OH	Cellulose	373k, 3hrs	Glucose	4.0%[a]	(Suganuma et al. 2008)
AC-SO3H	Cellulose	423k, 24hrs	Glucose	61.0%[c]	(Pang et al. 2010)
BC-SO3H	Cellulose	373k, 1hrs MW(350W)	Glucose	19.8%[c]	(Wu et al. 2010)
BC-SO3H+COOH+OH	MCC	423k, 8hrs	Glucose	60.68%[b]	This work

[a]Catalyst: 0.300 g; cellulose:0.025 g; distilled water: 0.7 g. [b]catalyst: 0.125 g; cellulose: 0.1 g; distilled water: 10g. [c]catalyst: 0.300 g; cellulose: 0.25 g; distilled water: 2.25 g.

Thus, from the comparison reasonable value of glucose yield was obtained using modified amorphous sulfonated biochar catalyst for the depolymerization of microcrystalline cellulose by hydrolysis (Table 4.10).

CHAPTER 5 CONCLUSIONS AND RECCOMENDATIONS

5.1 CONCLUSIONS

A promising heterogeneous catalyst for depolmerization of cellulose has been prepared through sulfonation of biochar, i.e., a by-product of pyrolysis of woody biomass, with 96% sulphuric acid. Applying a stronger sulfonation procedure by using stronger sulfonation reagent (concentrated sulphuric acid) and higher mass ratio of sulphuric acid (10:1) to biochar resulted in higher hydrolysis activity. The high catalytic activity for the reaction can be attributed to the ability to adsorb β-1, 4 glucan, the large effective surface area in water, and SO_3H groups tolerable to hydration in the carbon material.

The main objective of this research was to synthesize high surface area, porous, and high acidity carbon-based catalysts that have high activities on hydrolysis reactions. All synthesized catalysts were physically and chemically characterized using bulk density, acid density, elemental analysis, FT-IR, XRD, SEM, and TGA. Structural study through Elemental Analysis and FT-IR spectroscopy suggests that biochar-based catalyst consists of polycyclic aromatic carbon sheets bearing three different acidic groups of phenolic, carboxylic, and sulfonic, and the catalyst is stable up to temperature of 250^{o}C.

The first major contribution was the synthesis of porous and high acid density carbon based catalyst using a pyrolysis method. Bamboo was used as a carbon precursor to produce the catalyst support, while pyrolysis at different temperatures 400, 450 and 500^{o}C produced an incomplete carbonized of catalyst support. Sulfonation of the bamboo char in a high concentrated sulfuric acid with or without sonication produced six different catalysts. In general, as pyrolysis temperature increases the total acid density of the catalyst also increases because total pore volume increased with increasing activation temperature, it was expected that the total acid density of the catalyst samples would increase, as the increase in the pore size would allow the sulfonic groups to be more easily incorporated onto the carbon matrix. Sulfonation with sonication produces a catalyst with a high acid density and sulfur content than the catalyst with stirring or without sonication. Based on the acid density and elemental analysis of the prepared catalyst, catalyst prepared at 500^{o}C with sonication was selected for testing the activity of the catalyst. The biochar-based (bearing-SO_3H) catalysts supported on pyrolysis bio-chars at temperature 500℃(i.e., BC-500-SO_3H with sonication) were tested for hydrolysis reaction as one of the major factor

that affects the reaction. The other reaction parameters affecting the yield of glucose were reaction temperature and reaction time to hydrolysis of cellulose has been studied. The outputs of the experiment conducted have been analyzed by employing Design-Expert 10.0.0, three-level with three-factor BBD, in which all samples were analyzed on absorbance value of the product using DNS method and the optimum value obtained from glucose yield of each samples. Based on the analysis of experimental results, it is found that all the process variables exhibited significant interaction effect on the yield of glucose. This shows that the capability of the design of experimental analysis in successfully capturing these effects. A reaction temperature of 149^{o}C, reaction time of 8hrs and catalyst to cellulose ratio of 1.469:1 results an optimal value of 61.898% yield of glucose.

5.2 RECCOMENDATIONS FOR FUTURE WORKS

For more research works on the development of this catalyst, using stronger functionalizing reagents such as fuming acids with higher percentage of free SO_3 or super acids is suggested. Moreover, further investigations on the effect of pyrolysis process conditions and feedstock on the catalytic activity of biochar would be useful.

According to the results of hydrolysis reaction under high temperature conditions, catalyst deactivation might be a possible reason for the reduction in the amount of catalytic activity, thus, investigations on this factor should be another important aspects of catalyst development.

Studying the kinetics of hydrolysis reaction and also finding the optimal temperature which favours all the consecutive reactions of hydrolysis would be another interesting aspect of catalyst development. All aforementioned biochar-based catalysts have been compared for depolymerization of cellulose activity.

Solid acids have many advantages over conventional liquid acids, in separation, recycling and being environmental benign. However, the mass transfer limitations between the insoluble polymers and heterogeneous catalysts are bottlenecks for the hydrolysis system. Conceptually, solid acids having higher specific surface areas, pore sizes/volume and strong acid sites are prone to exhibit better performances in the reaction. Further investigations on the biochar-based catalyst have been conducted for the effect of sulfonation time (5 and 15 hours) and surface area development on the hydrolysis activity.

Further study on improvement of the depolymerazation process parameters of effect of water addition on reducing sugar yield, the recycle of the carbon catalyst was tried to study its activity and stability and the effect of rpm on the yield of glucose is also suggested.

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APPENDICES

APPENDIX A Proximate analysis calculation

1. Volatility matter (bamboo)

Sample I.

A: Mass of crucible, cover, and sample, g: 22.7585g

B: Mass of crucible and cover, g: 21.7577g

C: mass of crucible, cover, and de-volatilized sample, g: 21.9443g

D: volatile matter content of as-received sample

Weight loss, % = (A-C)/ (A-B)

= (22.7585-21.9443)/ (22.7585-21.7577)

= 0.814*100%= 81.4%

Sample II.

A: Mass of crucible, cover, and sample, g: 24.3258g

B: Mass of crucible and cover, g: 23.3256g

C: mass of crucible, cover, and de-volatilized sample, g: 23.5102 g

D: volatile matter content of as-received sample

Weight loss, % = (A-C)/ (A-B)

= (24.3258-23.5102) / (24.3258-23.3256)

= 0.815*100% = 81.5 %

Sample III.

A: Mass of crucible, cover, and sample, g: 22.6135 g

B: Mass of crucible and cover, g: 21.6124 g

C: mass of crucible, cover, and de-volatilized sample, g: 21.8122g

D: volatile matter content of as-received sample

Weight loss, % = (A-C)/ (A-B)

= (22.6135 -21.8122) / (22.6135-21.6124)

= 0.800*100% = 80 %

2. Moisture content of bamboo dust (0.5-1.4mm)

Sample I

A. Wt. of empty crucible : 36.7273g

B. Wt. of bamboo dust : 1.0000g
C. Wt. of bamboo dust and crucible after drying : 37.6242g

Moisture content, % = 100- [(C-A)/B]*100%

= 100% - [(37.6242-36.7273)/1.0000]*100%= 10.31%

Sample II

D. Wt. of empty crucible : 35.3544g
E. Wt. of bamboo dust : 1.0005g
F. Wt. of bamboo dust and crucible after drying :36.25 30g

Moisture content, % = 100- [(C-A)/B]*100%

= 100% - [(36.25 30-35.3544)/1.0000]*100%= 10.18%

Sample III

A. Wt. of empty crucible : 34.8694g
B. Wt. of bamboo dust : 1.0009g
C. Wt. of bamboo dust and crucible after drying :35.7761g

Moisture content, % = 100- [(C-A)/B]*100%

= 100% - [(35.7761-34.8694)/1.0009]*100%= 90.6%

3. Ash content of Bamboo

Sample 1

A. Mass of crucible before burning: 17.0090g
B. Mass of sample : 1.0007g
C. Mass of sample + Crucible after burning: 17.0246

Ash content amount = [((C-A)/B)*100%]

= [((17.0246-17.0090)/1.0007)*100%= 1.56%

Sample 2

D. Mass of crucible before burning: 17.4094g
E. Mass of sample : 1.0008g
F. Mass of sample + Crucible after burning: 17.4245

Ash content amount = [((C-A)/B)*100%]

= [((17.4245-17.4094)/1.0008)*100%]= 1.51%

Sample 3

G. Mass of crucible before burning: 16.8616g
H. Mass of sample : 1.0010g
I. Mass of sample + Crucible after burning: 16.8789

Ash content amount = [((C-A)/B)*100%]

= [((16.8789-16.8616)/1.0010)*100%]= 1.73%

APPENDIX B Experimental and box- Behnken design tables and figures

Table B-1: Absorbency readings were taken at 540nm of 5 samples and the blank with known glucose concentration.

Cuvette no.	Concentration of glucose, mg/ml	Absorbance @540nm, O.D
1	0.2	0.500
2	0.4	1.278
3	0.6	1.878
4	0.8	2.400
5	1.0	2.800

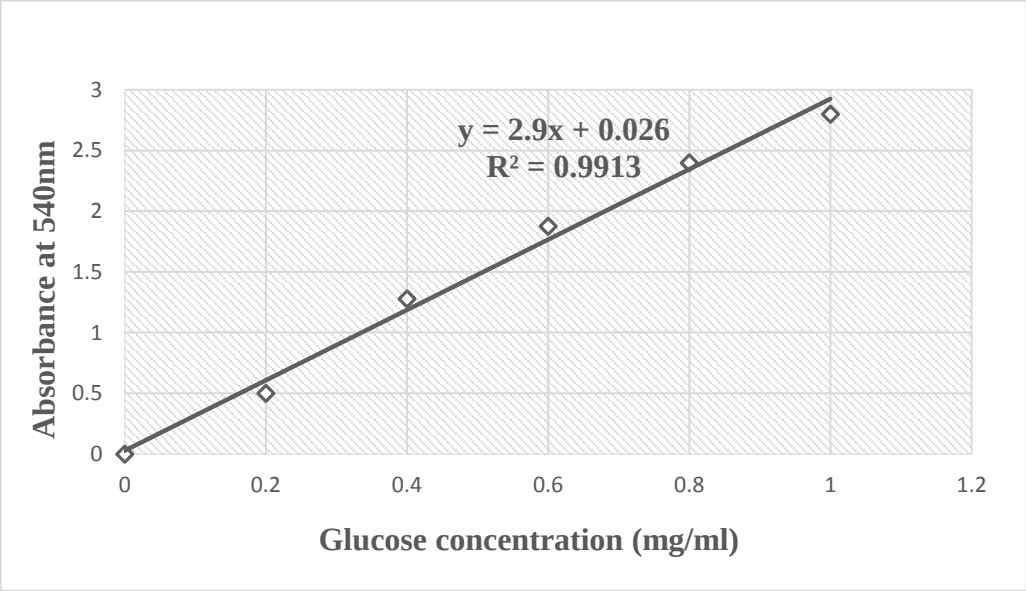

Figure B-1: Standard curve of Concentration of Standard Glucose versus Absorbency at 540 nm wavelength

Table B-2: the conversion of microcrystalline cellulose in the hydrolysis reaction

		Factor 1	Factor 2	Factor 3		
Std	Run	A:Temperature	B:Time	C:Catalyst to cellulose ratio	Mass sample after hydrolysed	Conversion of MCC
		℃	hrs		gram	%
1	1	120	4	1.25:1	0.0650	35.0
2	3	150	4	1.25:1	0.0450	55.0
3	4	120	8	1.25:1	0.0569	43.1
4	8	150	8	1.25:1	0.0420	58.0
5	7	120	6	1.00:1	0.0664	33.6
6	6	150	6	1.00:1	0.0578	42.2
7	15	120	6	1.50:1	0.0611	38.9
8	9	150	6	1.50:1	0.0440	56.0
9	11	135	4	1.00:1	0.0589	41.1
10	14	135	8	1.00:1	0.05510	44.9
11	13	135	4	1.50:1	0.0531	46.9
12	5	135	8	1.50:1	0.0460	54.0
13	12	135	6	1.25:1	0.0515	48.5
14	2	135	6	1.25:1	0.0510	49.0
15	10	135	6	1.25:1	0.0500	50.0

Table B-3: The yield of the glucose in the hydrolysis of microcrystalline cellulose

		Factor 1	Factor 2	Factor 3		Response 1
Std	Run	A:Temperature	B:Time	C:Catalyst to cellulose ratio	Absorbance at 540nm	Yield of glucose
		0C	hrs	unit	O.D	%
1	1	120	4	1.25	0.487	45.88
2	3	150	4	1.25	2.548	52.60
3	4	120	8	1.25	0.776	47.12
4	8	150	8	1.25	2.572	60.50
5	7	120	6	1	0.598	46.42
6	6	150	6	1	0.625	54.49
7	15	120	6	1.5	0.503	47.294
8	9	150	6	1.5	2.558	58.12
9	11	135	4	1	0.635	48.92
10	14	135	8	1	1.244	52.564
11	13	135	4	1.5	1.663	49.967
12	5	135	8	1.5	2.357	57.49
13	12	135	6	1.25	1.725	55
14	2	135	6	1.25	1.728	55.84
15	10	135	6	1.25	1.748	55.96

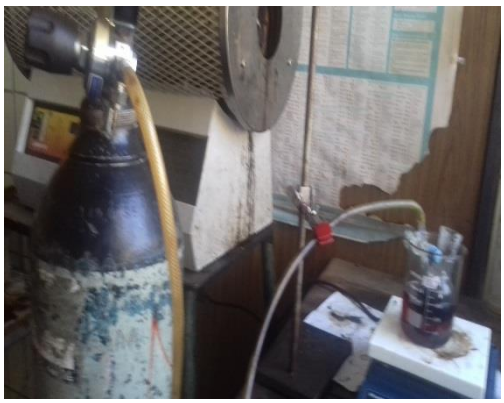

Figure B-2: Equipment used during preparation of catalyst

Figure B-3: Equipment used during characterization of the catalysts

Figure B-4: Experimental setup for hydrolysis reaction

APPENDIX C Crystallite size analysis

Crystallite size analysis using **Scherrer's formula**:

$$Crystallite\ size\ (average\ in\ \text{Å}) = K\,\lambda\,/\,(FWHM * cos\,\theta)$$

Where

K is the so-called "Scherrer constant" (typically =0.94 for FWHM of spherical crystals with cubic symmetry),

λ is the wavelength of the radiation and

θ is the diffraction angle of the peak.

The formula is based on the FWHM values (FWHM = Full Width at Half Maximum) of the peaks. The data where read from XRD match!3 graph of the MCC.

$$\lambda = 1.51874\text{Å}\ at\ Cu - Ka$$

2θ=22.28° at maximum peak height of 1000

FWHM= 0.16

$$Crystallite\ size\ \left(average\ in\ \text{Å}\right) = \ 0.94 * \frac{1.51874}{(0.16\ *\ cos\ 11.14)} = 62\text{Å}$$

APPENDIX D Simplified Infrared Correlation Chart

	Type of Vibration	Frequency (cm^{-1})	Intensity
C-H	Alkanes (stretch)	3000-2850	Strong
	$-CH_3$ (bend)	1450 and 1375	medium
	$-CH_2-$ (bend)	1465	medium
	Alkenes (stretch)	3100-3000	medium
	(out-of-plane bend)	1000-650	Strong
	Aromatics (stretch)	3150-3050	Strong
	(out-of-plane bend)	900-690	Strong
	Alkyne (stretch)	~3300	Strong
	Aldehyde	2900-2800	weak
		2800-2700	weak
C-C	Alkane not interpretatively useful		
C=C	Alkene	1680-1600	medium
	Aromatic	1600 and 1475	Weak
C≡C	Alkyne	2250-2100	medium
C=O	Aldehyde	1740-1720	Strong
	Ketone	1725-1705	Strong
	Carboxylic Acid	1725-1700	Strong
	Ester	1750-1730	Strong
	Amide	1670-1640	Strong
	Anhydride	1810 and 1760	Strong
	Acid Chloride	1800	Strong
C-O	Alcohols, Ethers, Esters, Carboxylic Acids, Anhydrides	1300-1000	Strong
O-H	Alcohols, Phenols		
	Free	3650-3600	Medium
	H-bonded	3500-3200	Medium
	Carboxylic Acids	3400-2400	Medium
N-H	Primary and Secondary Amines and Amides		
	(stretch)	3500-3100	Medium
	(bend)	1640-1550	Strong
C-N	Amines	1350-1000	Medium
C=N	Imines and Oximes	1690-1640	Weak
C≡N	Nitriles	2260-2240	Medium
X=C=Y	Allenes, Ketenes, Isocyanates, Isothiocyanates	2270-1950	Strong
N=O	Nitro ($R-NO_2$)	1550 and 1350	Strong
S-H	Mercaptans	2550	Weak
S=O	Sulfoxides	1050	Strong
	Sulfones, Sulfonyl Chlorides, Sulfates, Sulfonamides	1375-1300 and 1200-1140	Strong

(Source: http://en.wikipedia.org/wiki/Infrared_spectroscopy_correlation_table**)**

APPENDIX E Bulk Density of Bio-chars and catalyst

Sample calculation for the determination of bulk density of the catalyst and bio-char

1. **Bulk density of bio-chars prepared at 400ºC**
   A. Mass of bio-char: 0.7834gm
   B. Volume of bio-chars: 2.733ml

$$Bulk\ density\ = \frac{weight\ of\ the\ samples\ (g)}{Volume\ of\ the\ sample\ (ml)} = \frac{A}{B} = \frac{0.7834}{2.733} = 0.2867 gm/ml$$

2. **Bulk density of catalyst prepared at 400ºC**

A. Mass of bio-char: 1.4145gm

B. Volume of bio-chars: 3.6ml

$$Bulk\ density\ = \frac{weight\ of\ the\ samples\ (g)}{Volume\ of\ the\ sample\ (ml)} = \frac{A}{B} = \frac{1.4145}{3.6} = 0.363 gm/ml.$$

Similarly for BC-450, BC-500, BC-450-SO_3H and BC-450-SO_3H. The measurement was repeated three times, with the average reported as the bulk density.

APPENDIX F Determination of Acid density of the catalyst

I. Determination of total acid density for BC-400-SO_3H

Sample calculation for the determination of total acidity

Number of moles of 20 mL 0.01 M NaOH (titrate)

$$= 0.01\frac{mol}{litre} \times 20ml \times \frac{1l}{1000ml} = 2 \times 10^{-4} moles\ NaOH$$

Number of moles of 2.1 mL 0.01 M HCl (titrant) (data obtained from the titrate analysis)

$$= 0.01\frac{mol}{litre} \times 2.1ml \times \frac{1l}{1000ml} = 2.1 \times 10^{-5} moles\ HCl$$

In a back titration analysis, the acid functional groups on the sample were neutralized with an excessive 20 mL 0.01 M NaOH to ensure a complete neutralization. Therefore, the total acidity of the sample can be calculated as:

$$= 2 \times 10^{-4} mol - 2.1 \times 10^{-5} mol = 0.179 mmol$$

The total acidity per gram of catalyst

$$= \frac{0.179 mmol}{0.05 gm} = 3.58 mmol/gm$$

Similarly for BC-450, BC-500, BC-450-SO_3H and BC-450-SO_3H. The measurement was repeated three times, with the average reported as the total acidity.

II. Sample calculation of SO_3H acid density for BC-400-SO_3H

Volume of 0.01 M NaOH (titrant) (data obtained from the titrate analysis): 2.7ml

SO_3H acidity per gram of the catalyst

$$= \frac{(volume\ of NaOH\ used\ for\ the\ titration * Normality\ of\ NaOH)}{Mass\ of\ catalyst} = \frac{2.7 \times 0.01}{0.05}$$

$$= 0.54 mmol/gm$$

Similarly for BC-450, BC-500, BC-450-SO_3H and BC-450-SO_3H. The measurement was repeated three times, with the average reported as the SO_3H acidity.